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ORIGINAL PAPER

Locomotion and fine structure of parapodia in *Myzostoma cirriferum* (Myzostomida)

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Abstract Most myzostomids are ectocommensals of crinoids on which they move freely. Their locomotion is ensured by five pairs of parapodia located laterally below their trunk. Each parapodium in *Myzostoma cirriferum* is a conical structure that includes a hook-like chaeta, replacement chaetae and an aciculum. Structure and ultrastructure of the myzostomid chaetae are similar to those of polychaetes: they are formed by a chaetoblast, which gives rise to microvilli where chaetal material is assembled on the outer surface. *Myzostoma cirriferum* walks on its host. It moves the anterior part, the posterior part or the lateral parts forwards but is able to rotate of 180° on itself. Its locomotion entirely depends on parapodial motions and not on trunk movements. Three pairs of muscles are involved in parapodial motions: parapodium flexor and parapodium extensor, aciculum protractor and aciculum retractor, and hook protractor with conjunctor. A functional model is proposed for explaining the global motion of a parapodium in *M. cirriferum* that may be extended to all ectocommensal myzostomids.

Keywords Myzostomida · Annelida · Parapodium · Chaeta · Locomotion

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Introduction

Myzostomids are minute, soft-bodied, marine worms that are all associated with echinoderms (Grygier 2000; Eeckhaut and Lanterbecq 2005). They are found in all oceans from subtidal to a depth of over 3,000 m. Most of them are ectocommensals of crinoids on which they move freely at the surface. The body of ectocommensals is totally adapted to crinoids and contrarily to most crinoid associates they are unable to move on the substrates that surround their hosts. Locomotion in ectocommensal myzostomids is ensured by five pairs of parapodia located laterally below their trunk. Each parapodium is a conical structure that includes a hook-like chaeta, replacement chaetae and an aciculum (Jägersten 1936). At present, the structure of myzostomid parapodia has been studied by light microscopy, mainly in *Myzostoma glabrum* Leuckart, 1842 (essentially the parapodial muscles; Graff 1877), and in *M. gigas* Lütken, 1875 (Stummer-Traunfels 1926; Jägersten 1936). As in polychaetes, chaetae are synthesized in separate epidermal follicles. The only difference that has been pointed is the presence of a manubrium at the apex of each aciculum in myzostomids that acts as a muff in which hook-like chaeta is guided (Jägersten 1936).

Recently, Hausen (2005) reviewed chaetae and chaetogenesis in polychaetes and made comparisons with the chaetae present in other taxa. He emphasized that the ultrastructure of chaetae in polychaetes, clitellates, echiurians and pogonophorans (syn. Siboglinidae) is identical and that there is no doubt that these structures are homologous. Chaetae described in juvenile octopods, a certain placophoran, brachiopods and bryozoans are probably not homologous structures (Hausen 2005). The ultrastructure of myzostomid chaetae has not yet been analysed in details except for their larvae (Eeckhaut et al. 2003). This paper aims at analysing the ultrastructure of the parapodia of the

European myzostomid *Myzostoma cirriferum* Leuckart, 1836, especially the chaetae and to compare them with those of the other taxa. We also describe the functioning of a parapodium and its use in the myzostomid locomotion.

Materials and methods

Adults of *Myzostoma cirriferum* Leuckart, 1836 (order of Proboscidea Jägersten, 1940; family of Myzostomatidae Beard, 1884; see Grygier 2000 for details), ectocommensal of crinoids, were collected with their host *Antedon bifida* (Pennant, 1777) (order of Comatulida; family of Antedonidae) by SCUBA diving at Morgat (Brittany, France) in May 2003. They were maintained in an open-circuit aquarium at the Observatoire Océanologique de Roscoff. Locomotion of *M. cirriferum* individuals was analyzed under binocular microscope (Leica MZ8). Movements of individuals were observed on their hosts and motions of parapodia were analyzed on individuals separated from their hosts and placed in Petri dishes. Individuals were also placed on different substrates (*Laminaria*, *Fucus*, sediment and cooked pasta of the same diameter than that of crinoid arm) and their reactions recorded. All observations were recorded on cassettes. Records were analyzed for (i) observing the movements of individuals, (ii) estimating the motion sequences of parapodia, and (iii) determining the parapodial motion. To study the motion sequences of parapodia and parapodial motions, 30 individuals, placed with their backside on the bottom of Petri dishes, were stimulated in touching their marginal cirri. For each individual, six cirri (the first, the fourth and the tenth pairs) were gently touched with small forceps and the resulting motions recorded.

For SEM observations, myzostomids were fixed in Bouin's fluid for 24 h, dehydrated in a graded ethanol series, dried by the critical point method (using CO_2 as the transition fluid), mounted on aluminium stubs, coated with gold in a sputter coater, and observed with a JEOL JSM 6100 scanning electron microscope. For observations with light microscope and TEM, myzostomids were fixed in a 3% solution of glutaraldehyde in cacodylate buffer (0.1 M, pH 7.8) for 3 h at 4°C. They were rinsed in the buffer and postfixed for 1 h with a 1% solution of osmium tetroxide in the same buffer. After a final rinsing in buffer, they were dehydrated in a graded ethanol series and embedded in Spurr resin. Serial transverse and longitudinal sections of parapodia, 1 µm thick, were performed with a glass knife using a Reichert Om U2 ultramicrotome and stained according to the method of Humphrey and Pittman (1974). Ultrathin, 70 nm thick sections were cut with a diamond knife using a Leica UCT ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a Zeiss LEO 906E transmission electron microscope.

