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Structural elements of the gills of the shore crab *Carcinus mediterraneus*

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ABSTRACT: Fine structural studies were conducted on the gills of the shore crab *Carcinus mediterraneus* using scanning electron microscopic techniques. The results obtained show the structural organization of crab gills from whole gills including spiny elements over the 150 lamellae to lamellar components such as cuticles, median shaft, marginal canal, afferent and efferent lamellar vessels and hemolymph cells. Enormous surface enlargement is accomplished by a variety of structural elements which allow rapid circulation of hemolymph. In the form of a relatively small organ, the gills fulfill all the necessary exchanges of specific molecules between the crab and its environment. Aggregations of ca 1-µm particles covering the outer cuticular surfaces are considered to be bacterial colonies of unknown properties and functions.

INTRODUCTION

During evolution, metazoans have developed organs serving a variety of specialized physiological functions. Crustacean gills are located in the interface between the organism and its typically aquatic environment, mediating exchange processes between the individual and its ambient medium. They are multifunctional organs fulfilling a complexity of metabolic roles, e.g. uptake of oxygen, excretion of carbon dioxide, active ion transport in osmoregulating species, acid-base balance of the hemolymph, and excretion of nitrogenous end products. These functions are interconnected to a high degree. Regarding the variety of metabolic roles, gills are comparatively unique organs within the animal kingdom.

Many aspects of the functioning of crab gills have been thoroughly investigated in the past decades. For a recent review, see Lucu (1990). However, descriptions of gill physiology will remain incomplete without knowledge of the structural matrix enabling the fulfillment of metabolic roles. In contrast to the large body of literature on biochemical and physiological aspects of crustacean gills, the literature dealing with the architecture and fine structure of this organ is comparatively scarce.

Following investigations on gill physiology in the osmoregulating shore crab *Carcinus maenas* (Siebers et al., 1986, 1988, 1989), the present work describes some structural elements of the gills of the shore crab *Carcinus mediterraneus* using scanning electron microscopical techniques.

MATERIALS AND METHODS

Adult male crabs *Carcinus mediterraneus* were caught by fishermen in the vicinity of the port of Lido in the lagoon of Venice. Here the salinity is 33 ± 2‰. Crabs were transferred to the laboratory and kept in aquaria. The sea water was filtered, and the crabs were fed with pieces of bovine heart three times weekly.

After removal of the carapace, one of the posterior gills, usually gill no. 7, was cut away from the body wall, blotted to near-dryness on paper tissues and transferred for 3 h at 4 °C to 2.5 % gluteraldehyde in 0.1 M Na^+ phosphate buffer, pH 7.2. The gill was subsequently rinsed three times for 30 min at 4 °C with 0.15 m Na^+ phosphate buffer, pH 7.2. Thereafter, the gill was transferred for 1 h to 1 % osmium tetroxide in 0.1 M Na^+ phosphate buffer pH 7.2 at 4 °C.

The gill was then rinsed four times for 4 min at room temperature with the phosphate buffer used for the second fixation, and dehydrated in graded solutions of ethanol. The ethanol concentrations used were 30 and 50 % for 2 min, 70, 85, 90, and 95 % for 5 min and 100 % for hours up to 1 day. Afterwards, the gills were dried repeatedly in a critical point (CPD) drying apparatus (Balzers) with liquid CO_2, which is completely mixable with ethanol, until the remaining ethanol was completely replaced.

Dried gills were coated with a 25 nm gold film in a S 150 A Edwards sputter coater apparatus and examined with a Cambridge Stereoscan 250 scanning electron microscope.

RESULTS

An intact posterior gill (no. 7) is shown in Figure 1. It consists of 150 flat lamellae which are lined at their outer edges with the marginal canals (see also Figs 2, 4–6). They belong to the system of lamellar vessels transporting within each platelet part of the circulating hemolymph from the afferent to the efferent vessel. On the lower right side, bulb-like enlargements of the marginal canals are seen (see also Fig. 4, lower right side). Between both sides of the lamellae the afferent vessel is seen in the centre of the gill.

