

The surface morphology of the ctenidia of *Spondylus spinosus* (Mollusca: Bivalvia) from Antalya Bay, Turkey

Deniz Aksit^{1,2}, Beria Falakalı Mutaf¹ & Ahmet Balcı¹

¹ Akdeniz University, Faculty of Aquatic Sciences and Fisheries, Antalya, Turkey.

² Corresponding author. E-mail: denizaksit@akdeniz.edu.tr

ABSTRACT. The surface morphology of the ctenidia of *Spondylus spinosus* Schreibers, 1793 was studied with light and scanning electron microscopy for comparison with the gill structures of other bivalves. The demibranch of *S. spinosus* is heterorhabdic, with the principal filaments at the descending lamellae and ordinary filaments at the ascending lamellae. The gill lamellae have a prominent gauze-like structure at their distal part, with numerous groups of eight ordinary filaments. They bear ciliary arrays on their frontal surfaces and ostia at their latero-frontal surfaces. Frequent cirral plates form regular interfilamentary junctions. The description of the gill structure of *S. spinosus* presented here can be used to derive implications for the correlations among the structure, habitat and mode of life of this species. At a particular stage of its adult life, *Spondylus spinosus* could be used as a subject for biomonitoring studies in natural and experimental environments.

KEY WORDS. Ciliary structures; ctenidia; morphology; scanning electron microscopy (SEM); spiny oyster.

Species of *Spondylus* Linnaeus, 1758, also known as spiny oysters, are the only bivalve molluscs of the family Spondylidae Gray, 1826. *Spondylus spinosus* Schreibers, 1793 is highly abundant on Mediterranean shores (ZENETOS *et al.* 2003), a habitat where these bivalves live attached to rocky substrates. As a result of evolutionary processes, the gills of *Spondylus* spp. perform many functions, collecting food particles and facilitating the dispersal of gametes in addition to their role in respiration, which includes establishing a water current in the mantle cavity as part of the circulatory system (GOSLING 2003). In brief, the gills consist of two ctenidia, each containing two demibranchs; each V-shaped demibranch has inner and outer components consisting of two lamellae with or without marginal grooves at their free end (GOSLING 2003).

The use of bivalve mollusks to assess national marine resources and their biological status has been the subject of many extensive studies (DOMOUHSIDOU & DIMITRIADIS 2000, DAVID & FONTANETTI 2005, USHEVA *et al.* 2006). In species of Bivalvia, the gills and digestive glands are the first organs exposed to water and its pollutants (DOMOUHSIDOU & DIMITRIADIS 2000). Despite the abundance of the genus in tropical and subtropical seawaters worldwide, very little is known about the basic biology and ecology of *Spondylus* spp. Although, some research on the reproductive biology of several species has been performed (VILLALEJO-FUERTE *et al.* 2002), very little information is available on the morphology of the organs of this bivalve (YOUNG 1973).

The main purpose of this study was to use light and scanning electron microscopy to provide detailed information on

the structure of the gill filaments of *S. spinosus*, as we believe that an understanding of this structure will serve as a tool of fundamental importance in biomonitoring studies.

MATERIAL AND METHODS

Spondylus spinosus individuals were collected from the littoral zone of the rocky shores of Antalya Bay (between 36°36'25.22"-36°53'04.26"N and 30°42'03.62"-31°46'31.30"E) on the southwestern coast of Turkey, a site where dense aggregations were found attached to the substrate. The individuals were extracted from rocks that were detached from the substrate by breakage using special sledgehammers during scuba diving excursions. All of the specimens were transported to the laboratory at the Faculty of Aquatic Sciences and Fisheries at Akdeniz University. Four specimens were washed, opened by breaking their shells and washed again in sea water.

The general morphology of the ctenidia of all the individuals was observed using stereo-light microscopy (LM). Undamaged areas of the gills were excised, and the dissected specimens were examined by LM and scanning electron microscopy (SEM). The gill lamellae were fixed in Bouin's solution for LM and in 2.5% glutaraldehyde in Sorensen's buffer (0.1 M, pH 7.3) for SEM. For LM, the specimens were cleared in xylene after rinsing in a graded-ethanol series and embedded in wax. Sections of 4-7 µm were stained with haematoxylin and eosin (H-E) (DRURY & WALLINGTON 1973). For SEM, the dehydrated and air-dried lamellae were mounted flat on aluminium stubs and coated with gold-palladium (AKSIT & FALAKALI

MUTAF 2011). We examined the lamellar morphology using a Zeiss Leo 1430 Scanning Electron Microscope at the Akdeniz University Medical School EM Unit (TEMGA).

