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Anatomy of the pallial tentacular organs of the scallop *Nodipecten nodosus* (Linnaeus, 1758) (Bivalvia: Pectinidae)

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ABSTRACT

Tentacular organs comprise a variety of body projections that have specialized functions in several invertebrate phyla. In bivalve mollusks, tentacles of the mantle margin serve sensorial and secretory functions involved in predator detection and interactions with the surrounding environment. However, their morphological diversity, detailed anatomy, and functional roles have only been scarcely investigated. Bivalves from the family Pectinidae are of particular interest in this context given the diversity of pallial tentacles, including distinct tentacle types arising on different mantle folds, and even eye-bearing tentacles. Combining several microscopy techniques, the present study investigates the anatomy of tentacular organs in postmetamorphic stages (juveniles and adults) of the scallop *Nodipecten nodosus* (Linnaeus, 1758). Scallop tentacles are formed shortly after metamorphosis, and except for pigmentation, they grow with no major morphological modifications. Tentacular organs of *N. nodosus* comprise eye-bearing and short and long tentacles from the middle mantle fold as well as velar tentacles from the inner fold. Although all tentacle types share a common basic structure (i.e., ciliated epithelium, peripheral muscle bundles, and a central nerve), they exhibit marked differences in ciliary distribution, epithelial secretory activity, and type of muscle fibers. Cilia distribution at the distal tip of sensory papillae represents a unique condition for the long tentacles from the middle mantle fold, and mucous secretion is restricted to the middle fold tentacles (except for eyestalks). Strikingly, velar tentacles and middle fold tentacles exhibit striated and non-striated myofibers, respectively. The data presented herein are discussed in light of the functional anatomy of the bivalve mantle margin.

1. Introduction

Involved in a plethora of functions, tentacular organs are widespread in invertebrates, displaying extensive morphological diversity. For instance, oral tentacles play vital roles in protection and food acquisition in cnidarians and bryozoans, and ciliated tentacles and palps are commonly present in suspension- and deposit-feeding polychaetes (e.g., Dauer, 1985; Gilmour, 1978; Nielsen and Riisgard, 1998; Shimizu and Namikawa, 2009). In Mollusca, the cephalic and buccal tentacles in gastropods and the brachial crown of cephalopods are probably the most familiar examples of tentacular organs (Künz and Haszprunar, 2001). Bivalve mollusks exhibit great diversity of tentacles on their mantle margin, usually associated with sensorial functions (Yonge, 1983), but these have only been scarcely explored so far.

Sensory organs in Mollusca comprise a myriad of complex structures, such as the camera-type eyes of cephalopods and rhinophores of opisthobranch gastropods (Serb, 2008; Wertz et al., 2006 Young, 1971). These include also a wide range of sensorial structures in bivalves, including simple and intricate organs. Most of these structures are associated with the mantle margin and act as mechano-, chemo- or photoreceptors, but our knowledge of their function and diversification is very limited. While some sense organs are sheltered inside the pallial cavity, e.g., the abdominal organ of pteriomorphian bivalves (Haszprunar, 1985), pallial tentacles are exposed structures in direct contact with the surrounding environment (Yonge, 1983). Photoreceptive organs have evolved independently in tentacular organs of several unrelated Bivalvia taxa (Morton, 2008; Serb and Eernisse, 2008). Eye-bearing tentacles are known to serve a variety of visually-influenced tasks, usually associated with light or movement detection and siphonal retraction, shell closure, or escape responses (Morton, 2008; Serb

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and Eernisse, 2008). Chemo- and mechanoreceptors are commonly found in the pallial epithelium of bivalves, also usually concentrated on the pallial tentacles (Fishelson, 2000). In bivalves with fused mantle margins (e.g., most Heteroconchia), tentacular structures are typically associated with the exhalant and inhalant apertures of siphons (e.g., Fishelson, 2000; Moueza and Frenkiel, 1974; Sartori and Mikkelsen, 2008). Besides sensorial functions, these siphonal tentacles prevent the entrance of large particles into the siphonal channel (Hodgson and Fielden, 1984; Narchi, 1972; Passos and Domaneschi, 2004). In bivalves with free mantle edges (e.g., most pteriomorphians), pallial tentacles may occur along its entire extension, but they exhibit considerable diversity in structure and distribution. In pinnids (pen shells, Pinnidae), for example, neither tentacles nor sensory structures occur on the middle mantle fold, but in *Pinna carnea* Gmelin (1791), a row of short tentacles occur at the posterior region of the prominent inner fold (Yonge, 1957). In oysters (Ostreidae) and most pteriids (Pteriidae), short tentacles are regularly distributed on both middle and inner folds, including branched tentacles in *Pinctada imbricata* Röding, 1798 (Tëmkin, 2006; Waller, 1975). In the Galeommatoidea (i.e., Bieler et al., 2010), the mantle commonly bears conspicuous pallial projections (Lützen and Nielsen, 2005), ranging from sensory ciliated papillae, like those observed on the middle mantle fold of *Mysella charcoti* Lamy, 1906 (Lasaeidae) (Passos et al., 2005), to specialized tentacles, as reported for *Galeomma layardi* Deshayes, 1856 (Galeommatidae) (Morton, 1973). In addition, secretory activity has been extensively reported for bivalve tentacles, representing an important aspect in the anatomy and roles of such structures (Yonge, 1983). Bivalves from the family Limidae (e.g., file clams) are notorious for the presence of long and extensible tentacles that are too large to be enclosed within the valves and may be used for swimming (Gilmour, 1967, 1963). Secretory cells are largely distributed in those organs, and associated with autotomy and release of distasteful mucous to avoid predators (Owen and McCrae, 1979).