For cLSM (confocal laser scanning microscopy) observations, myzostomids were fixed in 4% paraformaldehyde in PBS (phosphate buffered saline, pH 7.4, 0.15 M). After several rinses with PBS for at least 1 h, individuals were preincubated for 1 h in PBS containing 0.25% Triton X-100 then incubated with phalloidin-TRITC in PBS/Twin 1%/BSA 3% for 150 min. The specimens were washed several times in PBS/Twin 1% mounted between two cover slips in Citiflour (Plano, Wetzlar). Preparations were analyzed with a confocal laser scanning microscope, Zeiss LSM 410.

Results

Locomotion in *Myzostoma cirriferum*

Movements of individuals on hosts

Myzostoma cirriferum adults are mainly located on the host's calyx where they move to meet other conspecifics during mating or to reach ambulacral grooves for feeding. Smaller adults are also found on the arms and pinnules while juveniles are exclusively found into the ambulacral grooves of pinnules where they grow until they are able to move. When disturbed by a light source, individuals move quickly (the fastest record was 4 cm in 3 s). They do not use their discoid trunk to move but only their parapodia to walk on the host's surface. Records analyses show that *M. cirriferum* (i) moves mainly the anterior part forwards, (ii) is able to move the posterior part or the lateral parts (left and right) forwards, and (iii) is able to rotate of 180° on itself. *Myzostoma cirriferum* is unable to move on the surrounding substrates or on any flat surface. The only materials where myzostomids move quite easily are some cooked pastas indicating that the texture of the substrates should be soft enough for chaetae to grip and that the substrates should have a certain curvature that fits the curved ventral side of myzostomids.

Parapodial motions

Only 23% of the cirrus stimulations induce parapodial motions. In 18%, induced parapodial motions were slow and concern only one parapodium that most often was located at the opposite side of the stimulated cirrus. In the last 5% (86 cases on 1,860 records), induced parapodial motion were fast and always concern more than one parapodium but never all the parapodia. When touching one right or left cirrus of the fourth pair, contralateral parapodia move: generally the third parapodium moves alone, the first and the fourth move together, and the second and the fifth move also together. Stimulations of the first anterior and the fifth posterior pairs of cirri induce motion in two to

three posterior and two to three anterior pairs of parapodia, respectively. Whether they are slow or fast, movements of each parapodium always occur in a plane that passes through the myzostomids at the level of the parapodial insertion and at the level of the myzostomid midpoint (intersection between the sagittal plane and the axis joining parapodia of the third pair). Parapodial motions thus occur in planes located 30°, 60° or 90° from the sagittal plane (Fig. 1). The trunk of individuals is slightly curved and the parapodial axis is such that they made an angle of 70° to −20° from sagittal plane (see Fig. 1).

Parapodium

A parapodium in adult *M. cirriferum* is a conical structure of ca 100 µm long and 30 µm wide at its base (Fig. 2a). A hook protrudes from its apex through a 5 µm wide opening (Fig. 2a). The length of the part of the hook visible externally is variable, depending on the state of protrusion. Observed length varied from 15 to 100 µm. Tissues that compose a parapodium are the epidermis, a parenchyma, muscles, nerves, and a chaetal apparatus. The epidermis and parenchyma are similar to those observed in the trunk,

