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Three-dimensional reconstruction of the musculature of *Cossura pygodactylata* Jones, 1956 (Annelida: Cossuridae)

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ABSTRACT

The musculature of adult specimens of *Cossura pygodactylata* was studied by means of F-actin labelling and confocal laser scanning microscopy (CLSM). Their body wall is comprised of five longitudinal muscle bands: two dorsal, two ventral and one ventromedial. Complete circular fibres are found only in the abdominal region, and they are developed only on the border of the segments. Thoracic and posterior body regions contain only transverse fibres ending near the ventral longitudinal bands. Almost-complete rings of transverse muscles, with gaps on the dorsal and ventral sides, surround the terminal part of the pygidium. Four longitudinal bands go to the middle of the prostomium and 5–14 paired dorso-ventral muscle fibres arise in its distal part. Each buccal tentacle contains one thick and two thin longitudinal muscle filaments; thick muscle fibres from all tentacles merge, forming left and right tentacle protractors rooted in the dorsal longitudinal bands of the body wall. The circumbuccal complex includes well-developed upper and lower lips. These lips contain an outer layer of transverse fibres, and the lower lip also contains inner oblique muscles going to the dorsal longitudinal bands. The branchial filament contains two longitudinal muscle fibres that do not connect with the body musculature. The parapodial complex includes strong intersegmental and segmental oblique muscles in the thoracic region only; chaetal retractors, protractors and muscles of the body wall are present in all body regions. Muscle fibres are developed in the dorsal and ventral mesenteries. One semi-circular fibre is developed on the border of each segment and is most likely embedded in the dissepiment. The intestine has thin circular fibres along its full length. The dorsal blood vessel has strong muscle fibres that cover its anterior part, which is called the heart. It consists of short longitudinal elements forming regular rings and inner partitions. The musculature of *C. pygodactylata* includes some elements that are homologous with similar muscular components in other polychaetes (i.e., the body wall and most parapodial muscles) and several unique features, mostly at the anterior end.

1. Introduction

Polychaetes are soft-bodied invertebrates, and their body shape and motion are provided by their muscular system (combined with hydro-skeleton). The degree of development of the musculature depends on the mode of life and locomotion. The arrangement of the muscular system might be an important systematic character (Rouse and Pleijel, 2001). Works by Storch (1968), Mettam (1967, 1971) were the first ones where detailed descriptions of polychaetes muscular system were done and were used for evolutionary reconstructions. Several papers, including few recent reviews (see Tzetlin and Filippova, 2005; Purschke and Müller, 2006), were devoted to the general architecture and ultrastructure of the musculature of polychaetes. Rapid progress in studying of the muscles began with the appearance of fluorescent staining of F-actin and consequent investigations using confocal laser scanning microscopes (CLSM) (Möllers and Müller, 2001; Tzetlin et al., 2002; Filippova et al., 2005, 2006, 2010; Müller and Worsaae, 2006; Rüchel and Müller, 2007). These studies revealed significant differences from the traditional view of the structure of polychaete musculature, for example, most of the investigated species do not possess complete rings of circular musculature. However, the number of polychaete taxa investigated with CLSM is still limited and does not represent the full range of morphological variety for this group.

Cossuridae is a small and poorly known family of benthic polychaetes. They are unique among polychaetes, having unpaired branchial filaments, a very unusual type of buccal apparatus, and their eversible buccal tentacles are attached to the dorsal side of the mouth cavity (Fig. 1) (Tzetlin, 1994). The phylogenetic position of Cossuridae in the modern polychaete system is not clear. They were assigned to the now abounded taxon Scolecida (Rouse and Pleijel,

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2001), but this placement was not confirmed by any subsequent studies (i.e., Struck et al., 2007; Zrzavý et al., 2009; Struck et al., 2011). Based on analysis of combined morphological and molecular (four nuclear, two mitochondrial genes) data, Cossuridae forms a clade with morphologically very different taxa – Paraonidae and Fauveliopsidae (Zrzavý et al., 2009).

In the present work, we investigated the muscular system of adult *Cossura pygodactylata* Jones, 1956 from the White Sea. The external morphology of this species was recently redescribed (Zhadan et al., 2012). Data on the ultrastructure of the body wall and gametogenesis of this species were provided by Rouse and Tzetlin (1997). According to their data, the cytoplasmic elements of the myocytes completely fill the space of the thoracal body cavity except for a small area in the dorsal region of the third chaetiger. Circular muscles are very poorly developed and could be observed only in the areas of inter-segmental fissures. In these regions, and where parapodial muscle complexes are absent, the dorsal and ventral bundles of longitudinal muscles on each side are fused. Oblique muscles are found in the regions of the parapodial complexes. There is a weakly developed dorsal blood vessel that connects to the intestinal vascular plexus. In the abdominal region, the body wall is thin, with a poorly developed muscle layer. The thickness of the muscular layer of the body wall varies from 0 to 5.0 μm. Circular muscles are poorly developed and could be observed only in intersegmental areas. There are areas in the body wall where muscles, both circular and longitudinal, are virtually absent. The arrangement of longitudinal muscle bands is basically the same as in the posterior thorax. In the present study, we extend and improve the characterisation of the muscular system of *C. pygodactylata* significantly.

