

The gill morphology of the date mussel *Lithophaga lithophaga* (Bivalvia: Mytilidae)

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Abstract: The purpose of the present research was to study the descriptive anatomy of the gill of the mussel *Lithophaga lithophaga*. Mussels were collected from 2 sites in Antalya Bay, Turkey. Observations using stereomicroscopy and electron microscopy showed that the gills of *L. lithophaga* consist of homorhabdic filaments, a characteristic of the mytilids. The lamellae have cavities at their laterals in addition to their prominent ciliary structure on the frontal and lateral surfaces. The latero-frontal cilia extend in pairs, one attached to the other with a membrane and showing a composite structure. The ascending and descending lamellae are fused by fan-like cilia groups and form a food groove at their ventral margins. Scabrous blocks at the abfrontal edges of the ascending lamellae appear to function as food graters. The relatively large food particles captured by the mussel are ground into smaller pieces to be ingested. These morphological features of the gills of *L. lithophaga* can be used for comparison with those of other congeners or mytilids.

Key words: Ciliary structures, date mussel, gill, scanning electron microscopy

1. Introduction

Lithophaga lithophaga L. 1758 (family Mytilidae), the date mussel, is a widespread species native to the infralittoral zones of the Mediterranean Sea, Atlantic Ocean, and Red Sea (Turner and Boss, 1962; Gargominy et al., 1999; El-Menif et al., 2007; Devescovi, 2009). The species is one of the principal boring organisms on any available calcareous substrate (Morton and Scott, 1980; Gargominy et al., 1999). Bivalve gills perform many functions. In addition to their respiratory role, they function to collect food particles and to facilitate the dispersal of gametes by establishing a water current in the mantle cavity (Gosling, 2003).

The morphological characteristics of any organ, such as the structure of the gills, are important for ecological studies, as well as for systematic and cladistic analysis. Although a number of previous studies have addressed the life history and ecological features of the members of the genus *Lithophaga* (Valli et al., 1986; Scott, 1988; Brickner et al., 1993; Galinou-Mitsoudi and Sinis, 1994–1995; El-Menif et al., 2007; Devescovi, 2009), including limited descriptions of the general anatomy of the genus (Turner and Boss, 1962; Simone and Goncalves, 2006), very little has been published on its ctenidial structure (Turner and Boss, 1962). Well-documented observations are available on other genera of Mytilidae: *Mytilus*, *Mytella*, and *Perna*

(Aiello and Sleigh, 1972; Domouhtsidou and Dimitriadis, 2000; Gregory and George, 2000; Gregory et al., 2002; David and Fontanetti, 2005; David et al., 2008).

The purpose of the present study is to investigate the descriptive and functional anatomy of the gill of *L. lithophaga* in order to understand the morphological adaptations and ecological characteristics of the species.

2. Materials and methods

2.1. Study area

Samples of *L. lithophaga* were collected from limestone rocks on the littoral of Antalya Bay (at 2 sites with coordinates 36°31'24.40"N, 30°33'06.67"E and 36°50'39.70"N, 31°04'12.68"E) on the southwestern coast of Turkey.

2.2. Materials

Dense aggregations of *L. lithophaga* were identified at the study sites. The organisms bored into the substrate to form these aggregations. Rocks were detached by breaking the blocks with special sledgehammers during scuba-diving in April 2010, and individuals were then extracted from the rocks. Two collections were made at each site, with 4 specimens in each case. All of the specimens were transported to the laboratory at the Faculty of Aquatic Sciences and Fisheries at Akdeniz University. Two specimens from each site were washed. They were opened

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by breaking their shells, washed again in sea water, and photographed with a stereomicroscope. The undamaged areas of the gills were excised.

2.3. Scanning electron microscopy

The gills of the specimens were fixed in Bouin's solution. After dehydration in ethanol/amyl acetate, the ctenidia were critical point-dried, mounted flat on aluminum stubs, coated with gold-palladium according to a method modified from David and Fontanetti (2005), and studied with a Zeiss Leo 1430 scanning electron microscope at the Akdeniz University Medical School EM Unit (TEMGA).