The following abbreviations are used in this report: (Af.bv) afferent blood vessel, (al) ascending lamella, (C.a) ctenidial axis, (cd) ciliary disc, (ce) ciliary epithelium, (dl) descending lamella, (Ef.bv.) efferent blood vessel, (H-E) haematoxylin and eosin, (mg) marginal gutter, (nce) non-ciliated epithelium, (of) ordinary filaments, (pf) principal filaments, (wt) water tubule, (lf) latero-frontal cilia, (fc) frontal cilia, (◀) ostia.

RESULTS

The ctenidia of *S. spinosus* represent the second largest gross anatomical structure of the visceral parts (Fig. 1), exceeded in size only by the single adductor muscle. Each of the ctenidia of *S. spinosus* is formed by two V-shaped demibranchs attached to a thick ctenidial axis, with the ascending lamella one-third shorter in length than the descending lamella (Fig. 2). The branchial haemolymph vessel was prominent (Fig. 3). There was no marginal food groove at the free distal end.

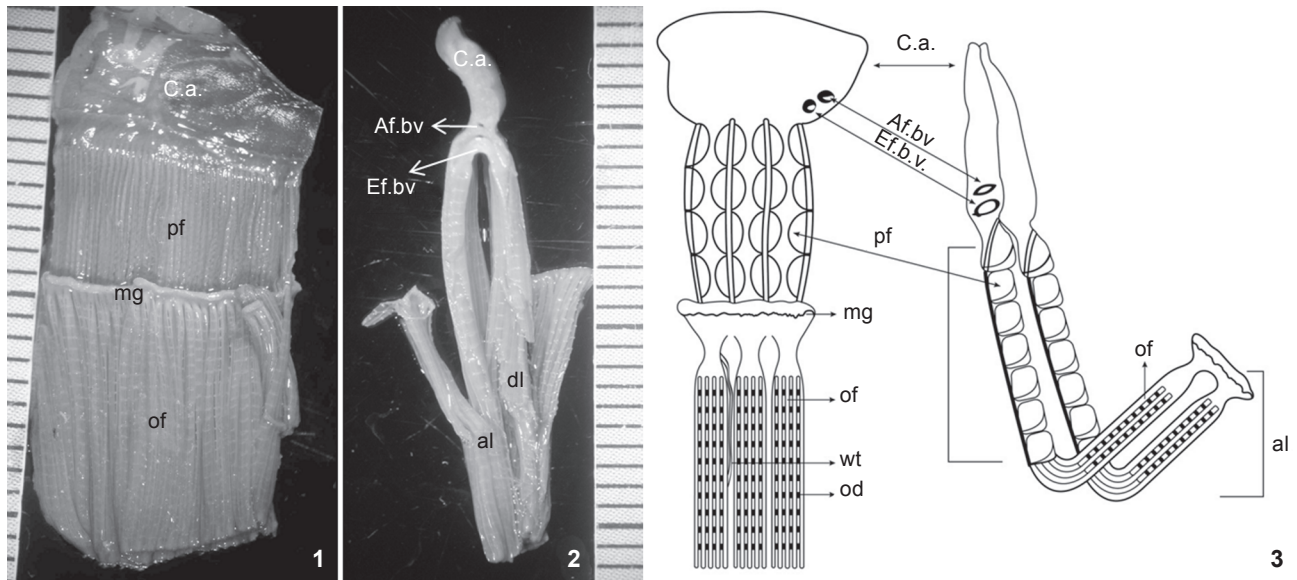
The filaments presented different appearances on the frontal surfaces of the ascending (al) and descending (dl) lamellae. The descending lamellae were thicker than the ascending, and the ascending lamellae differed from the descending by the presence of minor lamellar subunits and transparent water tubules (Fig. 3). The filaments of this fillibranch gill were heterorhabdic in appearance (Figs 1-6).

On the descending lamella, thick principal filaments were present, consisting of two different components: a straight outer part and spirally wound cord-like inner pieces (Figs 4-6). The straight branch was covered with a thick ciliary sheet, and the wound portion contained more than two pieced folds, which appeared to fold backwards, assuming a thick pocket-like appearance (Fig. 6). No interlamellar junctions were observed.