2. Materials and methods

Specimens were collected by an epibenthic dredge in Kandalaksha Bay near the White Sea biological station of Moscow State University (66°52′ N 33°19′ W) from muddy sediment at 30–60 m depth in September 2011 and October 2012. Samples were washed using seawater in a 100 μm mesh and sorted with a stereomicroscope. Specimens were relaxed in an isotonic $MgCl_2$ solution and fixed with a 4% paraformaldehyde solution in 0.1 M phosphate buffer for 4 h in a refrigerator (4 °C) with constant mixing. After fixation, worms were washed three times for 20 min in PBS during constant stirring. Then, specimens were placed in Phallacidin 607 or Phalloidin 488 (Sigma), diluted 1:70 in 0.1 M PBS for 1 h in the refrigerator (4 °C) with constant stirring. After staining, animals were washed 3 times for 20 min with 0.1 M PBS during constant stirring and placed into a drop of 0.1 M PBS on a glass coverslip coated with Poly-L-lysine (Sigma) to enable adhesion to glass. They were then dehydrated in ascending isopropyl alcohol solutions (70% – 85% – 95% – 100% – 100%) and cleared using Murray Clear (MC – benzyl benzoate, with benzyl alcohol (2:1)), MC1–MC2–MC3. Worms were placed in each solution for 30 s. Specimens were then mounted in MC between two cover slips and placed in a refrigerator (4 °C) overnight. After staining with Phallacidin or Phalloidin, the slides were kept in the dark. Examination of the muscular system was conducted using a Nikon A1 confocal laser scanning microscope (CLSM) with laser emission at 514 nm and 488 nm for Phallacidin and Phalloidin, respectively. Three-dimensional (3D) reconstructions were built using Imaris software.

The nomenclature used here was introduced by Filippova et al. (2005); abbreviations for the body sections—prostomium (pr), branchial filament (bf), buccal tentacles (bt), pharynx (ph), parapodia (pp), body wall (bw), are followed by the orientation as well as the location of the muscles, e.g., "bw/dl" indicates "body wall, dorsal longitudinal muscles". Furthermore, all muscles oriented perpendicular to the anterior–posterior body axis are termed "transverse muscles". Only transverse fibres encircling the entire body as a complete ring outside the longitudinal musculature (supralongitudinal) will be called "circular muscles". To keep the abbreviations short, a character for "muscle" is generally omitted.

3. Results

3.1. Design of the body wall

The body wall consists of four bands of longitudinal muscles (two dorsal (bw/dl) and two ventral (bw/vl)) and the weakly developed external circular (bw/c) or transverse (bw/t) muscles, mainly found in the abdominal segments. Intersegmental (bw/io) and segmental (bw/so) oblique muscle fibres are developed on the ventral side of the thoracal region (Fig. 2A–G). Transverse fibres (bw/t) located under longitudinal muscles connect the dorsal and ventral longitudinal bands in the anterior part of the body (Fig. 2D). Bracing muscle fibres were not found.

The anterior (thoracal) region has the most developed musculature: thick bands of longitudinal muscles (bw/dl, bw/vl), transverse fibres (bw/t) on segmental borders and oblique bundles (bw/io, bw/so) on the ventral side (Fig. 2A, B, E and G). The middle (abdominal) region is less muscularised: longitudinal bands become thinner, and there are no oblique bundles, but well developed circular muscle fibres (bw/c) on the segmental borders are present (Fig. 2C and D). In the posterior region, longitudinal and transverse (bw/t) elements are developed, and transverse muscle fibres dominate (Fig. 2F).

Outer transverse muscle elements do not form a complete cylinder. No circular muscle fibres were found in the thoracal region of the body, and only 3–7 thin transverse fibres going between dorsal and ventral longitudinal bands were observed in each segment in the inter-segmental zone. True circular muscle fibres (bw/c) were found in the abdominal segments; 8–10 relatively thick fibres run around the body on the segmental borders and discontinue only near the ventral nerve cord (Fig. 2C). No complete circular muscle fibres were found in the posterior part of the body – only transverse fibres that end on the ventral longitudinal bands. They are developed mostly on the segmental borders where 3–5 fibres are observed; individual thin fibres are found also on the segments (Fig. 2F). Thicker transverse fibres with small gaps on the dorsal and ventral sides form an external sphincter (es) on the pygidium (Fig. 2F).