3. Results

The gills of *L. lithophaga* are a very prominent anatomical structure. A double set of gills on each side of the body effectively divides the mantle cavity into chambers. The inner and outer demibranchs are approximately the same size (Figures 1a and 1b). Each demibranch has parallel filaments ridged on their distal portions (Figure 1c).

The filaments have a homorhabdic shape. The ctenidial axis of the gill has a spinal ridge with a rough, ciliated surface. A prominent canal is present at the dorsal end of the axial region, and dentation is present at the lower edge

(Figures 2a and 2b). Many particles, especially protozoa, appeared to be trapped within the canal (Figures 2b and 2c). The descending lamellae were not different from the ascending lamellae. They were attached lengthwise at intervals, showing the eulamellibranch gill (Figure 2a).

The lamellae appear as a grille in an abfrontal view and as a thick ciliary cover from the frontal side (Figure 3a). A plica formation outlines a cavity between the latero-frontal and lateral arrays of cilia along the lamella. Lengthwise channels extend from the top to the bottom of the filaments. The food groove is clearly visible at the free-folding end of the filaments. Two-leveled ciliary discs are present at regular intervals on the ascending and descending lamellae and serve to interlock the filaments (Figures 3b and 3c).

The free ends of the filaments bulge slightly around the food groove and are characterized by marginal cilia. The ciliary arrangement forms a short extreme portion with a thick cover from an internal view. Each arrangement has a fan of cilia at its base. Ascending and descending lamellae, connected by those fan-like cilia groups, extend onwards as 2 layers from the end piece (Figure 4a). The filaments are attached by recurring ciliary junctions. The