Bivalves from the family Pectinidae (i.e., scallops) have an enlarged mantle margin notable for its complex structures. Numerous tentacles are distributed along the entire margin, being divided into two categories: tentacles from the middle mantle fold and tentacles from the inner fold (Dakin, 1909). The first type includes numerous tentacles, some of them being differentiated into pallial eyes at irregular intervals. The second type consists of short tentacles located at the edge of the pallial curtain, i.e., the inner mantle fold (or velum). Scallop tentacles are known to be involved in the perception of the substrate and detection of predators by mechanical and chemical stimulation, with subsequent triggering of behavioral responses (Wilkens, 2006). Considering the extensive diversity of tentacular form and function exhibited by pectinids, scallops may represent suitable models for investigations on tentacle structure and function in bivalves. Therefore, we investigated tentacle anatomy in the scallop *Nodipecten nodosus* (Linnaeus, 1758) to explore anatomical variation in tentacle types of Pectinidae, and to gain insight into their possible functions. Given the plethora of studies focused on the scallop optical system and eye anatomy (e.g., Audino et al., 2015), in the case of eye-bearing tentacles we restrict our analysis to the eyestalk.

2. Material and methods

Specimens of *N. nodosus* were obtained at the scallop farm Institute of Eco-Development from Baía de Ilha Grande (IED-BIG), Rio de Janeiro, Brazil. Fragments from the mantle margin were removed from juvenile (about 4 mm length; few weeks after metamorphosis) and adult (about 8 cm length) individuals, which were anesthetized for 2 h by addition of 7.5% $MgCl_2$ prior to fixation. General morphology was observed using a Zeiss Stemi 2000-C stereomicroscope and images were captured using a Zeiss AxioCam MRc (Carl Zeiss, Jena, Germany).

2.1. Scanning electron microscopy (SEM)

After fixation in a modified Karnovsky solution (2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, adjusted to 1 Osm with sucrose; Marian, 2012), post-fixation was performed for 30 min in 1% OsO_4 in buffer solution, followed by 15 min in 1% tannic acid in buffer solution, and additional 15 min in a fresh solution of 1% OsO_4 at 4 °C. Then, samples were dehydrated in graded ethanol series (60%, 70%, 80%, 90%, 95%, and three changes of 100% ethanol, with at least 1 hour per step), critical point dried using CO_2 as intermediate in a Balzers CPD 030 (Electron Microscopy Sciences, EUA), mounted on stubs, coated with gold in a Balzers SCD 050 sputter coater (Electron Microscopy Sciences, EUA), and observed in a Zeiss DSM 940 (Carl Zeiss, Oberkochen, Germany).

2.2. Histology

Specimens were fixed for 3 h at 4 °C in a modified Karnovsky solution. Then, pallial margin fragments were dehydrated in a graded ethanol series, embedded in glycol-methacrylate resin (Leica Historesin Kit), and sectioned using a Leica RM2255 microtome (Leica, Wetzlar, Germany). Serial sections of 3 μm were stained with hematoxylin and eosin (HE) for basophilic (generally, nuclei) and acidophilic structures, respectively. In addition, sections were stained with toluidine blue and basic fuchsin (TB), a method that stains almost all cell components. Alcian Blue (AB) and periodic acid-Schiff (PAS) were applied as standard histochemical methods to visualize acidic and neutral mucopolysaccharides, respectively. Digital images were captured using a Nikon eclipse 80i microscope equipped with a Nikon DS-Ri1 camera (Nikon Instech Co. Ltd, Kawasaki, Japan).

2.3. Immunocytochemistry and confocal laser scanning microscopy

Juvenile specimens were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (PB) for 1 h, and stored in PB containing 0,1% NaN_3. For F-actin staining, fragments of the mantle margin were permeabilized in PBS containing 2% Triton-X 100 (PBT) overnight and incubated in a 1:40 dilution of Alexa Fluor 488 phalloidin (Molecular Probes) in PBT for 24 h at room temperature in the dark. For neuronal staining, samples were incubated in 6% normal goat serum in PBT (block-PBT) overnight at room temperature. Then, primary antibodies, i.e., anti-serotonin raised in rabbit and anti-α-tubulin raised in mouse, were applied at a concentration of 1:400 in block-PBT overnight. Specimens were rinsed several times in block-PBT prior to application of a secondary antibody (goat anti-rabbit Alexa Fluor 488 and goat anti-mouse Alexa Flour 633, Molecular Probes) at a concentration of 1:200 for 24 h in the dark. Nuclei were stained by adding a 1 μL drop of 4′, 6-diamidino-2-phenylindole (DAPI; Invitrogen) in conjunction with secondary antibody or phalloidin incubation. Subsequently, samples were washed three times in PBS, mounted in Fluoromount G (Southern-Biotech, Birmingham, Alabama) on standard microscope slides, and analyzed using a Leica TCS SP5 II confocal laser scanning microscope equipped with the software Leica Application Suite Advanced Fluorescence (LAS AF), Version 2.6.0 (Leica Microsystems, Wetzlar, Germany). Confocal image stacks were recorded with 0.3 μm step size along the z-axis and 3D reconstructions were created using the software Imaris Version 4.1 (Bitplane, Switzerland).