Longitudinal muscles are well developed; they form two dorsal (bw/dl) and two ventral (bw/vl) bands (Figs. 2A, B, E, 6A and 7A). They start at about the middle of the prostomium; small parts separate from the dorsal band and join to the ventral band in the peristomium (Figs. 2A, 6A and 7A). The anterior most part of the dorsal bands is narrow and widely separated on the prostomium. On the peristomium, bands become wider and almost merge, forming an entire layer. On the level of the branchial filament (bf) they separate again, stretching in parallel close to each other along the body and joining in the pygidium (Fig. 6A). Ventral bands are separated more widely than dorsal bands and run in parallel collocation along the whole body and join in the pygidium (Fig. 2E and F). In addition, a thin median ventral bundle (bw/vml) goes between the ventral bands from the lower lip to the pygidium (Figs. 2G, 4A and 9D). This bundle is very thin in the thoracal region and is well developed in the abdominal and posterior parts of the body; it joins to ventral bands in the pygidium (Fig. 2F). In bigger specimens, one more median bundle (vm) was observed above and parallel to the medial ventral bundle. This bundle most likely comprises the ventral mesenterium (Fig. 9D).

Oblique muscle fibres are strongly developed in the anterior part of the body (Figs. 2G, 3A, B, 4A, B and C). Intersegmental oblique bundles (bw/io) go from the ventral median longitudinal bundle

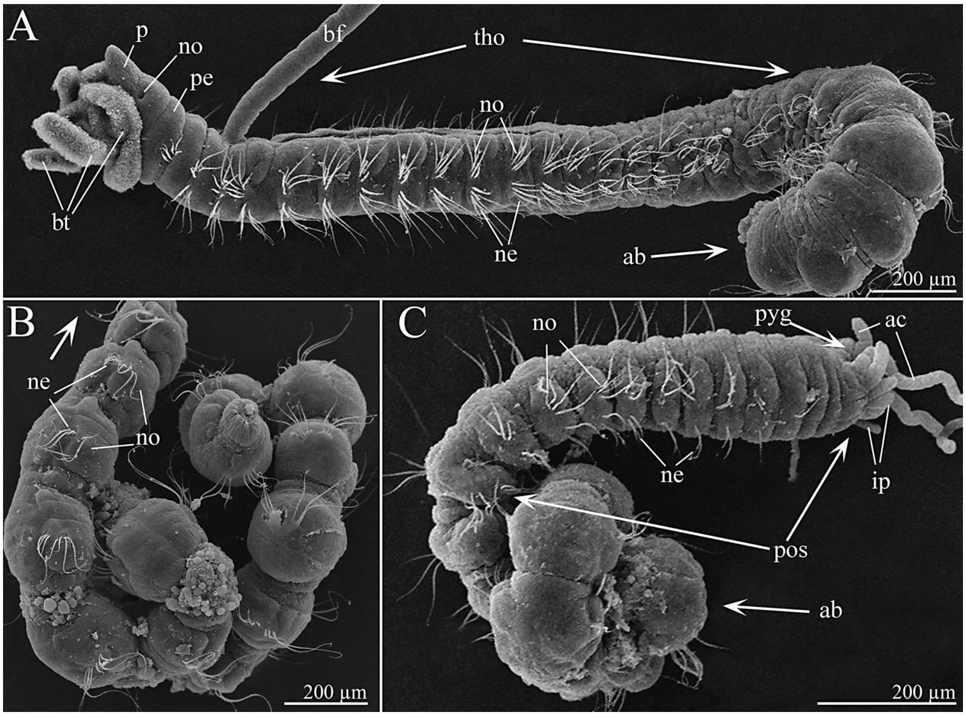

Fig. 1. SEM micrographs showing body regions. (A) Anterior end, left lateral view, buccal tentacles (bt) everted; note prostomium (p), peristomium (pe), branchial filament (bf), thorax (tho), abdomen (ab). (B) Middle (abdominal) body region, left lateral view, anterior end marked by arrow. (C) Posterior end, left lateral view, showing abdomen (ab), posterior region (pos), pygidium (pyg), anal cirri (ac), intercirral processes (ip). *Abbreviations*: notopodia (no), neuropodia (ne).

(bw/vml) on the border of the segments backward to the next segment (Figs. 2G, 3A, B and 4A, C). Smaller segmental bundles (bw/so) go from the same point to the body wall of the same segment close to the parapodia (Figs. 2G, 3A, B and 4C). Oblique muscle fibres are almost completely absent in the middle and posterior parts of the body. In some specimens, very thin bundles go from the ventral body wall to the parapodia of the same segment (segmental oblique muscle fibres (bw/so)). Semi-circular bundles start from the intersegmental oblique muscle fibres on the segmental borders and form incomplete rings around the intestine (Figs. 3A–D and 9C). We suppose that these bundles (ms) comprise the dissepiments.