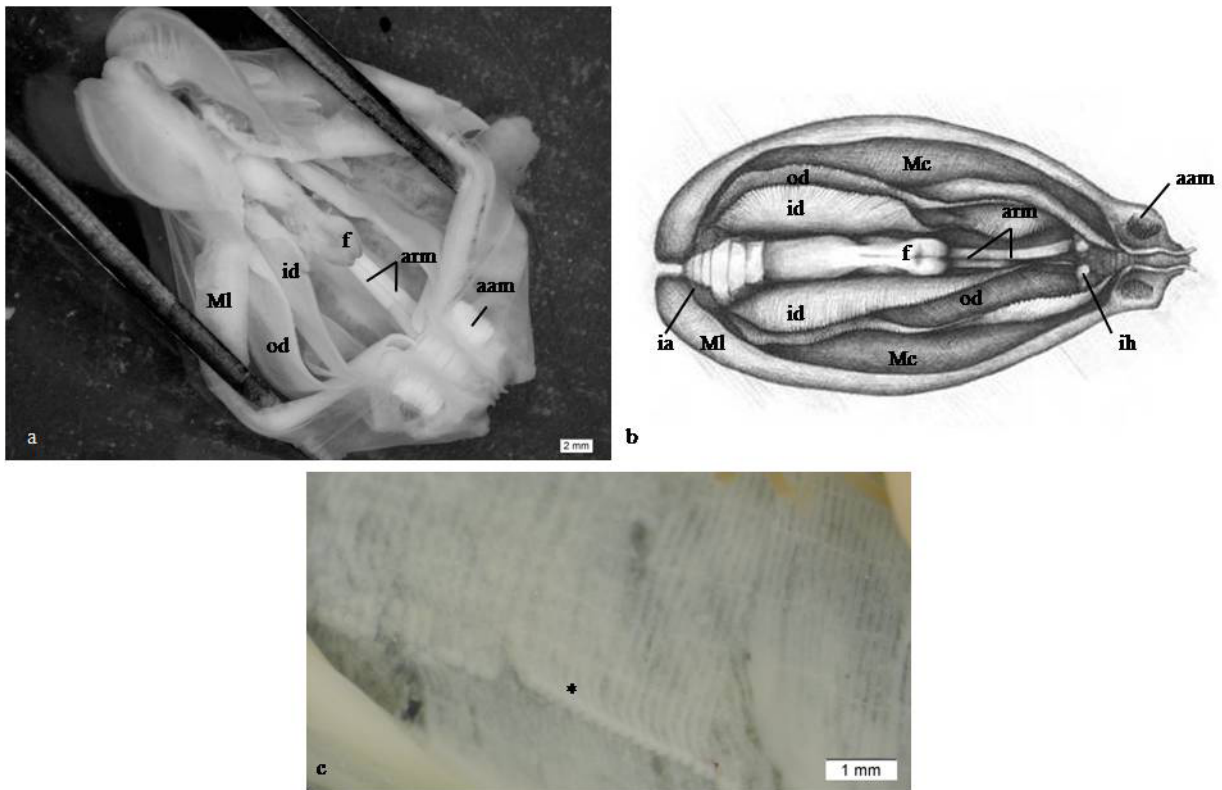


Figure 1. a. General anatomy of *L. lithophaga*. b. Schematic view of the gills showing very prominent double sheets within the mantle cavity. c. A ridged demibranch (*). Ml: mantle layers, Mc: mantle cavity, f: foot, ea: excurrent aperture, ia: incurrent aperture, aam: anterior adductor muscle, arm: anterior retractors, ih: inner hemipal, od: outer demibranchs, id: inner demibranchs.

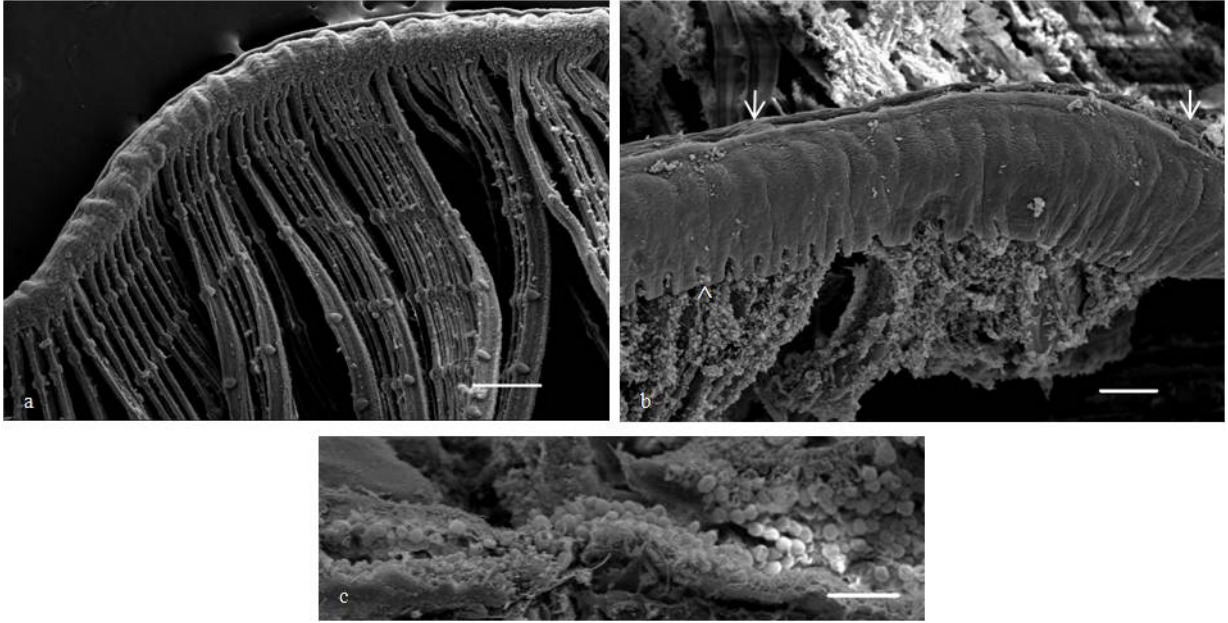


Figure 2. Axial region of the ctenidia. **a.** A narrow axis and thin filaments of the gill of *L. lithophaga*. **b.** A prominent canal, full of food material (arrows) at the dorsal end of the axial region and a dentation at the lower edge (arrowhead). **c.** Protozoa trapped in the axial canal. Scale bar: a = 200 µm, b = 100 µm, c = 50 µm.