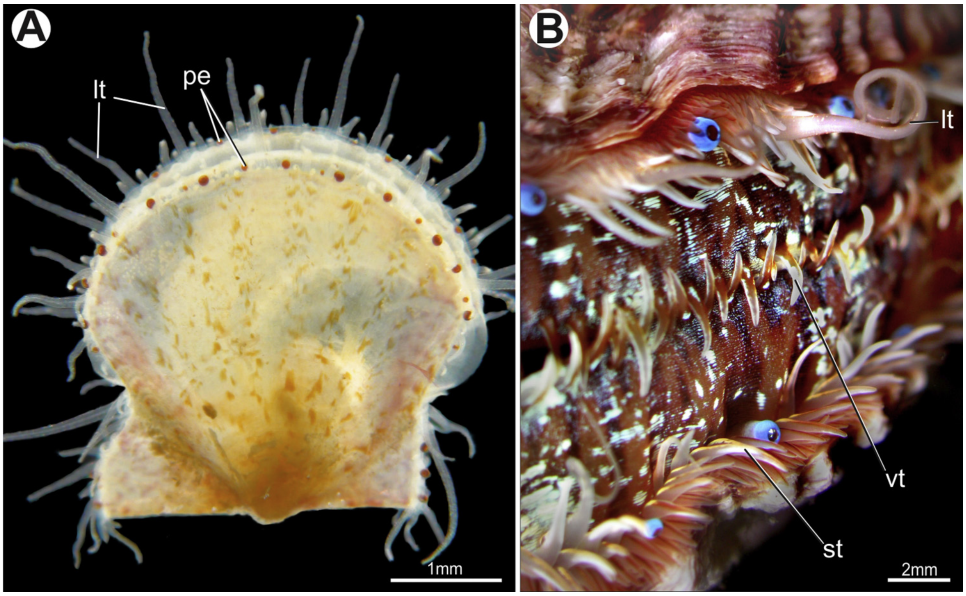

Fig. 1. *Nodipecten nodosus*. (A) Postmetamorphic juvenile scallop with extended tentacles from the middle pallial fold. (B) Detail of the adult mantle margin displaying different tentacular organs. Abbreviations: lt, long tentacle; pe, pallial eye; st, short tentacle; vt, velar tentacle.

3. Results

Tentacle organs emerge as conspicuous, translucent projections of the middle and inner mantle folds in postmetamorphic juveniles of *N. nodosus* (Fig. 1A). Whereas middle fold tentacles are generally elongated with tapered ends, the eye-bearing ones are short and differentiate their distal regions into an ocular apparatus (Fig. 1B). The inner fold tentacles consist of short projections at the edge of the inner mantle fold (Fig. 1B). From the early emergence of the juvenile stage until the adult stage, tentacle anatomy remains similar throughout development, except for the increase in size, muscular density and pigmentation (Fig. 1B).

3.1. Middle mantle fold eyestalks

Pallial eyes are easily noticeable by their blue pigmentation in *N. nodosus* (Fig. 1B). While distal optic components comprise unique structures in the scallop's anatomy, the eyestalk shares great similarity with adjacent middle-fold tentacles. The eyestalk is a short peduncle filled by connective tissue and a pair of longitudinal muscle bundles (Fig. 2A). The epithelium ranges from cuboidal to columnar cells, usually pigmented (Fig. 2A), and covered by microvilli (Fig. 2C), but without gland cells. The distal epithelium exhibits scarce tufts of cilia (Fig. 2B and C), while the proximal surface of the eyestalk is more densely ciliated, with several sparse ciliary tufts (Fig. 2D).

3.2. Middle mantle fold tentacles

Middle fold tentacles are divided into two types: long and short tentacles. Long tentacles are extensible projections occurring at irregular intervals along the middle mantle fold (Fig. 1A and B; Fig. 3A and B). In live animals, these tentacles slowly extend to interact with the surrounding environment and can rapidly contract when the animal is disturbed. The long tentacle's epithelium displays a gradual increase in ciliary density from proximal to distal regions (Fig. 3B). At the distal third of the tentacle, tufts of cilia occur at the tip of papillary projections (Fig. 3C). Ciliary tufts are also found interspersed between these projections (Fig. 3C). At the proximal surface, however, ciliary tufts are fewer and sparsely distributed (Fig. 3D). Short tentacles (Figs. 1B and 3E) are far more numerous and their length slightly exceeds the eyestalk's length. In this case, papillary projections are absent, and dense ciliary tufts are distributed over the entire tentacular surface (Fig. 3F).

Long and short tentacles are anatomically similar. They bear several thin muscles and a central, branched nerve embedded within the connective tissue (Fig. 3G). Their epithelium has numerous secretory cells with large basal content with strong affinity for Alcian blue and PAS, which suggests intense acid mucopolysaccharide secretion (Fig. 3H, I). Both tentacle types are also innervated by long projections from the circumpallial nerve. Tentacular nerves exhibit strong serotonin- (Fig. 4A, C) and α-tubulin-like (Fig. 4B and C) immunoreactivity, and run from the circumpallial nerve at the mantle margin to the tentacular tip. The tentacular nerve is directly connected to the ciliated cells from the epithelium by thin neuronal projections, as revealed by α-tubulin and serotonin markers (Fig. 4A–C). Tentacular muscles originate from the pallial muscular system associated with the mantle margin (Fig. 5A). Surrounding the nervous axis, numerous bundles of longitudinal muscles run along the entire tentacle extension (Fig. 5A and B). Additionally, short transverse fibers are present (Fig. 5B). Both longitudinal and transverse muscle bundles in the middle fold tentacles are apparently formed by non-striated myofibers (Fig. 5B).