3.1.1. *Musculature of the pygidium (Fig. 2F)*

Dorsal longitudinal bands (bw/dl) merge on the dorsal side and ventral longitudinal (bw/vl) and median (bw/vml) bands merge on the ventral side of the pygidium. Transverse fibres are well developed and discontinue by a narrow gap only on the dorsal and ventral sides; they form external sphincter (es). Anal cirri do not contain any muscular elements.

The branchial filament contains two longitudinal muscular bundles (bf/l), anterior and posterior. They are not fused or connected with the body musculature. Circular, spiral and transverse elements were not found (Figs. 2A, 5D and 8D).

3.2. *Musculature of the parapodia*

The parapodial complex (pp) consists of the following three groups of muscle bundles:

1. Chaetal retractors (pp/r). A single bundle goes from the median longitudinal band to the base of the chaetal bundle (Figs. 3A, B and 4A, E, G).
2. Chaetal protractors extend from the chaetal base to the body wall. Notopodial protractors (no/p) are more complex than neuropodial. They consist of a bundle going to the next segment along the ventral side and coming to the base of next parapodia, a bundle going backward to the outer parapodial muscle fibres and a bundle going up and anteriorly to the outer parapodial muscle fibres (pp/e) (Figs. 3A, B and 4A, E, G). Neuropodial protractors (ne/p) connect to the ventral body wall of the same segment (Fig. 4B and G).
3. External parapodial muscle (pp/e) fibres run from longitudinal bundles to the body wall close to the chaetae. Dorsal external muscle (pp/de) fibres extend from segmental borders forward and backward to notochaetae (Fig. 4D, E and G). Ventral external muscle (pp/ve) fibres stretch from segmental borders forward and backward to the neurochaetae (Fig. 4F, E and G).

3.3. *Prostomium and peristomium*

Dorsal and ventral longitudinal bands run to the prostomium (p), connecting at about the middle of its length (Figs. 5A, C, D, 6A, D and 7A). The anterior most part of the prostomium bears two rows, each consisting of 5–14 strait, strip-like, dorso-ventral bundles (p/dv) running vertically between the dorsal and ventral walls of the prostomium; their number increases with the size of the worm (Figs. 2A, 5C, 6A, C and 7A, B). The posterior most pair of these bundles is wider and longer than the others.

The paired ventral rostral muscle fibres (p/vr) start from the ventral longitudinal bands, each by two roots, and go forward and upward, where they join each other (Figs. 6B–D and 7B). The similar but thinner pair of dorsal rostral muscle fibres (p/dr) start from the dorsal longitudinal bands and extend forward and downward, where they merge with the ventral rostral bundles (Fig. 6C and B). From the point where the ventral rostral muscle fibres join, a pair of bundles going to the nuchal organs starts (the retractor muscles of the nuchal organ (nu)) (Figs. 6C and 7B). Anteriorly from these bundles, a pair of bundles going upward and downward is located close to the ventral rostral muscle fibres, but is not attached to them (Figs. 6C and 7B). These dorso-ventral bundles are located at some distance from the anterior most ones and are less straight. Two bundles located more posteriorly than the retractor muscles of the