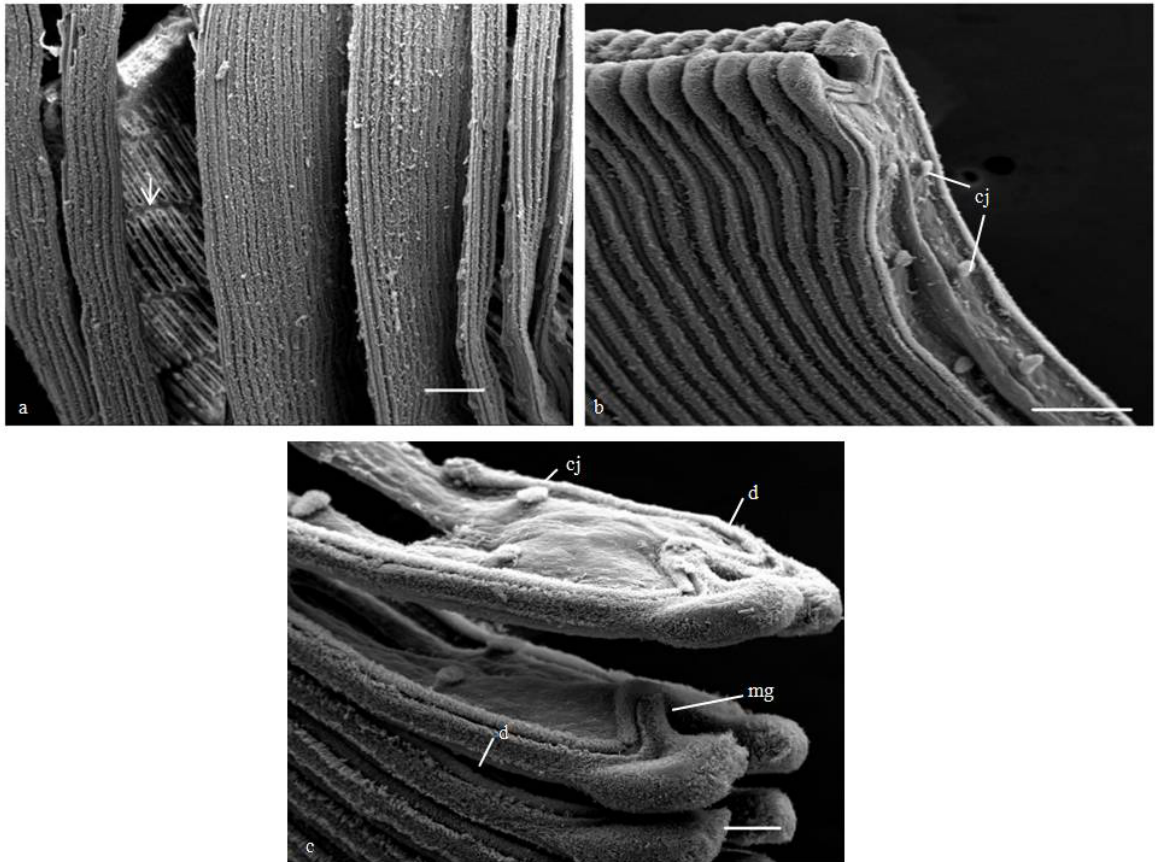


Figure 3. **a.** Frontal and abfrontal (arrow) views of the demibranches of *L. lithophaga*. **b.** Canal formation and food grooves are very prominent at the ventral end of the filaments. Leveled connective discs occur at regular intervals along the filaments; cj: ciliary junction. **c.** Enlarged free end with marginal groove (mg) and duct (d) on the surface. Scale bar: a = 200 µm, b = 100 µm, c = 40 µm.