3.3. Inner mantle fold tentacles

The tentacles distributed along the edge of the inner fold (Fig. 1B) are also referred to as "velar" or "guard tentacles" (e.g., Beninger and Pennec Le, 2006). They are pigmented projections, and their general structure resembles that of the short middle fold tentacles, including a ciliated epithelium and a central nerve as well as muscles embedded within the connective tissue (Fig. 3J). In addition, innervating nerves exhibit strong serotonin and α-tubulin signals, similar to the middle fold tentacles, with tentacular nerves passing through the inner fold from the circumpallial nerve towards the velar tentacle distal tip (Fig. 4D and E). Nevertheless, there are some key differences between the tentacles from both folds. An important difference concerns the lack of secretory activity in the velar tentacles, not detected by any staining method applied (i.e., Alcian Blue and Periodic Acid-Schiff staining). In velar tentacles,

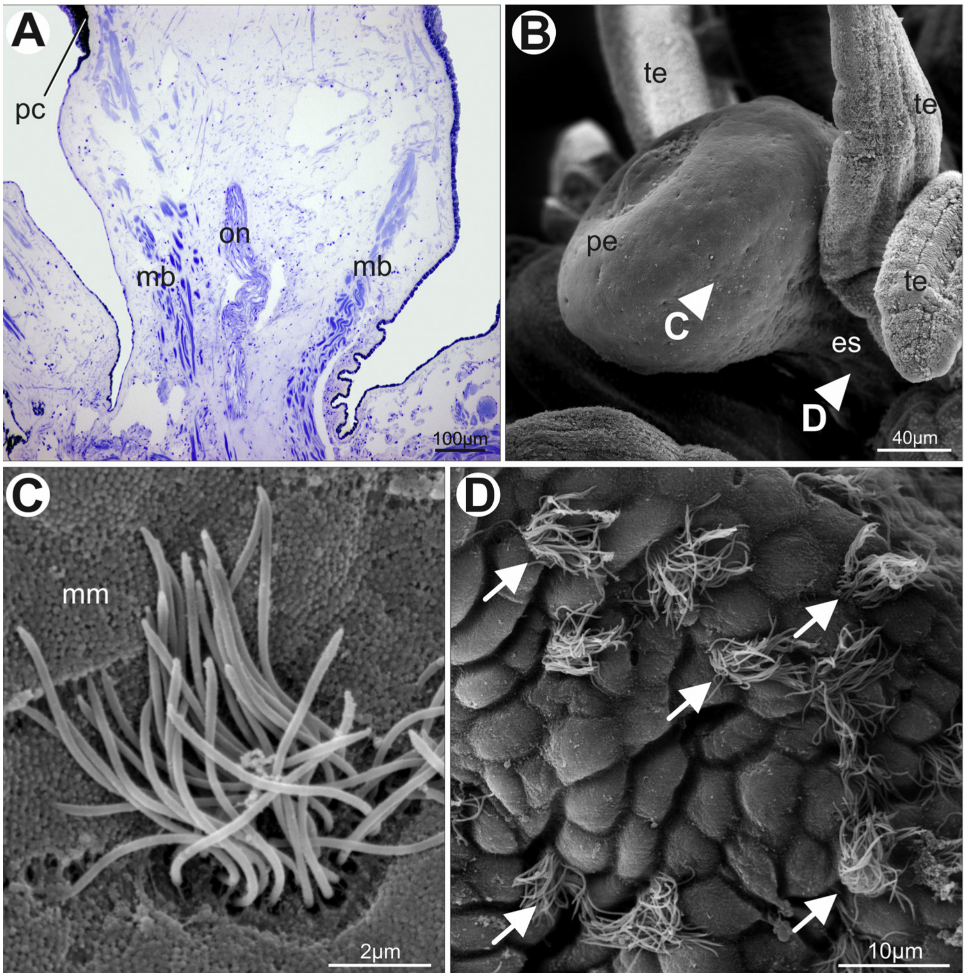

Fig. 2. Eye-bearing tentacles from *Nodipecten nodosus*. (A) Longitudinal section through the eyestalk showing internal muscular and neural components. Toluidine blue and basic fuchsin. (B) General view of the pallial eye as revealed by scanning electron microscopy. (C) Detail of a ciliary tuft surrounded by microvilli on the distal eyestalk epithelium of B. (D) Detail of sparse ciliary tufts (arrows) on the proximal eyestalk of B. Abbreviations: es, eyestalk; mb, muscle bundles; mi, microvilli; on, optic nerve; pc, pigmented epithelial cells; pe, pallial eye; te, tentacle.

cilia are present in less dense and more sparsely distributed tufts than in the short tentacles of the middle fold (Fig. 3K). Velar tentacle musculature is formed mainly by longitudinal fibers, transverse muscles being less numerous (Fig 5C). More strikingly, longitudinal fibers are evidently composed of striated myofibers (denoted by the non-stained Z-bands observed in samples stained for actin; Fig. 5C), in contrast to the apparent non-striated muscular structure exhibited by middle fold tentacles (Fig. 5B).