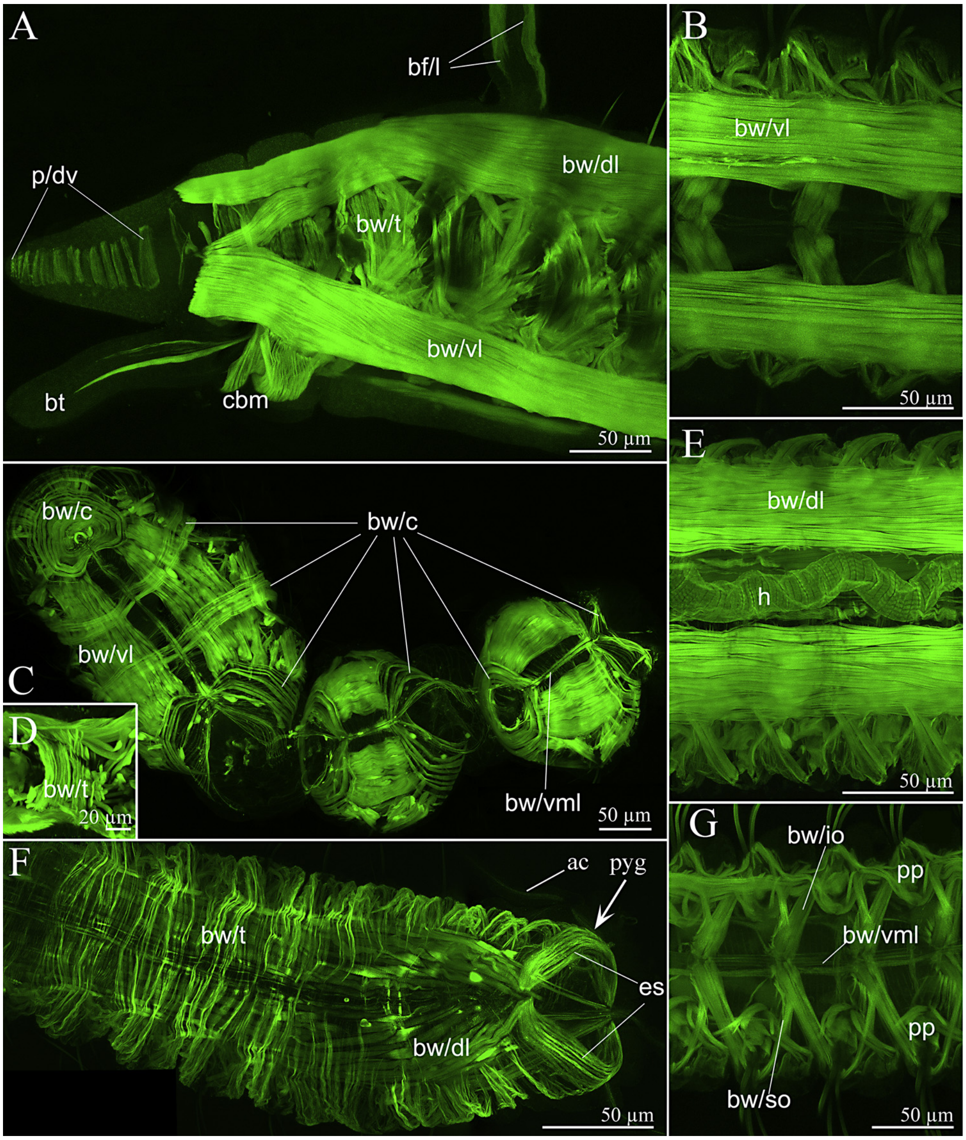

Fig. 2. CLSM micrographs showing phalloidin staining of body wall musculature, 2D projections. (A) Anterior end, left lateral view, buccal tentacles (bt) everted; note prostomial dorso-ventral bundles (p/dv), circumbuccal muscle fibres (cbm), dorsal (bw/dl) and ventral (bw/vl) longitudinal bands, transverse fibres (bw/t), and longitudinal muscle fibres of branchial filament (bf/l). (B) Thorax, ventral view showing ventral longitudinal bands (bw/vl). (C) Abdomen, ventral view showing ventral longitudinal bands (bw/vl), ventromedial longitudinal muscle (bw/vml) and circular muscles (bw/c) on the segmental borders. (D) Thorax, lateral view showing transverse fibres (bw/t) connecting dorsal and ventral longitudinal bands. (E) Thorax, dorsal view showing dorsal longitudinal bands (bw/dl) and heart (h). (F) Posterior end, dorsal view, showing dorsal longitudinal bands (bw/dl), transverse fibres (bw/t), pygidium (pyg), anal cirri (ac) and external pygidial sphincter (es). (G) Thorax, ventral view; ventral longitudinal bands are removed to show intersegmental oblique bundles (bw/io), segmental oblique bundles (bw/so), parapodial muscle complexes (pp), and middle ventral longitudinal bundle (bw/vml).

nuchal organ fibres (nu) stretch dorsally. Between their bases runs a short transverse muscle (p/t) fibre connecting the ventral and rostral muscle fibres (Figs. 6C, D and 7B). The transverse bundle comprising an upper lip (ul) connects the basal parts of the ventral longitudinal muscles (bw/vl) (Figs. 5A, B, 7B and 8B).

The buccal muscle complex includes the upper and lower lips (Figs. 5A, B, 6A, B, 7B and 8A, B). The upper lip (ul) contains only transverse muscle fibres. It has two groups of retractors (ul/r): (1) three bundles of retractors going from the upper lip muscle fibre to the anterior parts of the ventral rostral muscle fibres and to a transverse bundle between them; (2) one more upper lip retractor, sometimes split into two bundles, extends from the transverse muscle fibre upward to the dorsal longitudinal bands (Figs. 6B, C, D, 7B and 8B).

The lower lip (ll) includes a transverse muscle bundle extending to the dorsal longitudinal bands (bw/dl), and the circumbuccal muscles (cbm). Circumbuccal muscles (cbm) consist of an external layer of thin transverse muscle fibres interconnecting ventral longitudinal bands. Internally from these transversal fibres, a second layer of four bundles of circumbuccal oblique fibres is located, which pull forward ventro-medially and stretch backward and upward to the dorsal longitudinal bands. The first pair is the widest, and the remaining ones are thinner. The median longitudinal band (bw/vml) starts from the posterior edge of the lower lip (Figs. 5A, B, 6A, B, 7B and 8A, B).