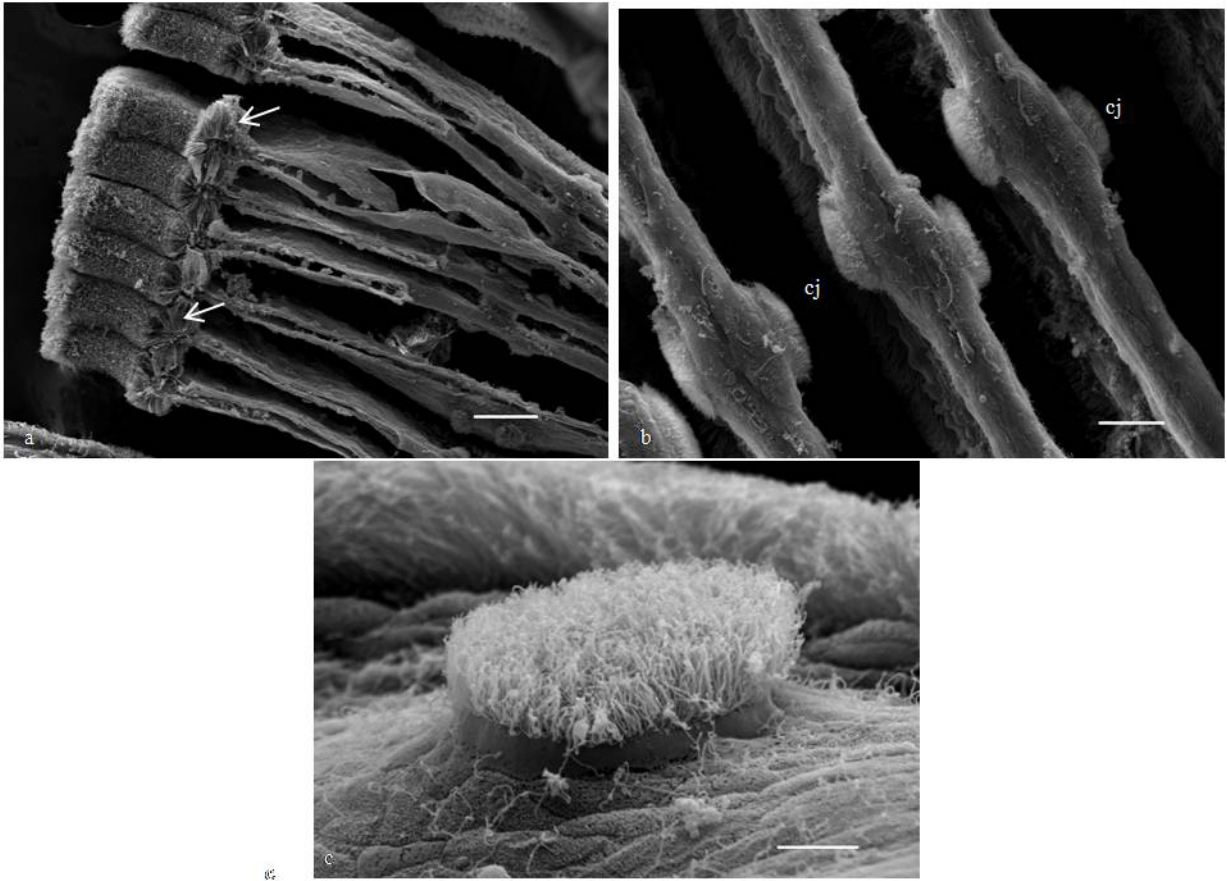


Figure 4. **a.** Inner sides of the food grooves of the filaments in *L. lithophaga* with a thick ciliary head and fan-shaped ciliary connection of the lamellae (arrows). **b.** Filaments attached by continual ciliary junctions (cj). **c.** Discs formed by condensed tufts of simple cilia. Scale bar: a = 60 μ m, b = 20 μ m, c = 6 μ m.

discs are formed by condensed tufts of simple cilia, which are rooted from a base extended from the latero-abfrontal surfaces of the lamellae (Figures 4b and 4c).