4. Discussion

Tentacular organs of *N. nodosus* comprise eye-bearing and short and long tentacles from the middle mantle fold, and velar tentacles from the inner fold. While short tentacles are regularly present in the pectinid middle pallial fold, the extensible, long tentacles observed in *N. nodosus* were previously detected in other scallops, such as *Pecten maximus* (Linnaeus, 1758) (Dakin, 1909) and *Argopecten irradians* (Lamarck, 1819) (Wilkens, 2006), although they were not reported for *Placopecten magellanicus* (Gmelin, 1791) (Drew, 1906). Such difference is intriguing, and deserves further studies to evaluate tentacle type and distribution in Pectinidae to understand functional roles and variation. Velar tentacles, similar to those described herein, are apparently more common, occurring on the edge of the inner mantle fold of *A. irradians* (Gutsell, 1931) and *P. magellanicus* (Moir, 1977). In Spondylidae, cemented bivalves phylogenetically close to Pectinidae (Alejandrino et al., 2011; Waller, 2006), the mantle margin exhibits great similarity to the general pectinid condition, including the presence of pallial eyes, short tentacles and a pigmented velum. Nevertheless, neither long tentacles nor a row of velar tentacles are present (Dakin, 1928; Viana and Rocha-Barreira, 2007). Short tentacles also occur on both middle and inner mantle folds of Ostreidae and Pteriidae (Tëmkin, 2006), which suggests a convergent condition of tentacle organization in Pectinidae. Notwithstanding, tentacle variation and distribution is still poorly understood across major bivalve groups, so speculations on tentacle evolution should be treated with caution at this time.

Eye-bearing tentacles are not exclusive to Pectinidae, but also occur in the phylogenetically distant families Cardiidae and Laternulidae (Adal and Morton, 1973). The eyestalk of *Cerastoderma edule* (Linnaeus, 1758) shares great similarity with the scallop eyestalk, including an epithelium covered by microvilli, peripheral muscle bands, and a central nerve (Barber and Wright, 1969). In contrast to the ciliated epithelium of scallop pallial eyestalks, ciliated receptor cells of *C. edule* and *Cochlodesma praetenue* (Pulteney, 1799) are restricted to the tip of the eye-bearing tentacles (Barber and Wright, 1969; Morton, 2008). Pallial eyes also occur at the base of the tentacles, as in *Ctenoides floridanus* (Olsson and Harbison, 1953) (Limidae), in which the pigment cups are located at the base of the middle mantle fold tentacles (Morton, 2000). Even though