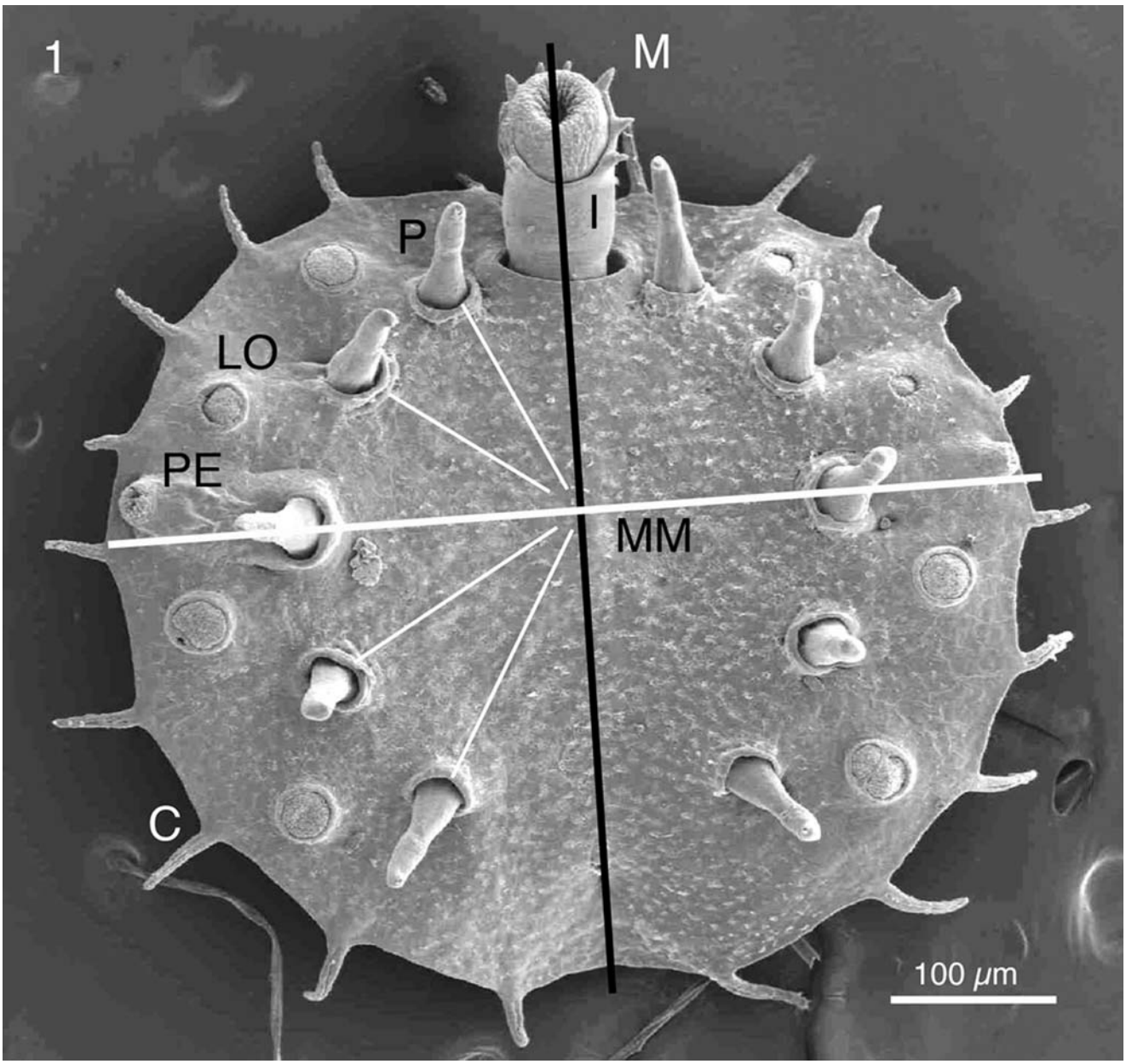


Fig. 1 Ventral view of *Myzostoma cirriferum* (SEM). *Black line* indicates sagittal axis; *thin white line* indicates axis joining parapodial base and the myzostomid midpoint; *thick white line* indicates axis joining the base of the parapodia of the third pair. *C* cirrus, *I* introvert, *LO* lateral organ, *M* mouth, *MM* myzostomid midpoint, *P* parapodia, *PE* penis

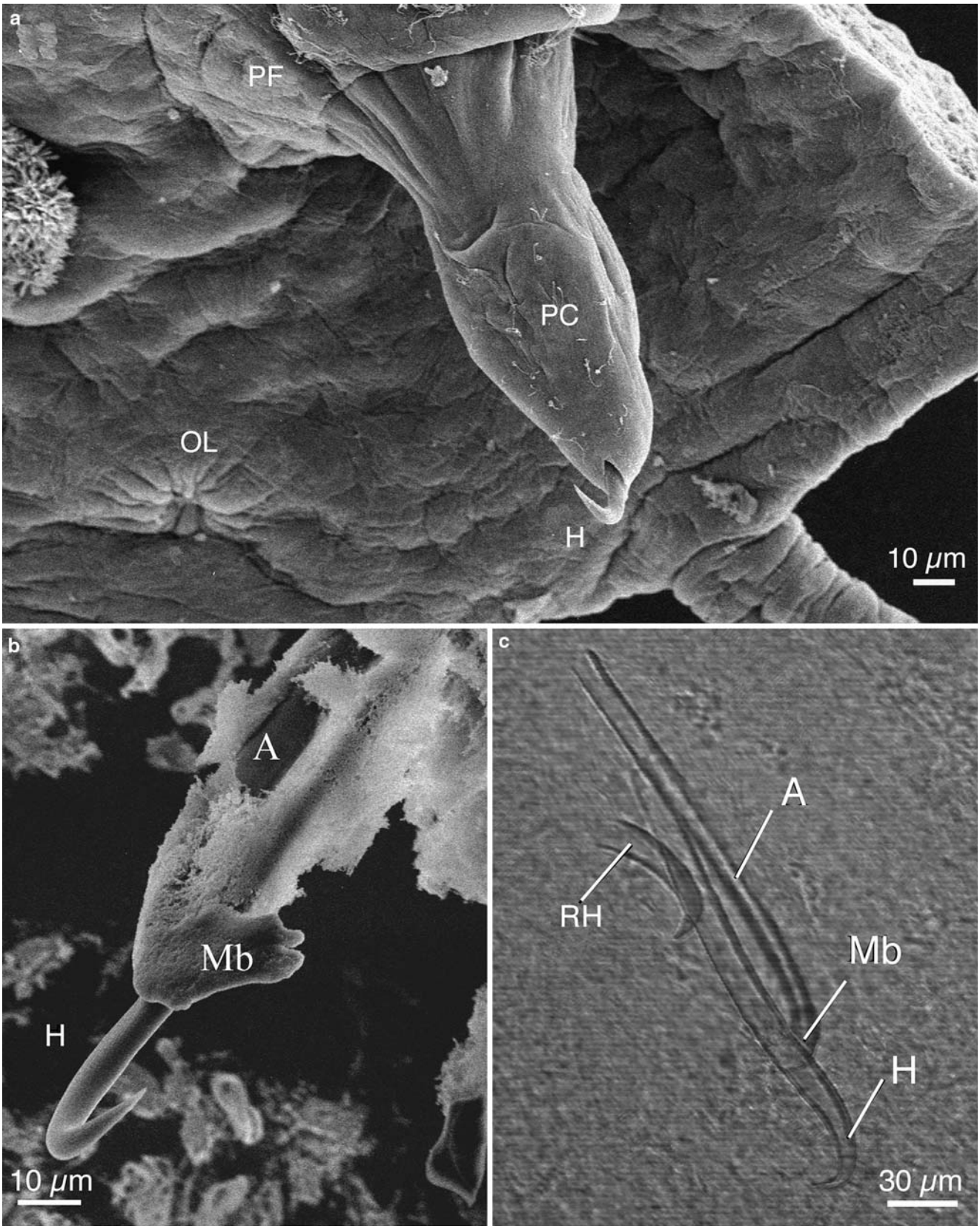


Fig. 2 Parapodium structure. Ventral view of a parapodium (SEM) (**a**) and details of the chaetae in SEM (**b**) and in optic microscopy (**c**). *A* aciculum, *H* hook, *PC* parapodial cone, *PF* parapodial fold, *Mb* manubrium, *OL* lateral organ, *RH* replacement hook

already described by Eeckhaut and Jangoux (1993). Differences observed here are that (i) the epidermis is extremely flat where muscles anchor (it measures no more than 200 nm), (ii) the parenchyma is low developed as muscles filled the most parts of parapodia. Parapodial nerves have been described in Müller and Westheide (2000).