Figure 2 shows the gill from the opposite side, exhibiting in its centre the efferent vessel. It is covered irregularly with spines (see also Figs 8–10). The insertion of the marginal canals of several lamellae into the efferent vessel is shown in Figure 3. In the troughs and infoldings between the insertion points dense assemblages of approximately 1-µm particles, presumably bacterial colonies, are obvious (see "Discussion").

A transversal section of a posterior gill no. 7 (Fig. 4) reveals the shape of an individual lamella. Its surface is covered by the cuticle which is characterized by a specific pattern of ripples. The photo shows the afferent vessel on the left and the efferent vessel on the right side. Both vessels are connected by the median shaft which separates the individual platelet into two halves. Note the bulb-like enlargements of the marginal canals on the lower right side.

Figure 5, an enlarged sector of Figure 4, shows the surface of a lamella with the marginal canal on the upper right side and the wave-shaped pattern of cuticular ripples. In the troughs between the ripples assemblages of approximately 1-µm particles are present, probably identical to the bacterial colonies in Figure 3.

Removal of the upper layer of epithelial cells reveals the interior of the gill platelet (Fig. 6). Right of the marginal canal (on the left side of the photo) the lamellar septum is

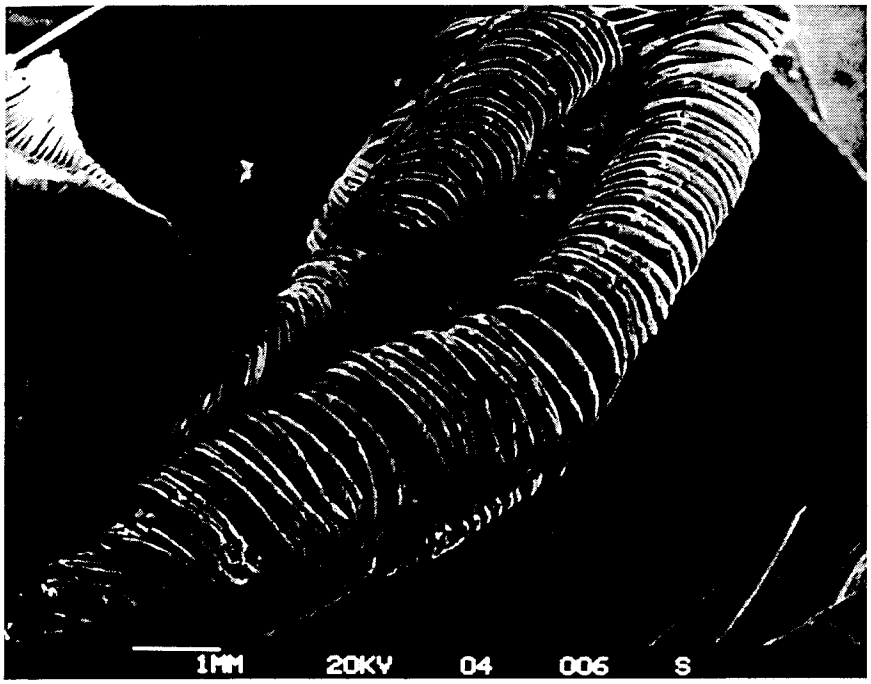

Fig. 1. Isolated gill no. 7 with its tip on the left side, showing the lamellae and the afferent vessel in the central part