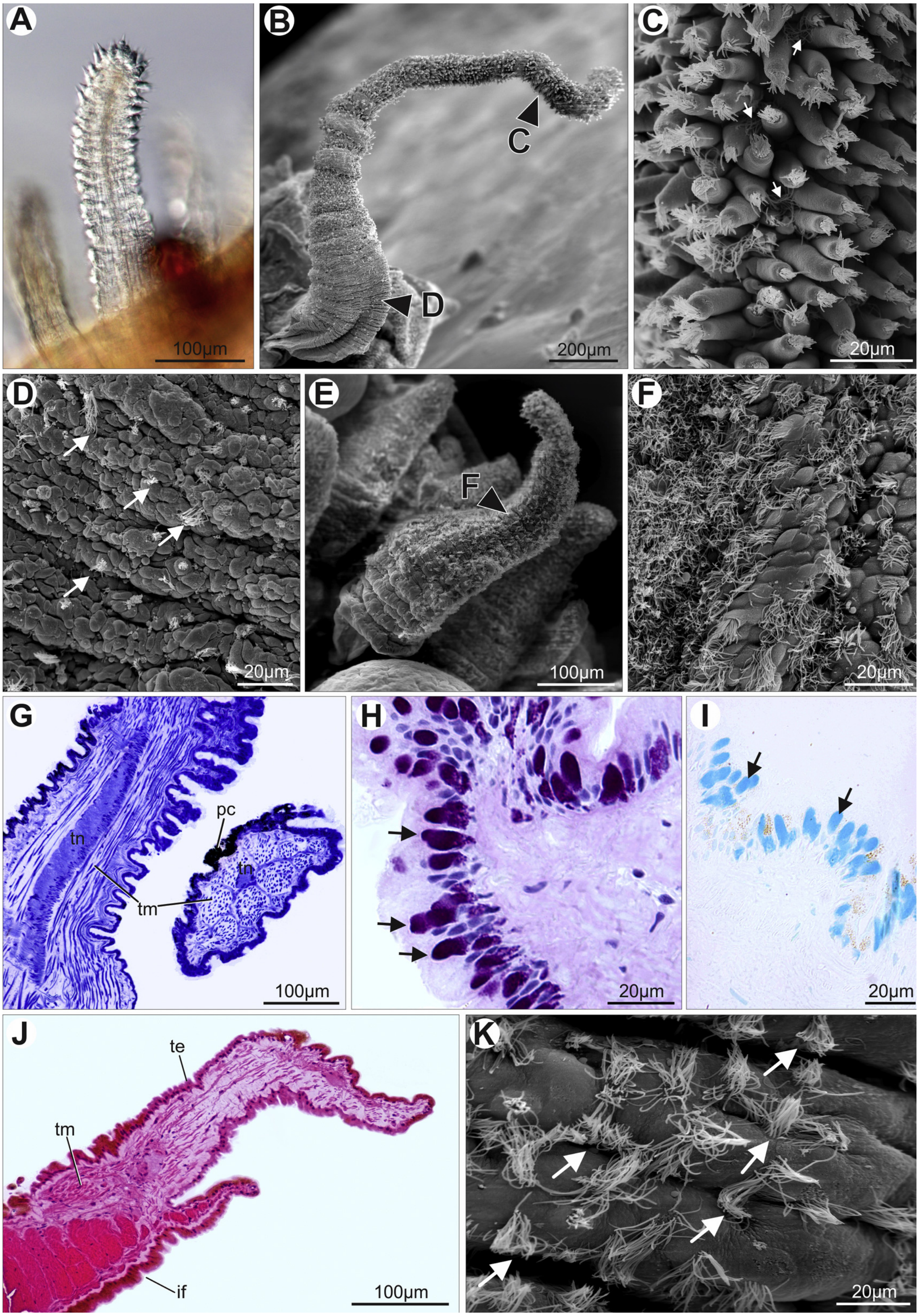

Fig. 3. Tentacles from the middle mantle fold (A–I) and inner fold (J and K) of *Nodipecten nodosus*. (A, G–J) Light micrographs. (B–F, K) Scanning electron micrographs. (A) General view of a long tentacle of a live specimen. (B) Long tentacle morphology. (C) Detail of the distal tentacular region of B, with cilia located on the tip of papillary projections and few ciliary tufts interspersed between the projections (arrows). (D) Detail of the proximal tentacular region of B, with sparse ciliary tufts. (E) Short tentacle morphology. (F) Detail of the tentacular surface of E, with dense distribution of ciliary tufts. (G) Longitudinal (left) and transverse (right) sections of short tentacles showing internal components such as peripheral muscles and a central nerve. Toluidine blue and basic fuchsin. (H) Secretory cells from the tentacular epithelium with PAS-positive content (arrows). (I) Secretory cells from the same tentacle of H, showing affinity also for Alcian blue (acid mucopolysaccharide content; arrows). (J) Histology of a velar tentacle. Hematoxylin and eosin. (K) Detail of the velar tentacle surface with sparse ciliary tufts (arrows). Abbreviations: if, inner mantle fold; pc, pigmented epithelial cells; te, tentacle epithelium; tm, tentacle muscle; tn, tentacle nerve.