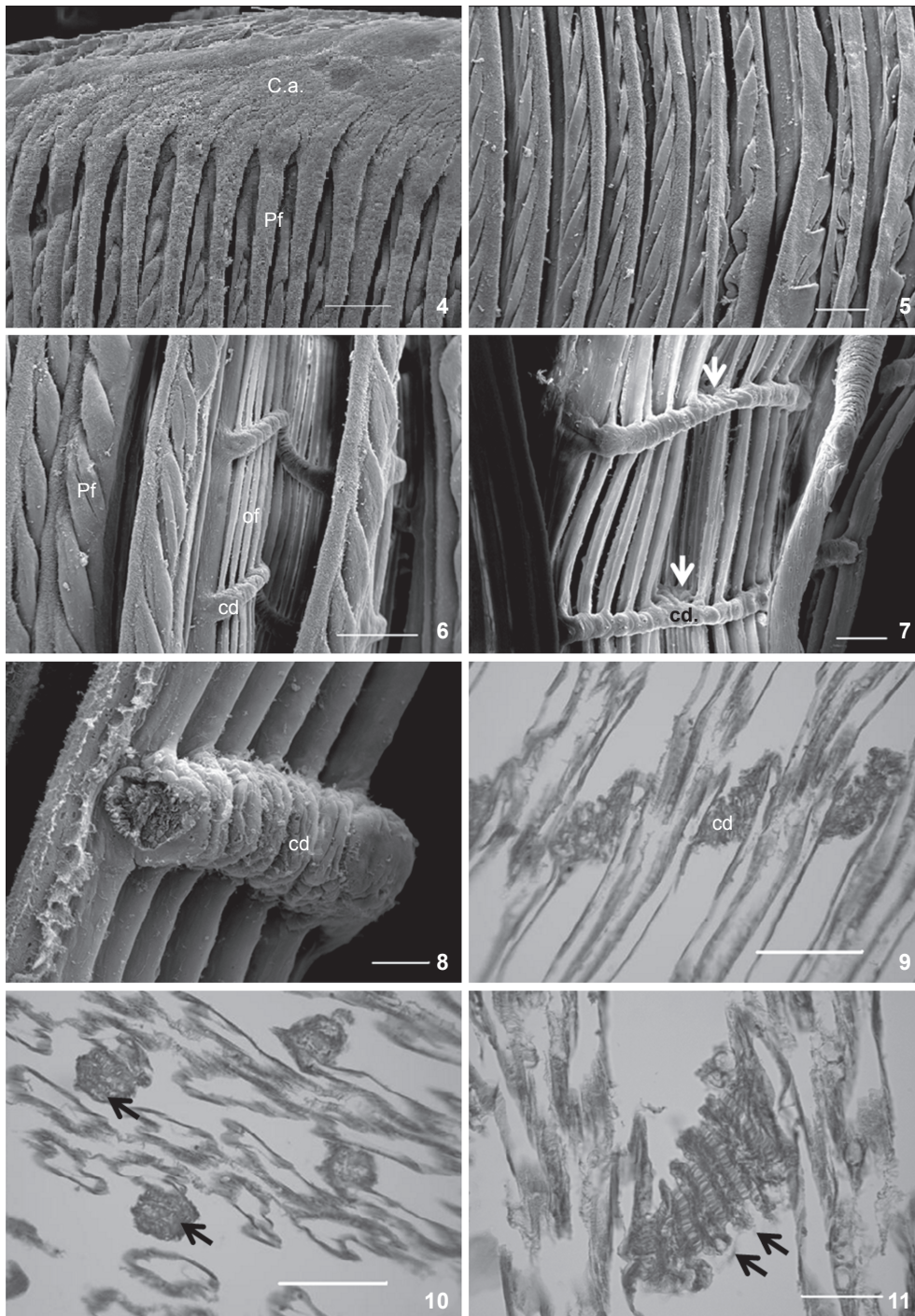
Ordinary filaments were the only components of the ascending lamellae located at the ventral part of each demibranch. Eight ordinary filaments formed the continuation of every principal filament formed by branching at the lower part of the demibranch. The ordinary filaments, resembling translucent channels, extended parallel and caused a fan-like expansion (Figs 6 and 7). Many ordinary filaments formed a group, and numerous groups were found in the terminal portion of the dorsal region with a flattened undulating edge, which was shaped into a marginal gutter by outwards folding.

Frequent ciliary discs connected each of the eight ordinary filaments to each other at regular intervals and extended in two directions (Figs 7 and 8). These interfilamentary connections showed a complex structure (Figs 9-11), with each connective disc consisting either of 5-7 cirral or microvillar plates, interlocked at the top with each other (Fig. 11). Their location on the surface epithelium was clearly visible under light and electron microscopy (Figs 8-11).

The frontal surface of the ordinary filaments showed a uniform array of cilia characterized by ciliary rootlets, whereas the abfrontal surfaces were covered with a non-ciliated epithe-



Figures 1-3. General appearance of the ctenidia of *Spondylus spinosus*: (1) ascending and descending lamellae attached at the ctenidial axis, forming a W shape; (2) frontal view of the principal and ordinary filaments; (3) schematic drawings of the ctenidia, with frontal (left) and side (right) views of the gill filaments. Scale: 1-2 = a ruler in mm, 3 = the drawing is merely to specify the structural definition and is not the actual size.