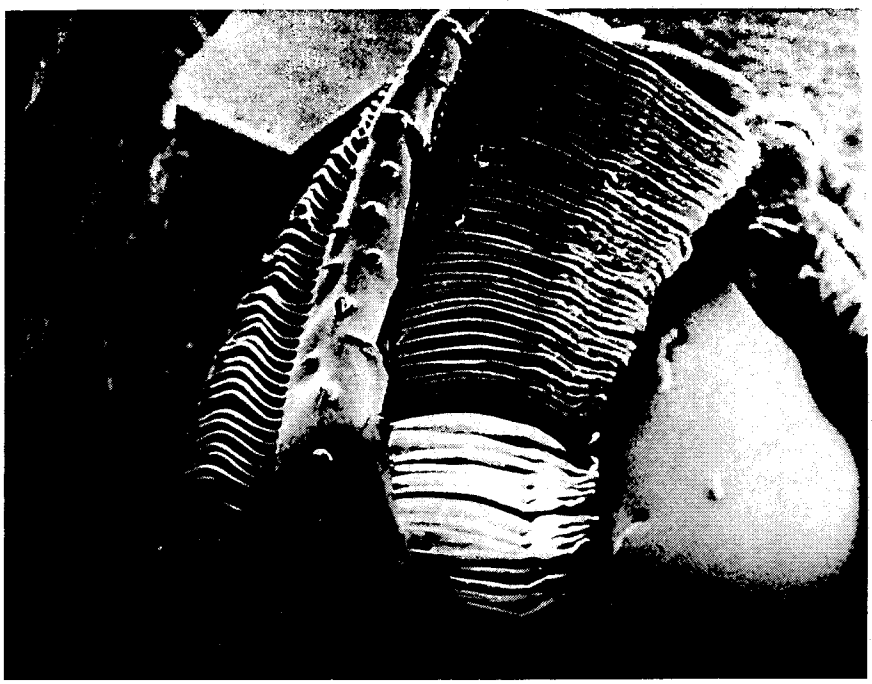

Fig. 2. Isolated posterior gill no. 7 with its lamellae and the irregular pattern of spines on the external surface of the efferent vessel

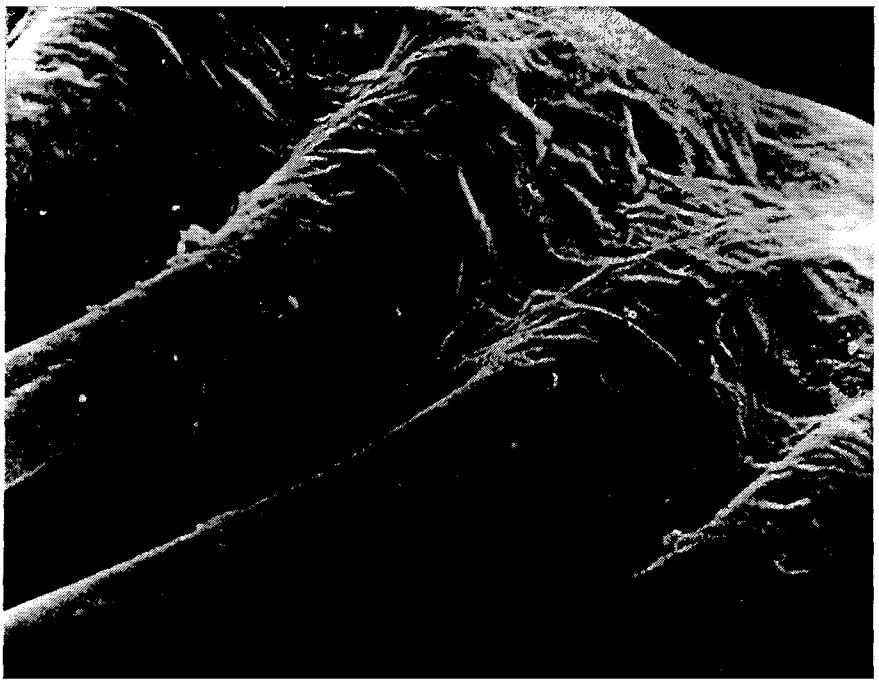

Fig. 3. Insertion of marginal canals of individual lamellae into the efferent vessel. Note bacterial assemblages especially between the insertions