Chaetae

The chaetal apparatus is a deep epidermal fold made of three chaetal follicles where three chaetae are located: a hook that protrudes to the exterior, an internal replacement hook and an internal aciculum (Fig. 2b, c). In adults, the hook is 250 µm long for 9 µm wide at its base (Fig. 2c). Its base is most often located in the myzostomid trunk except when the hook is totally protruded. The aciculum is a rod of 200 µm long for 9 µm wide at its base (Fig. 2b, c). It is also deeply inserted in the myzostomid trunk and never protrudes to the exterior. At its apex, the aciculum enlarges and forms a plate of ca 15 µm high, the manubrium, which is its point of contact with the hook (Fig. 2b, c). At the base of the aciculum stands a replacement hook of ca 70 µm long and 9 µm wide at its base (Fig. 2c). Hook and replacement hook are always closer to the sagittal plane of the myzostomids than the aciculum.

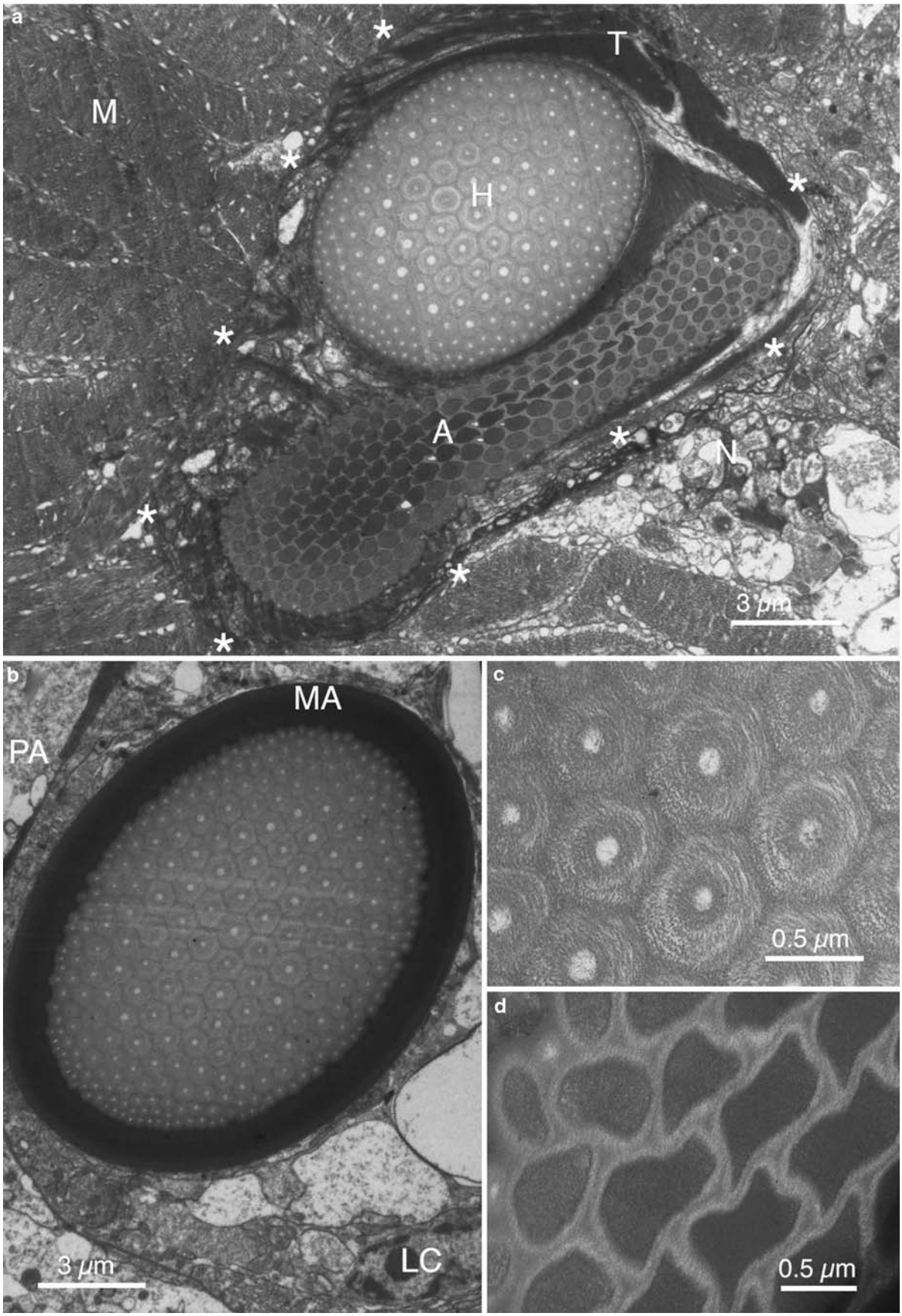
In transversal section, a hook-like chaeta appears as an ovoid structure (9 µm wide and 12 µm long) made of a central core including 235–290 hexagonal clear alveoli and a peripheral electron dense enamel layer (Fig. 3a, b). Alveolus size decreases from the centre of the core to its periphery, the length between to opposite angles being of 0.2–1 µm for peripheral and central alveoli, respectively (Fig. 3a, b). Each alveolus is made of a fibrillar material that surrounds a central channel of 50–250 nm in diameter (Fig. 3a, b, c). The electron dense enamel layer measures 100 nm, where it contacts muscles (Fig. 3a), and up to 1 µm thick everywhere else (Figs. 3b, 4a). It is composed of fibrillar layers of different electron density (Fig. 3b). The innermost layer is 100 nm thick and is the only one that is present at the level of muscle junctions (Fig. 3a). When sections are close to the basal part of the shaft, central channels of the alveoli are each completely filled by a microvillus emanating from a basal chaetoblast (Fig. 4b). In longitudinal sections, the base of the hook appears to be made of parallel rows of large microvilli that are surrounded by a thin fibrillar material (Fig. 4b). Microvilli are themselves filled with the fibrillar material (Fig. 4b).