The ciliary covers of the filaments show different cilia formations from the frontal side. The frontal and lateral cilia are much shorter than the latero-frontal ones (Figure 5a). The latero-frontal cilia extend in pairs, one attached to the other with a membrane, and show a composite structure (Figure 5b). These cilia branch bidirectionally along their axis and appear to be very effective functionally in maintaining the water flow and collecting pieces of food. Among many particles aggregated by the mucus secretion, solitary protozoa were observed on the frontal cilia (Figure 5c).

The length of the filaments varies from place to place. Three layers with a brick-like pattern are formed by blocks with scabrous surfaces at the abfrontal edges of the ascending lamellae, primarily adjacent to the marginal groove (Figure 6a) and in the slits between the lamellar junctions. These blocks appear to function as food graters,

and particulate material aggregated with mucus adhered to their surfaces (Figure 6b).

However, the abfrontal surfaces of the descending lamellae bear many infrequently occurring ostia at scattered locations on the gill (Figure 7).

4. Discussion

The general architecture and the arrangement of the filaments of the gills in *L. lithophaga* are partially similar to those documented for other mytilids, such as *Perna perna*, *Mytella falcata*, and *Mytilus edulis* (Beninger and St Jean, 1997; Gregory and George, 2000; David and Fontanetti, 2005). Among the first drawings of the ctenidia of *L. lithophaga*, that furnished by Turner and Boss (1962) shows that the prominent 2 sheets of the outer and inner demibranches are very similar.

In many mytilid species, ciliation covers not only the frontal surface but also the lateral surfaces, and scattered cilia are recorded on the abfrontal surface of the filaments (Gregory and George, 2000; David and Fontanetti, 2005).