Fig. 4. Transversal section of a posterior gill (no. 7) showing the surface of an individual lamella, the afferent vessel on the left and the efferent vessel on the right side connected by the median shaft. Note the marginal canals at the edges of the lamellae and the pattern of ripples on the lamellar surface

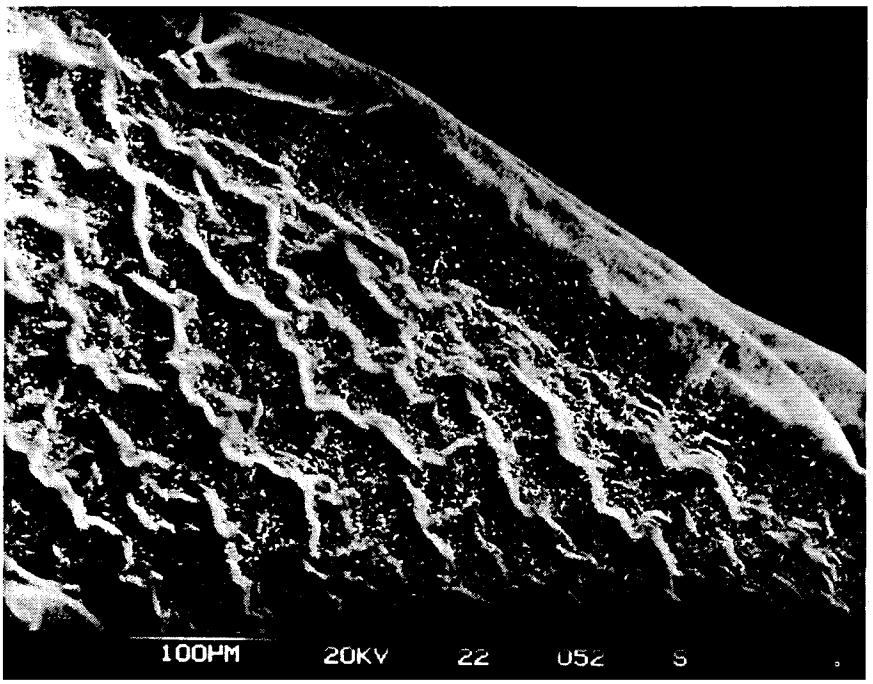

Fig. 5. Sector of Fig. 4 showing the pattern of ripples on the lamellar surface and bacterial assemblages in the spaces between the ripples. The marginal canal is seen on the upper right side