Whereas the ultrastructure of the replacement hook is similar to that of the hooklike chaeta, the ultrastructure of the aciculum is a bit different. Firstly, only the innermost layer of the electron dense mantle covers the central core of the shaft (Fig. 3a). Secondly, alveoli are not exactly hexagonal but irregular in shape (Fig. 3a, d). Finally, the central channels of the alveoli are filled with an electron dense granular material at the level of the manubrium (while they are empty everywhere else) (Fig. 3a, d).

The three chaetae are located in extracellular spaces that meet to form a unique channel that opens to the exterior at the apex of the parapodia. Extracellular spaces are lined by two types of cell: chaetoblasts and lateral cells. Each chaeta is flanked by one chaetoblast at its base and few lateral cells on its flanks (Fig. 4a). Chaetoblasts are present in juveniles and adults where chaetae are fully developed and they appear in both as flat cells with microvilli. There is no sign of intense metabolic synthesis in the cytoplasm of chaetoblasts and lateral cells. There is no sign of vesicular transportation in the cytoplasm or at the base of microvilli. Lateral cells look similar to chaetoblats but they do not possess microvilli (Fig. 4a). At the level of muscular junctions, tonofilaments fill lateral cells and adhere to the chaetae on one side and to the basal lamina on the other side thanks to hemidesmosomes (Fig. 4c). Manubrium consists in lateral cells that are totally filled by tonofilaments.

Parapodial muscles

Analyses of serial semi-thin sections revealed that each parapodium includes six chaetal muscles that contact the chaetae by one of its extremity (Figs. 5, 6). In addition to these chaetal muscles, parapodia also include muscle fibers that do not contact chaetae. Some of these fibers come from the myzostomid ventral muscle that starts below the nerve cord and runs to the apex of the parapodium. Contraction of these muscle fibers results in the shortening of the parapodial cone. Other muscle fibers are epidermal. They are similar to those located in the trunk or in the introvert already described by Eeckhaut and Jangoux (1993). In parapodia, they form epidermal muscular rings located each 50 µm from the apex to the base of the parapodium. Their contraction results in the lengthening of the parapodial cone. The six chaetal muscles form three pairs (Fig. 6). The first pair is made of the parapodium flexor and extensor (Fig. 6). Both are made of unidirectional fibers (i.e., all fibers run in the same direction) and anchor to the manubrium. They attach either to the ventral epidermis located in the sagittal plane of the myzostomid (parapodium flexor) or to the dorsal epidermis (parapodium extensor). The second pair of muscles is made of the aciculum retractor and protractor. The aciculum retractor is made of unidirectional fibers that anchor to the base of the aciculum on one hand and to the dorsal epidermis on the other hand. The aciculum protractor is made of pluridirectional fibers that radiate from the base of the aciculum and anchor to the epidermis of the parapodial fold. The third pair of muscle is made of the conjunctor and the hook protractor. The conjunctor is made of unidirectional fibers that anchor to the base of the hook on one hand and to the base of the aciculum on the other hand. The


a
T
M
H
A
N
3 µm
b
MA
PA
3 µm
LC
c
0.5 µm
d
0.5 µm

◀ **Fig. 3** TEM views of the chaetae. Transversal section through the hook and aciculum at the level of the manubrium (**a**); transversal section through the middle part of the hook (**b**); details of the alveoli of the hook (**c**) and aciculum (**d**). *Asterisks* indicate the limit of the chaetal follicle. *A* aciculum, *H* hook, *LC* lateral cells, *MA* mantle, *PA* parenchyma

hook protractor is made of pluridirectional fibers that radiate from the base of the hook and anchor to the epidermis of the parapodial fold.

Analyses of the cLSM preparations revealed the musculature in the trunk of individuals. Musculature that is put in evidence includes mainly muscle fibers of about 1 to 2 µm thick that run transversally to the individual sagittal plane, from the anterior to the posterior parts of the trunk (Fig. 5a). These fibers surround the internal part of the trunk and certainly correspond to the epidermal muscle fibers described in Eeckhaut and Jangoux (1993). Parapodial muscles that well appeared in preparations are the parapodium flexors: there are five pairs of muscles of about 10 µm thick and almost 300 µm long that end at the level of the nerve chain (Fig. 5a, b). The protractors (aciculum and hook protractors) measure about 5 µm in thickness and 100 µm in length. They end at the level of the parapodial folds.

Discussion

Ectocommensal myzostomids walk on the surface of their hosts. They do not crawl or swim as it is the case for polychaetes, the group to which myzostomids are generally related. Myzostomids have no true segments and they consequently cannot use coelomic cavities to lengthen or shorten body parts to move (Eeckhaut and Lanterbecq 2005). They also lack strong longitudinal muscles that could allow them to swim as some polychaetes do.