3.3.1. *The buccal tentacles*

Between 4 and 10 eversible buccal tentacles (bt) are developed in specimens having 21 and more chaetigers; the number is always even. Each tentacle contains one thick fibre and one thin longitudinal muscle fibre but no transverse or circular fibres (Figs. 5A, D, G and 7A–D). Longitudinal fibres merge into two thick

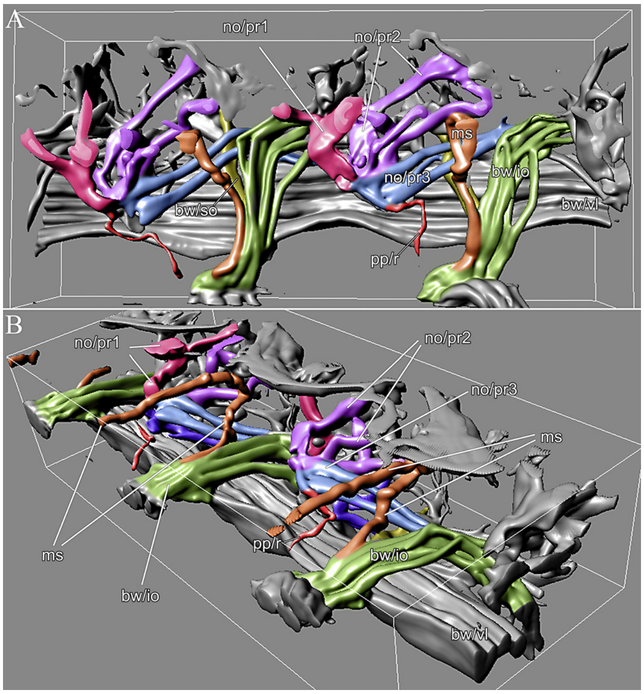

Fig. 3. 3D reconstructions of the right side of the anterior (thoracal) body region in the area of chaetigers 5–6; view from dorsal side, dorsal longitudinal bands removed to show (coloured) parapodial complex (only notopodial muscles are shown) and oblique muscle bundles. (A) Dorsal view. (B) Oblique posterior view (rotated by approximately 30 degrees). *Abbreviations*: bw/io, intersegmental oblique bundles; bw/so, segmental oblique bundles; bw/vl, ventral longitudinal bands; ms, semi-circular muscle of dissepiment; no/pr 1–3, notopodial protractors 1–3; pp/r, parapodial retractors. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

bundles in the basal part of the tentacles – the retractors of the buccal tentacles (bt/r). These bundles diverge and join to the right and left dorsal longitudinal bands at the level of the 4th chaetiger. In the retractors, tentacle muscle fibres are arranged in one vertical row. One half of the tentacle is attached to the left longitudinal band, and the other half is attached to the right longitudinal band (Figs. 5E, F and 8A, D).

3.4. *Musculature of internal organs*

Body cavity. Longitudinal bundles run from the dorsal and ventral side of the gut; they comprise the dorsal and ventral mesenteries (vm) (Fig. 9D). Dissepiments are developed in the thoracal region, where semi-circular muscle fibres (ms) surround the gut. These muscle fibres originate in the intersegmental oblique muscle fibres (bw/io) (see above) (Figs. 3A, B, 4A, C, D and 9C).

3.4.1. *Musculature of the digestive system*

The pharynx (ph) is soft, axial and eversible. It is also asymmetrical: the dorsal side is much longer than the ventral side and transformed into buccal tentacles. The ventral part is represented by a low fold containing longitudinal (ph/l) and transverse (ph/t) muscle elements; the latter are observed only present in the distal part of the pharynx. The dorsal part contains mostly thin longitudinal fibres (ph/l). The basal parts of the tentacles are connected with short transverse fibres (ph/t) (Figs. 5D and 8A, B).

The gut has circular musculature (i/c) along its entire length. Circular fibres are thin and do not form a complete layer (Fig. 9A).

3.4.2. *Heart*

The dorsal blood vessel has a thick muscular wall in its anterior part, from about 4 to 15 segments. We refer to this muscular part the heart (h). The outer heart musculature is represented by longitudinal muscle fibres with cross striations forming short regular rings (Figs. 2E and 9A). Internally from them, muscular transverse septae are located on every second ring of longitudinal muscle fibres (Fig. 9B).

4. Discussion

4.1. *Design of the body wall*

In general, the body wall of *C. pygodactylata* is organised in a pattern that is typical for polychaetes. Longitudinal muscles arranged in four bands are common for Phyllodocidae, Glyceridae, Ampharetidae, Nerillidae, Chrysopetalidae, and many other taxa (Tzetlin and Filippova, 2005). In addition, a ventral median bundle situated above the ventral cord was described for Chrysopetalidae, Nereididae, Hesionidae, Nerillidae, Dorvilleidae, and Capitellidae (Purschke and Müller, 2006).