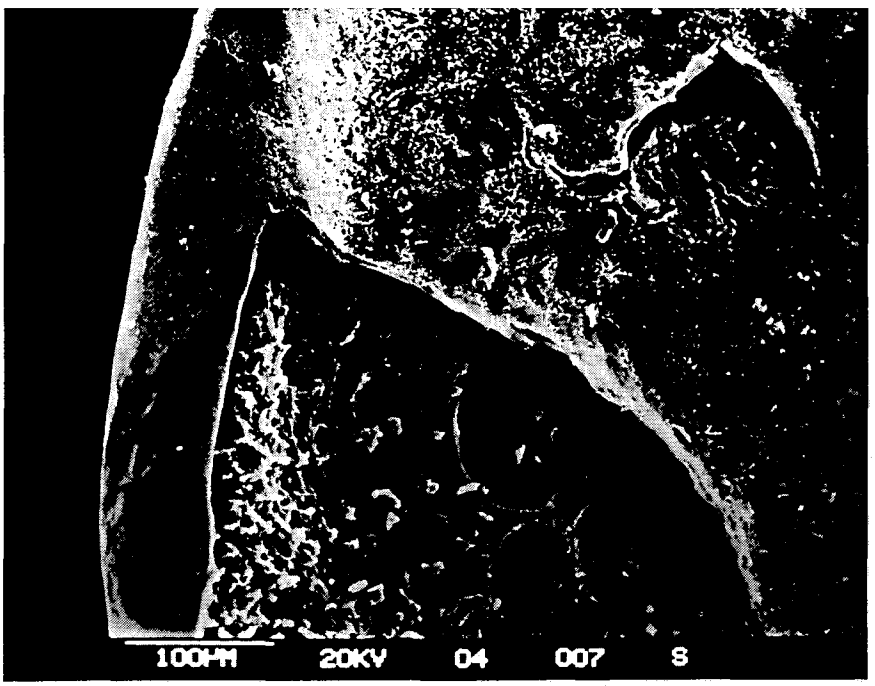

Fig. 6. Sector of Fig. 4 with the upper cellular layer broken away revealing the partially perforated lamellar septum with single hemolymph cells on its surface. On the left side the marginal canal is visible

seen with several openings allowing hemolymph passage. In agreement with Taylor & Taylor (1986) the light particulate structures of the surface of the lamellar septum are identified as hemolymph cells. They are clearly discernible from the considerably smaller particles seen as dense layers on the cuticle on the upper right side of the photo.

The longitudinal section shown in Figure 7 shows the appearance of a multi-folded organ. The large flat layers of the foldings reveal the nature of the gill platelet which is formed by a double layer of epithelial cells. The two folded systems are separated by the median shaft (from upper left to middle right side of the photo).

Fig. 7. Longitudinal section of a posterior gill (no. 7) showing both layers of each lamella. The surface layers of neighbouring lamellae are connected, thus building a system of multiple foldings

Figure 8 shows a sector of the efferent vessel covered with two spines which are inserted on a cone-shaped basis. Between the spines and on the surface of the cone-shaped basis the pattern of cuticular ripples is obvious, and on the upper left side some bacterial aggregations are seen. Removal of the spines on the efferent vessel allows a view on the network of tube-like structures forming the basis of the spines (Fig. 9). They are considered to be a sturdy mechanical skeleton.

The view into the efferent vessel (Fig. 10) reveals a round canal covered, and thus stabilized, with circular tissue elements. The light particles are more or less deformed hemolymph cells.