Locomotion in Myzostomida

Our observations made on living *Myzostoma cirriferum* put in evidence that: (i) the individuals move the anterior part, the posterior part or the lateral parts forwards; (ii) it is able to rotate of 180° on itself; (iii) its locomotion relies entirely on parapodial motions and not on trunk movements; (iv)

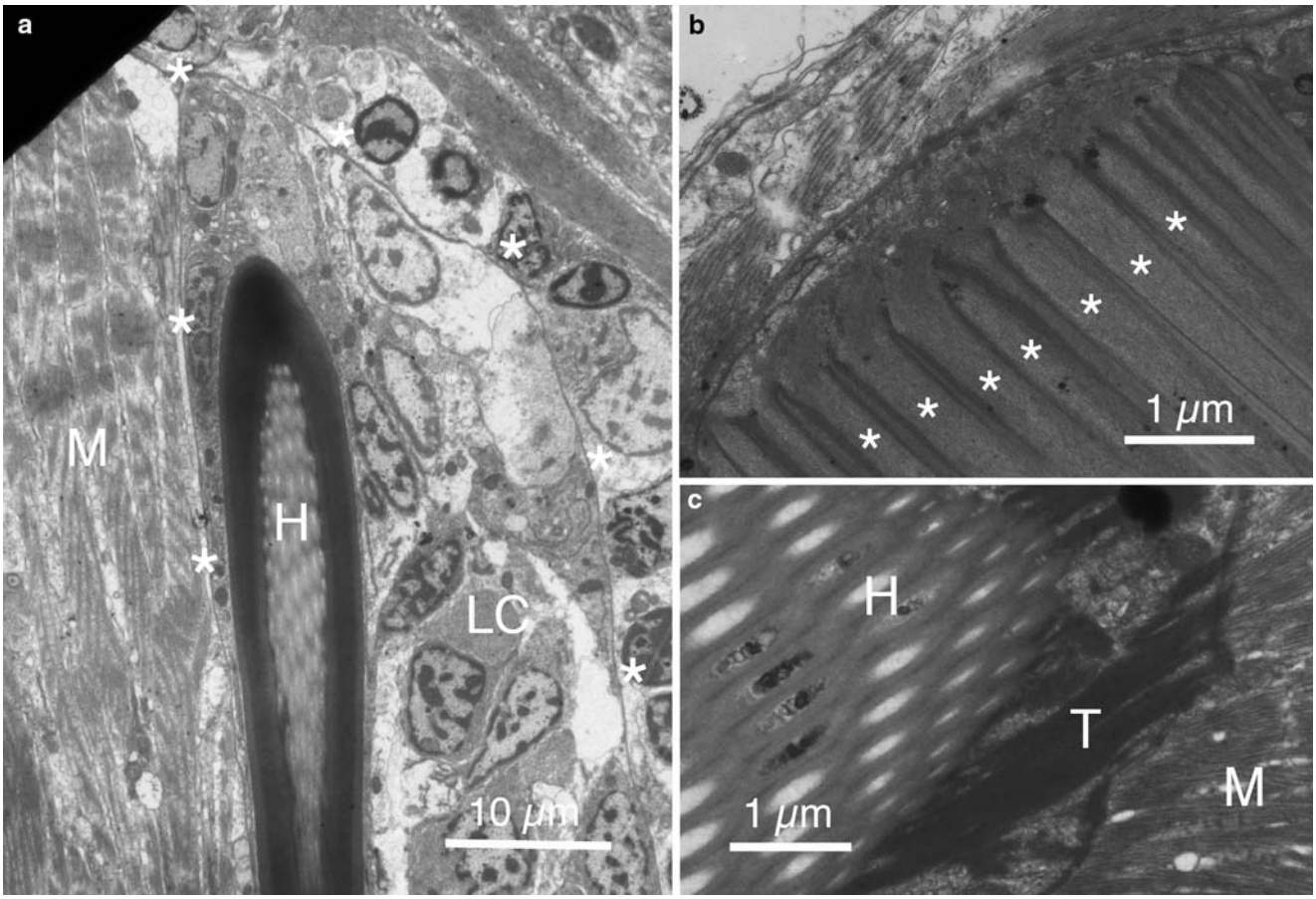


Fig. 4 TEM views of chaetae and their cells. Longitudinal section through the hook with its associated lateral cells and muscle (**a**); basal part of the hook in adult myzostomid (**b**); details of microvilli observed in the alveoli and the tonofilaments anchoring muscles to the hook (**c**). *Asterisks* indicate limits of the chaetal follicle in **a** and microvilli in **b**. *H* hook, *LC* lateral cell, *M* muscle fibers, *T* tonofilament