Figures 4-11. SEM and LM of the ctenidia of *Spondylus spinosus*: (4) proximal region of a demibranch, with principal filaments extending from the axis; (5) view of the principal filaments at different axes; (6) ordinary filaments located behind the principal filaments at the distal region of the demibranch; (7) ordinary filaments attached by continual ciliary junctions, with connective discs at regular intervals and extended inwardly (arrows); (8) ciliary discs interlocked with each other; (9) ciliary junctions between ordinary filaments (LM/H-E); (10) cross-section at the connection area (arrow) (LM/H-E-H); (11) ciliary junction and connective disc consisting of 5-7 cirral plates (black arrows) interlocked with each other (LM/H-E). Scale bars: 4-6 = 200 μ m, 7 = 60 μ m, 8 = 30 μ m, 9-10 = 100 μ m, 11 = 50 μ m.

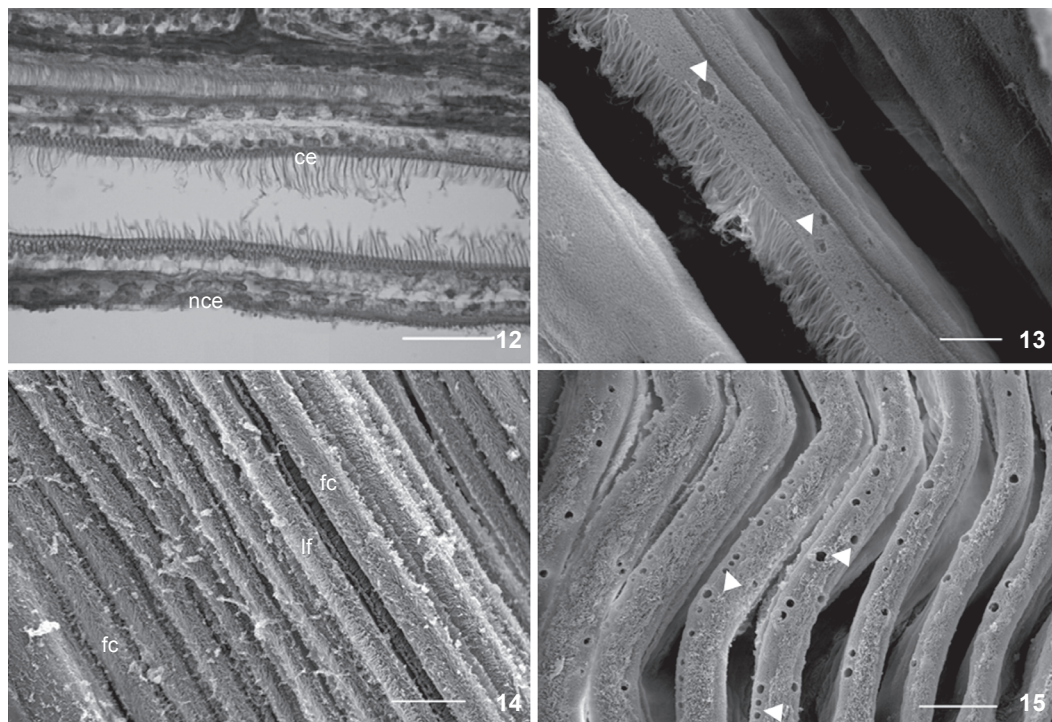
lium consisting of microvilli (Figs 12 and 13). The latter cells were characterized by large nuclei. A frontal view of the ordinary filaments showed abundant ciliary cover. The composite microcilia with an elongated latero-frontal array were clearly evident when the filaments were inflated and expanded into the interfilamentary spaces (Fig. 14). No ciliar differentiation was detected. All the cilia appeared to be simple cilia. Next to the ciliary band, an irregular number of ostia were observed on the lateral surfaces (Fig. 15). No mucus strings or layers or any lesional morphology that could be associated with environmental stress were observed in any part of the gill lamellae.

DISCUSSION

Light microscopy revealed that the general structure of the gills in *S. spinosus* was similar, in part, to that described for other bivalves, such as Mytilidae and Pectinidae (BENNINGER & ST JEAN 1997, GREGORY & GEORGE 2000, BENNINGER & DECOTTIGNES 2008). In brief, the ctenidia of *Spondylus* spp. were described as *Pecten* type (Type B1b), without a marginal groove (ATKINS 1937a,b). The general morphology observed in the present study was in accordance with previous descriptions. However, the ascending lamella of *S. spinosus* was found to be two-thirds

the size of the descending lamella, which is shorter than that described for *S. americanus* (YOUNGE 1973).