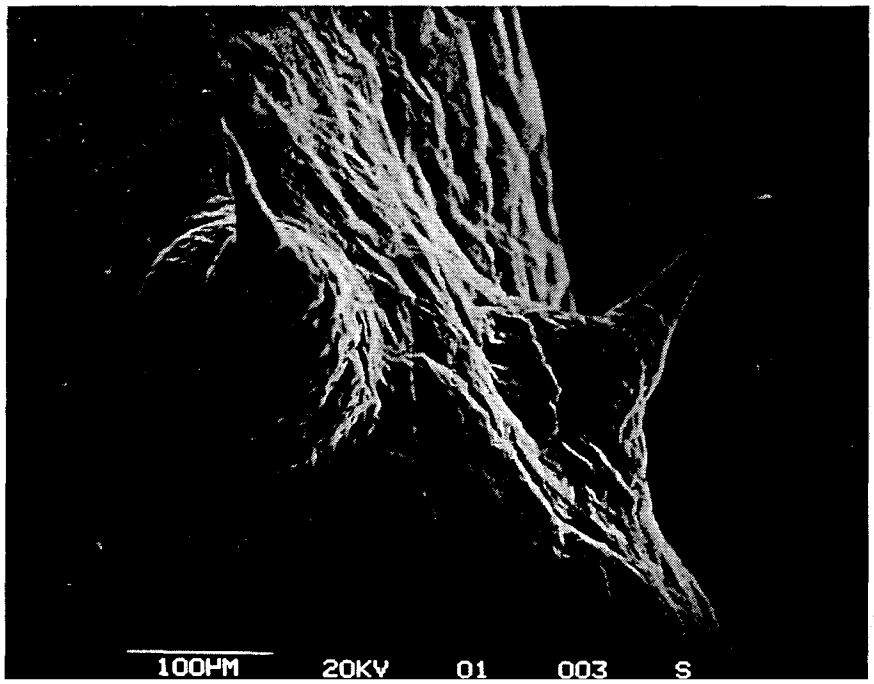

Fig. 8. Enlargement of spines located on the efferent vessel as shown in Fig. 2

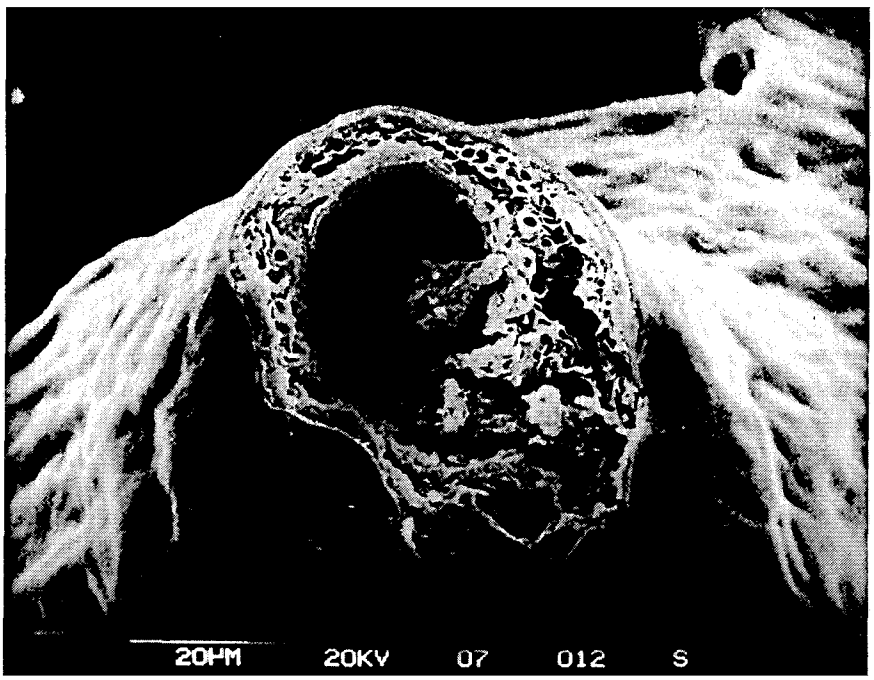

Fig. 9. Section of epithelium of the efferent vessel showing the insertion point of the spine

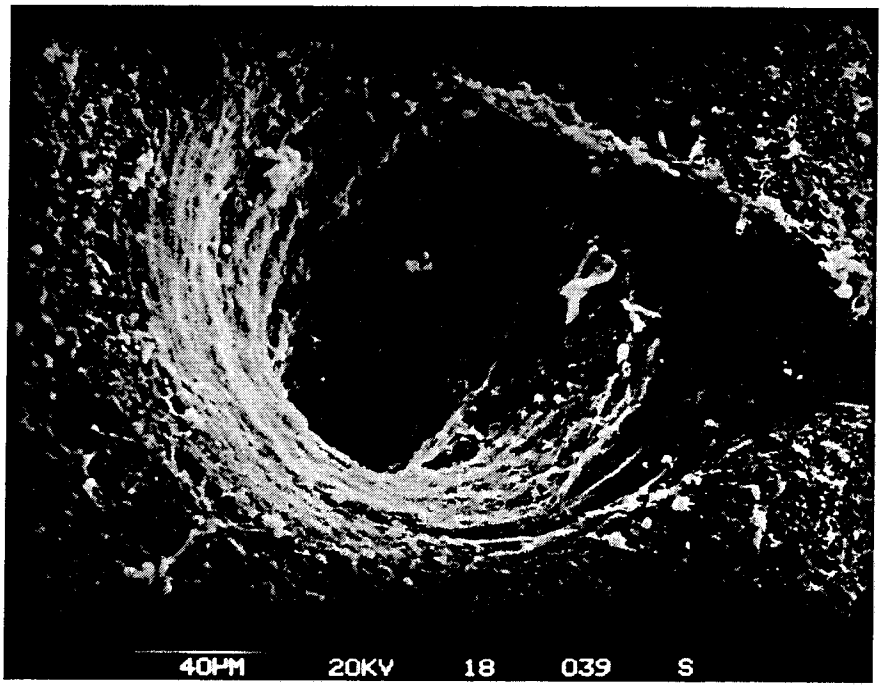

Fig. 10. Enlargement of the insertion of a spine seen from the interior of the efferent vessel. Several hemolymph cells are present on the internal surface of the vessel