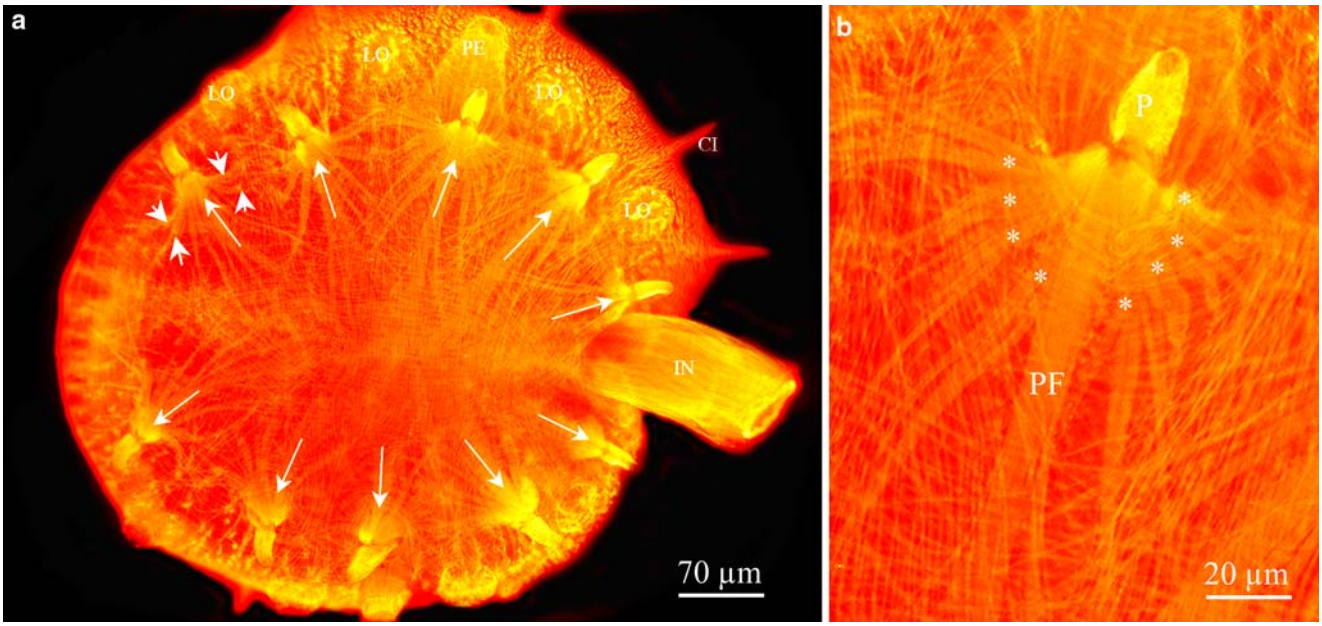


Fig. 5 cLSM views of *M. cirriferum* (**a**) and details of a parapodium (**b**). *CI* cirrus, *IN* introvert, *LO* lateral organ, *P* parapodium, *PE* penis, *PF* parapodium flexor. *Small* and *long arrows* in **a** show protractors and parapodial flexors, respectively. *Asterisks* shows protractors

the five pairs of parapodia do not work together at the same time; (v) the main parapodial motion is directed to the myzostomid ventral midpoint and implies a flexion of the parapodial cone followed by a subsequent return to a normal position, (vi) the parapodial cone always moves by 30°, 60° or 90° relative to the myzostomid sagittal plane. *Myzostoma cirriferum* thus walks on its host due to the ventral position of the parapodia. The ten parapodia are located by pair on two opposite latero-ventral arcs, which implies that all parapodia cannot act all together at the same time. The two first pairs and the two last pairs should act when the individuals move forward the anterior part and the posterior part forwards, respectively. The parapodia of the left and the right side (some or all) should be solicited when the myzostomids move right and left, respectively.

Stummer-Traunfels (1926) nomenclature and proposed function for parapodial muscles differ to some extent from our analysis (Table 1). He proposed the following actions for parapodial movement: at first the hook apparatus is protracted (using protractores uncini and protractores suffulcri) which also leads to the extension of the parapodium. Then the direction of the hook is regulated through unilateral contraction of particular retractores laterales and protractores suffulcri. The hook will be then pushed forward and hooked with help of the retractor internus and musculus centralis. Now the hook and the complete hook apparatus will be retracted through retraction of the parapodium through its own musculature and, together with the retraction of the musculus centralis, this results in the movement of the myzostomid body. Contraction of the retractor externus pulls the hook out of the substratum.

Our analysis suggests that three pairs of muscles are involved in parapodial motions: parapodium flexor and parapodium extensor, aciculum protractor and aciculum retractor, and hook protractor with conjunctor (Table 1; Fig. 6). When all these muscles are relaxed, the hook is retracted into the parapodial cone with just its tip emerging from the parapodial opening (Fig. 6a). The first action should be the contraction of the aciculum and hook protractors: they pull both chaetae towards the tip of the parapodial cone, the hook being projected towards the substratum (i.e., the host surface) (Fig. 6b). While these two muscles remain contracted, the parapodium flexor contracts and the hook penetrates into the substratum (Fig. 6c). The individual is subsequently pushed forwards along the axis joining the base of the parapodium and the myzostomid midpoint. Then, while the protractors are still contracted, the parapodium flexor relaxes and the parapodium extensor contracts to remove the hook from the substratum and the parapodium returns to its initial position (Fig. 6d). Finally, the two protractors relax and the aciculum retractor with the conjunctor contract to pull the two chaetae to their initial position (Fig. 6e).

Myzostoma cirriferum individuals are also able to rotate of 180° on themselves. These movements are possible if the parapodia do not act at the same time. Hooks might bend from their axis when a few (and not all) muscle fibers of the hook protractor (that radiate around the base of the hook) contract.