The peculiar character of cossurids is a conspicuous differentiation of the organisation of the muscular system in different body regions: oblique muscle fibres are developed only in the thoracal region and circular fibres are found only in the abdomen. We did not find circular elements on the segmental borders of the thorax, as described by Rouse and Tzetlin (1997). They most likely misinterpreted transverse elements connecting dorsal and ventral longitudinal bands. An unusual characteristic of C. pygodactylata that has not been described before in other polychaetes is an external sphincter on the pygidium, consisting of transverse fibres with gaps on the ventral and dorsal sides.

True circular muscle fibres were found only in the abdomen of *C. pygodactylata*. This region is characterised by a very thin body wall and often is autotomised. Presumably, circular fibres that develop on the segmental borders provide closing the body cavity in the course of fragmenting. Little is known about the biology of cossurids, so we do not know how often or under which circumstances body fragmenting occurs in natural conditions. We found some specimens with regenerating posterior ends, but never with a regenerating head. This finding suggests that autotomy is not used for asexual reproduction. A more likely explanation is that the posterior end autotomises as a result of a predator attack. The posterior region also lacks circular fibres. The absence of circular fibres has been reported for many polychaete taxa, such as Aphroditidae, Chrysopetalidae, Magelonidae, Nephtyidae, Nerillidae, Opheliidae, Oweniidae, Phyllodocidae, Pisionidae, Polynoidae, Protodrilidae, Sigalionidae, and Spionidae (reviewed in Purschke and Müller, 2006). Circular fibres are important for annelids that burrow by means of peristaltic movements in comparatively hard substrates (Purschke and Müller, 2006). According to phylogenetic analysis by Struck et al. (2011) most of these taxa (Arenicolidae, Maldanidae, Capitellidae, Clitellata) belong to clade Sedentaria, but Glyceridae and *Parapodrilus* (Dorvilleidae) possessing circular fibres (Purschke and Müller, 2006) are typical errant group; true circular fibres are also present in Oweniidae and Dinophilidae that are most probably placed in the basal part of the annelid tree (Struck et al., 2011). Therefore circular muscles can both evolve independently in these taxa or be secondary lost in most errant polychaetes. Cossuridae are not represented in Struck et al. (2011) analysis, but they are typical sedentary worms as they lack head appendages and podial lobes. To our opinion presence of circular fibres in abdominal region in *C. pygodactylata* does not have significant phylogenetic importance.

The oblique muscle fibres on the ventral side are also common in many polychaetes. However, in *C. pygodactylata*, oblique muscle fibres are developed only in the thoracal region and some of these muscle fibres extend from one segment to the next. This condition was has not been described for any other polychaete. The