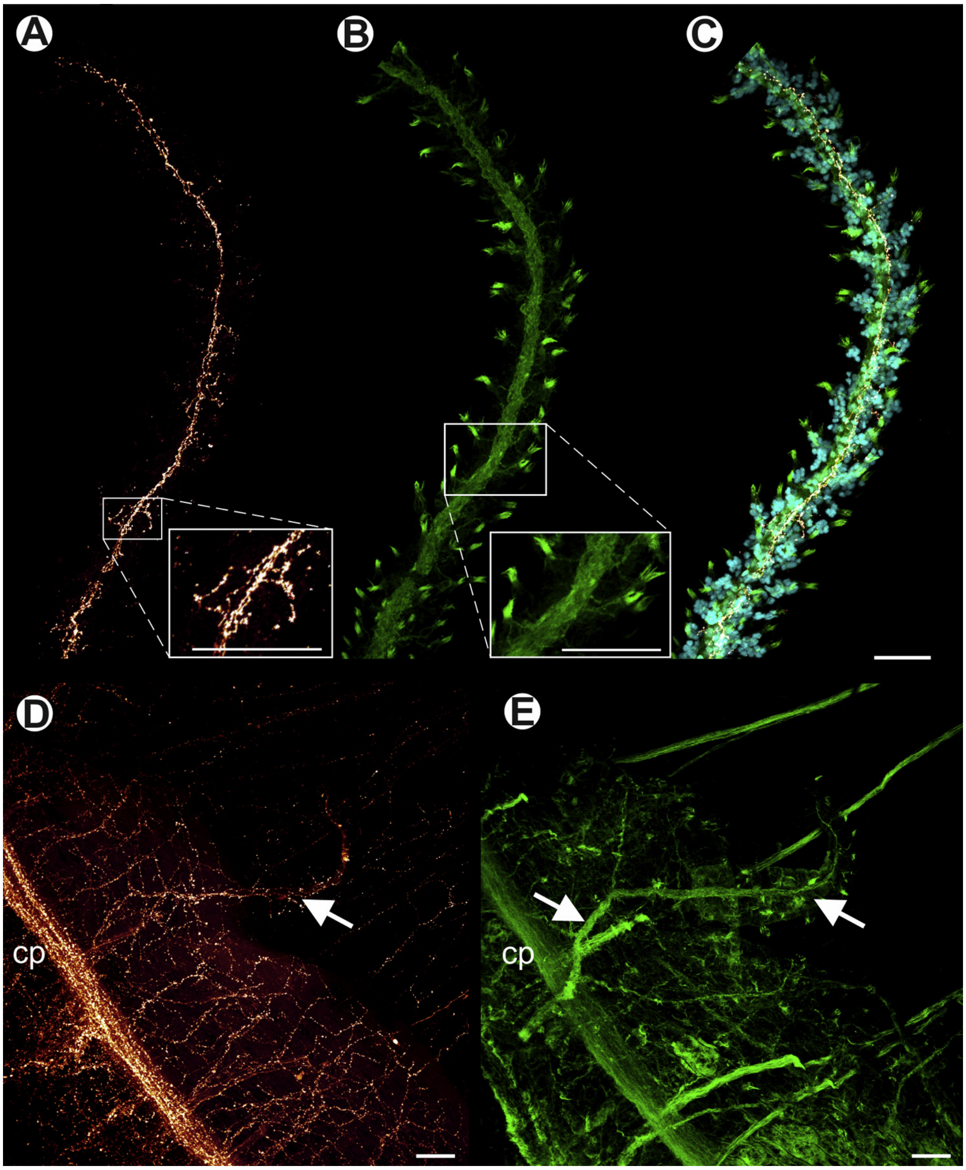

Fig. 4. Nervous organization in tentacles from the middle mantle fold (A–C) and inner fold (D and E) of *Nodipecten nodosus* as revealed by immunocytochemistry combined with confocal microscopy. (A) Serotonergic immunoreactivity in the tentacular nerve; neuronal projections towards epithelial cells are shown in detail in the inset. (B) Immunoreactivity to α-tubulin in the tentacular nerve; neuronal projections towards epithelial cells are shown in detail in the inset. (C) Overlay of serotonin, α-tubulin and nucleic acid staining in the tentacle. (D) Serotonin-like immunoreactivity in the velar tentacle nerve (arrow), which goes through the inner mantle fold and connects to the circumpallial nerve. (E) Immunoreactivity to α-tubulin in the velar tentacle nerve (arrows). Abbreviation: cp, circumpallial nerve. Scale bars: 30 μm.

mechanisms of tentacle differentiation into pallial eyes have not been determined, the convergent evolution of the optic apparatus in tentacles highlights the potential for diversification and the high plasticity of tentacular organs in the Bivalvia.

The general anatomy of the scallop tentacle consists of a tentacular nerve surrounded by muscles and connective tissue. Tentacle extension is generated by hydrostatic pressure from the haemolymph, while retraction is produced by contraction of longitudinal muscles (Beninger and Pennec, 2006). Transverse muscles might be involved in an array of tentacular movements, although their functions are uncertain. To our knowledge, the present study is the first to detect differences in tentacle muscle types between middle and inner mantle folds. Whereas tentacles from the middle pallial fold of *N. nodosus* are apparently formed only by non-striated myofibers, velar tentacles evidently possess striated fibers, similar to those observed in the musculature from the inner fold. Further studies applying transmission electron microscopy would be required to confirm the non-striated pattern observed in middle fold tentacles. Such a difference may be associated with functional divergences between tentacle types in this species. In scallops, striated muscles, like those forming the major portion of the posterior adductor muscle, are known to perform rapid and repeated contractions (Chantler, 2006; Millmax and Bennett, 1976). The presence of striated fibers in the inner fold tentacles may represent a developmental consequence of the intense growth of striated musculature in the inner fold itself, which is capable of rapid contractions associated with pseudofeces expulsion and swimming behavior (Yonge, 1981). Alternatively, smooth muscles, like those forming the middle fold tentacles, provide a catch-contraction capability, defined by long-term force maintenance with low energy requirements (Chantler, 2006; Hoyle, 1964; Ruegg, 1971). Therefore, our results suggest that the development and distribution of myofibers might represent a key feature to understand the roles and diversity of tentacular structures in scallops.

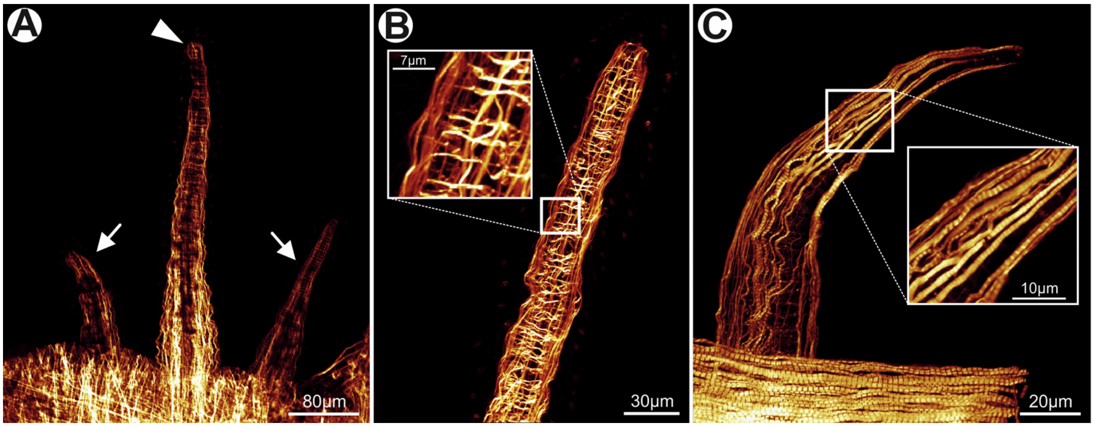

Fig. 5. Muscular organization of tentacles from the middle mantle fold (A and B) and inner fold (C) of *Nodipecten nodosus*, as revealed by phalloidin staining combined with confocal microscopy. (A) General muscular organization in short and long tentacles (arrows and arrowhead, respectively). (B) Musculature of a long tentacle, with transverse and longitudinal fibers (both apparently non-striated) shown in detail in the inset. (C) Velar tentacle musculature with transverse and striated longitudinal muscles shown in detail in the inset.