In many bivalve species, ciliation covers both the frontal surface and the lateral surfaces of the gill, and scattered cilia were described on the abfrontal surface of the ordinary filaments (DUFOUR & BENINGER 2001, DAVID & FONTANETTI 2005). Only the frontal surface showed a ciliary cover on the ordinary filaments of the gills of *S. spinosus*. AIELLO & SLEIGH (1972) suggested that the absence of ciliation provided a space for the eu-latero-frontal cilia to perform their movement, particularly in *Mytilus edulis* Linnaeus, 1758, whereas others have suggested that the lack of ciliation served to increase the surface available for the absorptive cells to obtain materials from the environment, as in *Mytella falcata* (Orbigny, 1846) (DAVID *et al.* 2008). Retention of the food particles in filter-feeding bivalves is performed either by the beating of cilia or the muscular control of ostia (GOSLING 2003). *Spondylus spinosus* appears to employ both mechanisms, exhibiting a sieve in many ostia next to the ciliary array. Oralwards currents could occur primarily in the dorsal channels, as described by YOUNGE (1973), and the prominent marginal gutter in *S. spinosus* could be proposed as an effective channel for particle movement because no marginal groove exists.



Figures 12-15. SEM and LM of the gill filaments of *Spondylus spinosus*: (12) general view showing the cellular organisation of the ordinary filaments, ciliated epithelium at only the frontal surface and prominent ciliary bases; (13) detail of a single filament with lateral cilia (the arrow indicates ostia); (14) detail of the ordinary filaments showing the ciliary cover; (15) frontal ciliary row on the inflated filaments and scattered ostia (◄) at the lateral surface. Scale bars: 12, 14-15 = 30 µm, 13 = 4 µm.

The regular distribution of ciliary junctions was similar to that found in certain species of Mytilidae, such as *Perna perna* Linnaeus, 1758 and *M. falcata* (GREGORY & GEORGE 2000, DAVID & FONTANETTI 2005), but several differences were also noted. The ciliary discs were located on either side of the ordinary filaments and extended laterally at eight filament intervals in *Spondylus spinosus*. The ciliary discs contained a smaller number of cirral plates and were, therefore, much more complex than those reported to occur in certain mytilid and pectinid species, such as *M. falcata* (DAVID & FONTANETTI 2005), *Modiolus barbatus* Linnaeus, 1758 (FALAKALI MUTAF *et al.* 2009) and *Placopecten magellanicus* (Gmelin, 1791) (MORSE & ZARDUS 1997), species in which only a single ciliary plate forms the disc. The distribution of the ciliary discs appears to serve predominantly to protect the filaments from hydrodynamic forces and to regulate the flow of water through the interfilamentary spaces, thus facilitating currents in the lower part of the demibranch in addition to those occurring in the dorsal channels.

Bivalve mollusks have been considered for monitoring aquatic habitats in many countries of the Northern and Southern Hemispheres, particularly well-settled species and those described as sentinel organisms in cases of pollution (GREGORY *et al.* 2002). In Turkey, only a few pilot projects of this nature have been conducted to establish a local assessment programme for marine pollution or to contribute to international surveys based on the study of sedentary species, such as *S. spinosus*. Indeed, gill morphology has been extensively used as an indicator organ of pollution (DOMOUHSIDOU & DIMITRIADIS 2000, GREGORY & GEORGE 2000, DAVID & FONTANETTI 2005, KOEHLER *et al.* 2008).

The results presented here provide data on the general structure of the gills of *S. spinosus*, with no pollution effect, for comparison in histopathological studies. Although increased mucus production on gill surfaces has been suggested to result from the accumulation of metallic or microorganismal pollutants (GREGORY *et al.* 2002, AKSIT *et al.* 2008), no trace of mucus was observed on the specimens studied.

In summary, *S. spinosus* have gills with heterorhabdic filaments, with the principal ones showing a complex structure. The ascending lamellae of the ordinary filaments, consisting of many minor lamellar subunits, are shorter than the thicker descending lamellae. Every eight ordinary filaments, connected by regular ciliary discs of 5-7 cirral plates, ended with a marginal gutter at the dorsal end. No marginal food groove at the free distal end was evident.

In conclusion, the external morphology of the ctenidia of *S. spinosus* can provide many useful features for evolutionary studies and can be the subject of biomonitoring studies within both natural and experimental contexts.

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