Myzostomid chaetae

Locomotion of myzostomids is unique within polychaetes. Our investigation of structure and ultrastructure of the

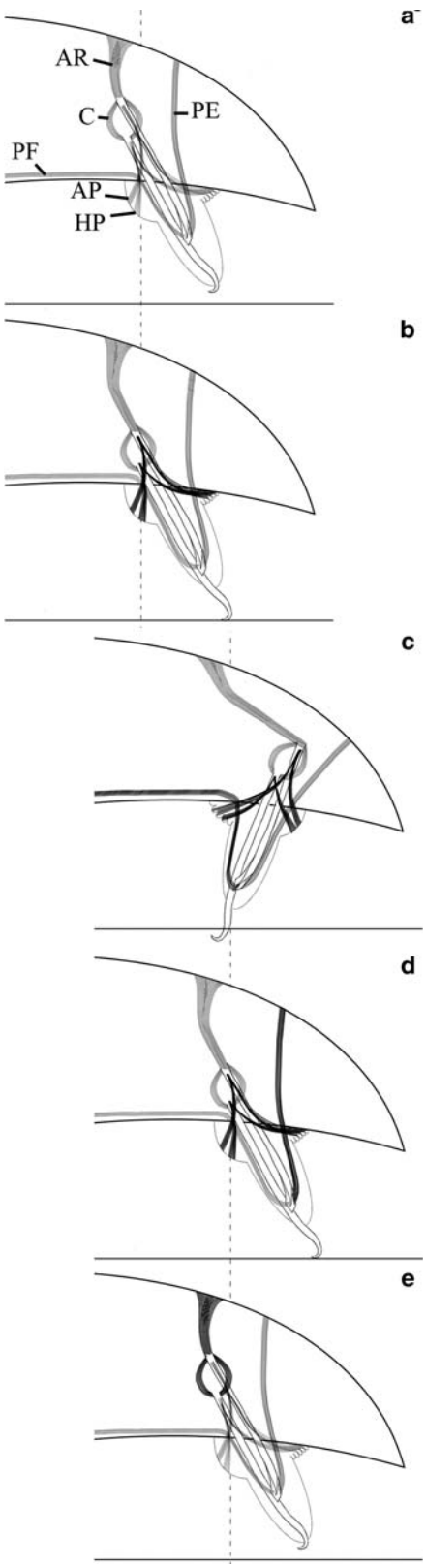


Fig. 6 Drawing of one parapodium showing the various actions (**a**–**e**) of parapodial muscles during the parapodial motion. Muscles in *grey* and *black* are relaxed and contracted, respectively. *AP* aciculum protractor, *AR* aciculum retractor, *C* conjunctor, *HP* hook protractor, *PE* parapodium extensor, *PF* parapodium flexor

Table 1 Similarities and differences between Stummer-Traunfels (1926)'s observations on the parapodial musculature of *M. gigas* and the present work made on *Myzostoma cirriferum*

Name	Stummer-Traunfels (1926)'s names	Starting point	End point	Biological function
Parapodium flexor	Musculus centralis	Manubrium	Ventral epidermis in the sagittal plane of the myzostomid	Parapodium flexion towards the myzostomid midpoint
Parapodium extensor	Own musculature of parapodium	Manubrium	Dorsal epidermis	Parapodium backwards movement towards the trunk margin
Aciculum retractor	Rectractores externii, R. internii	Base of the aciculum	Dorsal epidermis	Aciculum and hook retraction
Aciculum protractor	Protractores sufflulcri	Base of the aciculum	Epidermis in the parapodial fold	Projection of the aciculum towards the parapodium tip
Conjonctor	Conjunctores breves, C. longi	Base of the hook	Base of the aciculum	Maintenance of the junction between hook and aciculum
Hook protractor	Protractores uncini	Base of the hook	Epidermis in the parapodial fold	Projection of the hook towards the substratum

chaetae revealed similarities between those known in polychaetes and myzostomids. The results are congruent with previous studies based on light microscopy (Jägersten 1936). As typical for annelid chaetae, myzostomid chaetae are formed by a chaetoblast, which gives rise to microvilli where chaetal material assembles on the outer surface. Different chaetae can be classified considering form and function. A special type of chaetae is the aciculum (called "Stützstab" in Jägersten 1936). This chaeta acts as a supportive structure and is seen as a synapomorphy of the polychaete taxon Aciculata (Rouse and Fauchald 1997), and myzostomids group within this taxon in the same cladistic analyses. Besides this, some other polychaete taxa not belonging to the Aciculata also possess acicula in their parapodia: Orbiniidae, Apistobranchidae, Chaetopteridae, and Psammodrillidae (Hausen 2005). Some authors discuss the possibility that the Aciculata might represent a paraphyletic group at the root of the annelid tree (Rouse and Pleijel 2006). This assumption implies that acicula were present in the last common ancestor of all annelids. Nevertheless, acicula are often used to link myzostomids to aciculate polychaetes. The presence of chaetae, however, is not only restricted to annelids (including echiurians and pogonophorans). Similar structures are also reported from a few juvenile octopods, a certain placophoran, brachiopods and bryozoans (Lüter and Bartolomaeus 1997; see Hausen 2005 for review). Even though all these taxa are lophotrochozoans, these chaetae-bearing taxa never form a monophyletic group when rigorous phylogenetic analyses are applied to infer their relationship. In particular, in various analyses of different morphological as well as molecular data sets even myzostomids nest outside Annelida (Haszprunar 1996; Zrzavy et al. 2001; Eeckhaut et al. 2000; Passamaneck and Halanych 2006). However, a recent analysis investigating mitochondrial genome data strongly supports an annelid affinity for myzostomids (Bleidorn et al. 2007). Nevertheless, if one accepts that taxa with chaetae do not form a monophyletic group, then chaetae either evolved convergently in lophotrochozoans or chaetae were plesiomorphic structures present at the stem of lophotrochozoans and got lost in different lophotrochozoan lineages.

Convergence of chaetae implies that a complex ontogenetic system (one chaetoblast per chaeta, microvilli responsible of chaetal channels, chaetal material added through the base of microvilli) appeared multiple times during animal history—an assumption which is not parsimonious from a morphological point of view. On the other hand, the presence of organisms able to synthesize chaetae at the base of extant lophotrochozoans could imply that these organisms were morphologically complex and that body simplification occurred many times during lophotrochozoan history.

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