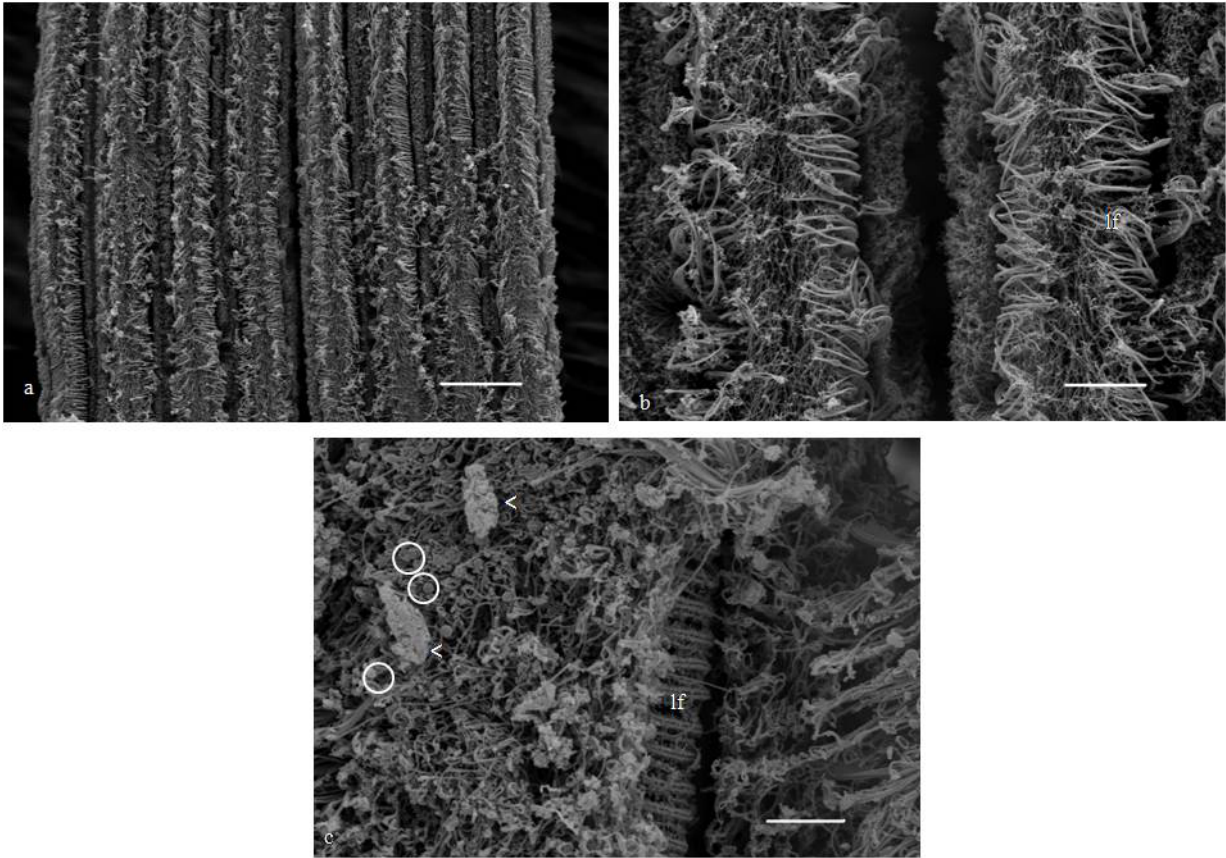


Figure 5. Ciliation on the filaments from the frontal view in the gill of *L. lithophaga*. **a.** Three very thick layers of cilia are prominent. **b.** Latero-frontal cilia in pairs. **c.** Duality in latero-frontal cilia and aggregated (arrowheads) or singly distributed (circles) food particles on the ciliary surface. lf: latero-frontal cilia. Scale bar: a = 40 μ m, b = 10 μ m, c = 4 μ m.

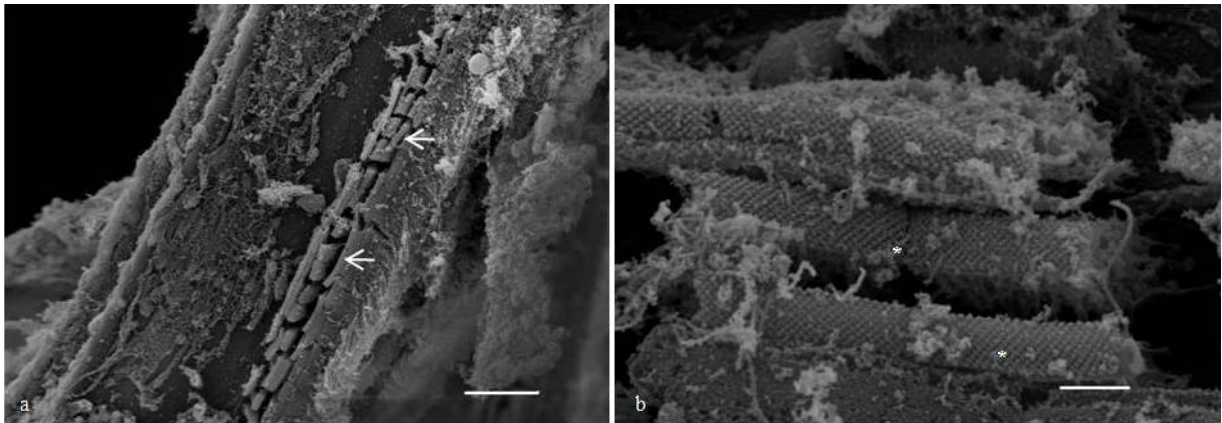


Figure 6. **a.** An ascending lamella showing a series of bricklike structures on the abfrontal edges (arrows) in the gill of *L. lithophaga*. **b.** Tissue blocks with a toothed plane on the surface (*). Scale bars = 20 μ m.