In a thorough description of central and peripheral nervous systems in bivalves, thin nerves from the circumpallial nerve of *Pecten maximus* were detected reaching tentacle structures of the middle mantle fold (Duvernoy, 1853). According to the same investigation, those organs were named "tactile" and "visual pedicles", due to their supposedly sensory functions (Duvernoy, 1853). The same innervation pattern was reported for *P. magellanicus* (Drew, 1906). Ciliated cells occurring on the epithelia of short and long tentacles of *P. maximus* were identified as receptors, emphasizing the sensory role of such pallial structures (Dakin, 1909). Serotonergic innervation was detected in the present study, confirming nervous activity in tentacles from the middle and inner folds, and a close connection of epithelial cilia and nerves by slender neuronal projections. Even though neuronal or muscular markers (combined with confocal microscopy) have never been used in studies on adult bivalves, this technique has already demonstrated great potential in understanding nervous organization in other molluscan taxa (Von Byern et al., 2012; Wertz et al., 2006; Wollesen et al., 2009). Consequently, our results represent a successful example applying such methods to anatomical investigations in bivalves, especially concerning muscular and sensory organs.

The long tentacles of *P. magellanicus* have their distal third organized in ciliated papillae, which radiate out from the central column (Moir, 1977). Similar to *N. nodosus*, ciliary tufts are located at the distal tip of these papillae, where they are supposed to act as mechanoreceptors, while another ciliated cell type is present between the papillae (Moir, 1977). At the proximal region of the tentacles, ciliated groups of cells are irregularly spread over the surface and their function is still debatable (Moir, 1977; present study). In the mussel *Brachidontes pharaonis* (Fischer, 1870) (Mytilidae), sensory ciliary tufts, similar to those present in the short and velar tentacles of *N. nodosus*, are spread among short kinetic cilia on the short tentacles (Fishelson, 2000). In infaunal bivalves, e.g., from the families Veneridae and Donacidae, sensorial cilia are commonly distributed on siphonal tentacles, but the dimension, structure and number of cilia are remarkably different from ciliary types observed in scallops (Ansell et al., 1999; Fishelson, 2000; Hodgson and Fielden, 1984). Tentacle ciliated receptors have also been analyzed in detail in *Limaria hians* (Gmelin, 1791), in which different ciliary tufts are in close association with gland cells which act as chemo- and mechanoreceptors (Owen and McCrae, 1979). In contrast to limid bivalves, no evidence has been reported in scallops for mucous secretion associated with sensorial reception in tentacles. Sensorial performance of pectinid tentacles has been studied in several behavioral and physiological contexts in an attempt to integrate stimulation and mantle margin response (Gutsell, 1931; Stephens, 1978). Previous behavioral experiments found that scallops extend their long tentacles towards slowly moving objects, e.g., sea stars, their natural predators (Buddenbrock and von Moller-Racke, 1953; Wilkens, 2006). These studies demonstrated that tentacle ciliated cells seem to serve as primary sensory receptors for tactile and chemical stimuli, triggering adjustments in pallial curtain movements, animal position, escape behavior, and shell closure (Wilkens, 2006).

Our results suggest similarities in the neural anatomy of velar tentacles and tentacles from the middle mantle fold in *N. nodosus*, including serotonergic innervation by a central nerve connected to the ciliated epithelial cells. Despite no evidence for differences in sensitivity between velar and long tentacles, both types are supposed to be able to detect tactile stimuli, but only the second one would detect substrate suitability and chemical stimulants from predators (Wilkens, 2006). Apart from sensorial functions, the secretory activity is clearly distinct between these tentacle types in *N. nodosus*. While short and long tentacles from the middle pallial fold exhibit intense mucous secretion, no secretory cell was detected in eyestalks and velar tentacles. Intense production of high-viscosity acid mucopolyssacharides was reported for the pallial epithelium of *P. magellanicus*, probably facilitating the passage of water over the mantle surface (Beninger and Pennec, 2006; Beninger and St-Jean, 1997). Mucous secretion in scallop middle fold tentacles might be associated with lubrication and cleansing of the mantle margin (a role possibly lost during eyestalk evolution), the function of velar tentacles being possibly restricted to tactile detection. In the pearl oyster *Pinctada margaritifera* (Linnaeus, 1758) (Pteriidae), the number of cells with affinity to PAS and Alcian blue decreases in the tentacles from the middle mantle fold in contrast to the inner fold (Jabbour-Zahab and Chagot, 1992), a condition also observed in *C. edule* (Richardson et al., 1981). Therefore, the abundance of mucous-secreting cells in the tentacles of *N. nodosus* may represent a particular condition of Pectinidae with respect to the functional anatomy of tentacular organs.

Comparative analyses of tentacular features in bivalves are still scarce, evoking the necessity of more studies to evaluate and test possible homologies and convergent evolution of tentacular traits. Furthermore, investigations on tentacle functional anatomy in the Bivalvia are still necessary to cover structural diversity and to provide substantial evidence to explain tentacle evolution in the group. The present paper should stimulate and serve as a basis for further attempts in understanding tentacular functional morphology and evolution in the Bivalvia.

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