DISCUSSION

Fine structural studies have been conducted on the gills of several decapod crustaceans such as *Callinectes* (Copeland & Fitzjarrel, 1968; Cioffi, 1984), *Gecarcinus* (Copeland, 1968), *Hemigrapsus* and *Pachygrapsus* (Wright, 1964), *Holthuisana* (Taylor & Greenaway, 1979), *Ocypode* (Flemister, 1959; Storch & Welsch, 1975), *Panulirus* (Strangeways-Dixon & Smith, 1970), *Penaeus* (Talbot et al., 1972; Foster & Howse, 1978), *Eriocheir* (Barra et al., 1983), *Uca* (Finol & Croghan, 1983), *Goniopsis* (Martelo & Zanders, 1986) and *Carcinus* (Taylor & Taylor, 1986; Goodman & Cavey, 1990). In only a few of these reports were scanning electron microscopic techniques employed.

The figures presented show a variety of structural elements specific of crustacean gills. The gill architecture is characterized by enormous surface enlargements allowing rapid movements of hemolymph over the internal surfaces and passages of ambient medium over the external surfaces. The gills thus represent in the form of a relatively small organ the technical solution developed during evolution to allow necessary exchanges of specific molecules between an organism with a hard cover and its environment.

The micrographs shown allow us to describe the flow of hemolymph in the gill. The gills of the shore crab *Carcinus mediterraneus* are composed of many repeated elements in the form of lamellae (Figs 1, 2, 4, 7) the area of which decreases in the direction of the tip. Two symmetrical half lamellae are connected by the median shaft (Figs 4, 7). The two

ends of the median shaft are represented by the afferent (Fig. 1) and the efferent (Figs 2, 3, 9, 10) vessel. Both vessels are connected at their base with the system of circulating hemolymph in the body cavity via a common opening in the lateral side of the carapace. The afferent vessel is directed to the body wall and the efferent vessel is located on the opposite side. The gill is oriented with the base in a lower position and the tip in an upper position.

The lamellae consist of two unicellular epithelia separating the ambient medium from the hemolymph (Fig. 7). Their outer sides are covered by the cuticles, three-layered structures released from the body together with the exuvia during the moulting process. Between the basal membranes of the two epithelial cell layers, a perforated lamellar septum is present (Fig. 6), and the hemolymph flow proceeds on both sides of it.

The circulating hemolymph enters the gill platelet via openings in the afferent vessel. The hemolymph flow within the gill proceeds within discrete canals of the internal lacunar system and the marginal canal of the platelet to reenter the body via valve-like openings of the afferent vessel (Taylor & Taylor, 1986).

The spines covering irregularly the efferent vessel are regarded as elements of considerable mechanical stability, however of as yet unknown physiological function. The presence of mattresses of small particulate elements on the cuticles (Figs 3, 5, 6, 8) has also been reported as "dark bodies on the outer surface of the cuticle" of the gills of the blue crab, *Callinectes sapidus*, by Copeland & Fitzjarrel (1968) and are considered to be "symbiontic algalike forms". They have also been observed in the form of dense aggregations of prolonged particles on the cuticles of the gills of the shore crab *Carcinus maenas* by Goodman & Cavey (1990) who considered them as "bacteria adhering to the epicuticle". Further work is deserved to clarify the nature of these particles, the coexistence with their hosts as well as their potential physiological role in gill functions.

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