The regular distribution of ciliary junctions, formed by simple cilia, is the same as that described in other species of Mytilidae: *Perna perna* (Gregory and George, 2000), *Mytella falcata* (David and Fontanetti, 2005), and *Modiolus*

barbatus (Falakali Mutaf et al., 2009). The aligned rows of ciliary discs with their evident distribution pattern in *Lithophaga* appear to function primarily to protect the filaments from hydrodynamic forces and to regulate the

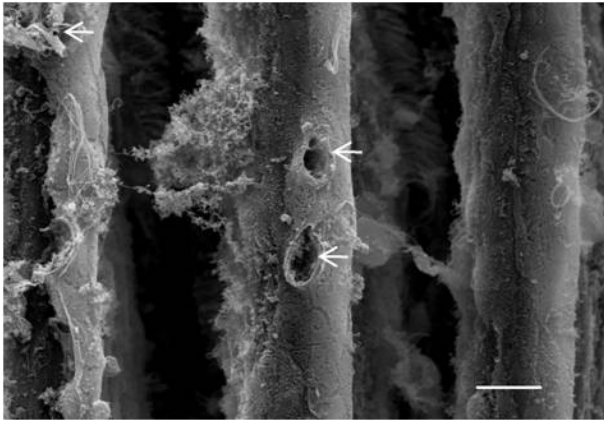


Figure 7. Ostia (arrows) distributed unevenly along the abfrontal surface of the lamellae. Scale bar = 10 μ m.

flow of water through the interfilamentary spaces. The mechanical adhesion of the ciliary junctions has previously been described in an ultrastructural study of the ciliary tips in scallop gills (Reed-Miller and Greenberg, 1982).

Because *Lithophaga* is permanently buried in the substratum after it settles, its gill has come to function efficiently to make the best use of the hydrodynamics of the immediate surroundings, as in other bivalves. Moreover, *Lithophaga* appears to use its gill to collect particles via 2 basic mechanisms, mechanical sieving and aerosol trapping, as described in many previous studies (Silverman et al., 1996; Dufour and Beninger, 2001). The ciliated protozoa accommodated in the axial canal of the ctenidia and observed in the present study appear to be an important component of the nutrition and feeding of *L. lithophaga*. A symbiotic ciliated protozoan has been described as dislodging from the ctenidia to the buccal region of *Placopecten magellanicus* (Beninger et al., 1988). The cavities formed by a ciliated-plial boundary and connected to the food groove appear to increase the supply of food particles through a controlled water flow. Atkins (1937) had earlier indicated that fine particles were transported via that pathway. The fan-like ciliary connection between the ascending and descending lamellae presumably serves to open and close the ventral

groove, which is involved in transporting food particles to the labial palp (Ward et al., 1993). The structure of the fused pairs of latero-frontal cilia in *L. lithophaga* is thought to be effective in water current, particle capture, and transport. This structure has been extensively described as a composite structure of 8–20 fused cilia in certain other bivalve species (Atkins, 1938; Ward et al., 1993; Silverman et al., 1996; David and Fontanetti, 2005).

The blocks with a scabrous surface present in the slits next to the marginal groove and between the tissue junctions of the lamellae are assumed to grate captured food particles that are large relative to the size of the aperture through which they must enter into smaller pieces to be molded with mucus and consumed by the mussel. Previous studies had not found any capability to grate food particles to finer dimensions, although the characteristics of the particles that are captured have been a subject of debate for many years. The results of this study suggest that the feeding mechanism of *L. lithophaga* involves the use of the gills for the collection of food particles. The particles are selected by size, and the larger particles are resized into ingestible forms.

In conclusion, the gill of *L. lithophaga* shows significant morphological features in addition to the characters common to mytilid bivalves. The pairing of the latero-frontal cilia has not been described previously in the anatomy of any other bivalve species, although no evidence has been available to highlight its significance. It appears reasonable to assume that the fan-like ciliary connection could be effective as a push-and-pull mechanism to control the food-holding capacity of the ventral food groove. The grater blocks appear to represent a distinctive character that is beneficial to the organism in its fixed location within the limestone. These possibly unique variations in the gill of *L. lithophaga* can be used for comparison with samples from different substrates that the species may inhabit with other congeners or mytilids.

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