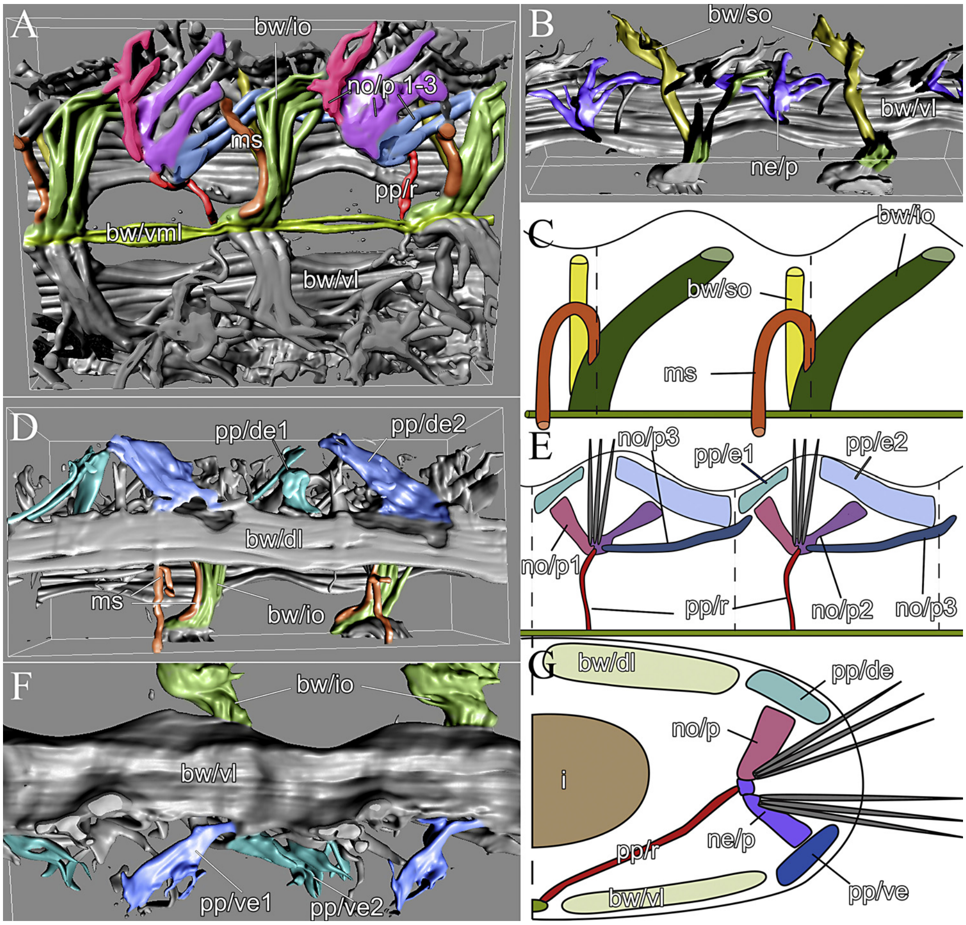

Fig. 4. Musculature of body wall and parapodia. A, B, D, F – 3D reconstructions of the anterior (thoracal) body region in the area of chaetigers 5–6. C,E,G – schemes. (A) Parapodial muscle complex, oblique muscles and ventral muscle bands. View from dorsal side, dorsal longitudinal bands removed. The notopodial muscle complex (no/p 1–3), parapodial retractors (pp/r), intersegmental oblique bundles (bw/io), semi-circular muscle of dissepiment (ms) and medial longitudinal ventral bundle (bw/vml) are coloured on the right side of the worm. (B) Segmental oblique muscles (bv/so), neuropodial protractors (ne/p) and ventral longitudinal bundles (bw/vl). View from the dorsal side, dorsal longitudinal bands, notopodial protractors and partly intersegmental oblique bundles removed. (C) Scheme showing basal parts of intersegmental (bw/io) and segmental oblique muscles (bw/so) and semi-circular muscle of dissepiment (ms). (D) Dorsal view, showing dorsal external notopodial bundles (pp/de1, pp/de2), intersegmental oblique bundles (bw/io), and semi-circular muscle of dissepiment (ms). (E) Scheme showing parapodial muscles, dorsal view. (F) Ventral view showing bases of intersegmental oblique bundles and neuropodial external muscles (pp/ve 1, 2). (G) Scheme of transverse section through chaetiger showing parapodial muscle complex, chaetae, dorsal (bw/dl), ventral longitudinal bands (bw/vl) and intestine (i). *Abbreviations*: bw/dl, dorsal longitudinal bands; bw/so, segmental oblique bundles; bw/vl, ventral longitudinal bands; ne/p, neuropodial protractors; no/p 1–3, notopodial protractors 1–3; pp/de, dorsal parapodial external muscles; pp/e, parapodial external muscles; pp/r, parapodial retractors; pp/ve ventral parapodial external muscles.

oblique muscle fibres in *C. pygodactylata* take part in maintaining of the shape of the body and in parapodial movements. It seems that only the anterior region is able to perform active movements, while abdominal and posterior regions are more passive. The exact mechanism of cossurid motion is unknown. Dorgan et al. (2006) supposed that they, like other inhabitants of mud burrows, move by crack propagation and use the elastic restoring force of sediment to aid their circular muscle fibres in elongating segments during peristalsis. The most probable in *C. pygodactylata* function of segments elongating is performed by transversal muscle fibres of anterior region, whereas circular muscles in abdominal region are used mostly during body fragmentation.

4.2. Parapodial musculature

The parapodia of Cossuridae lack podial lobes as well as in other sedentary taxa, and chaetae emerge directly from the body wall. A parapodial muscular system of this type has never been studied before. However, the musculature of cossurid parapodia has many typical elements that have been previously described for the parapodia of other polychaetes (Mettam, 1967, 1971; Storch, 1968; Tzetlin and Filippova, 2005; Purschke and Müller, 2006), including chaetal retractors and protractors and an external parapodial muscle fibre going from the longitudinal bundles to the body wall, which is likely to be homologous with parapodial wall muscle fibres of other polychaetes. This fact supports the hypothesis by Struck et al. (2011) on ground pattern of annelids that has developed parapodia. In Sedentaria parapodia are secondary reduced but present study shows cossurids have quite developed parapodial musculature despite of reduction of podial lobes. The oblique muscle fibres, which are involved in parapodial movement, have also been described for other polychaetes.

4.3. Architecture of the anterior end

The musculature of the prostomium of polychaetes is very variable and difficult to classify (Filippova et al., 2005, 2006, 2010). It is therefore not surprising that the prostomium of *C. pygodactylata* contains many unique features. The fact that the dorsal and ventral longitudinal bands run to the middle of the prostomium is unusual. In other polychaetes, longitudinal muscle fibres of the prostomium are described only from the ventral side and are rather thin. Paired

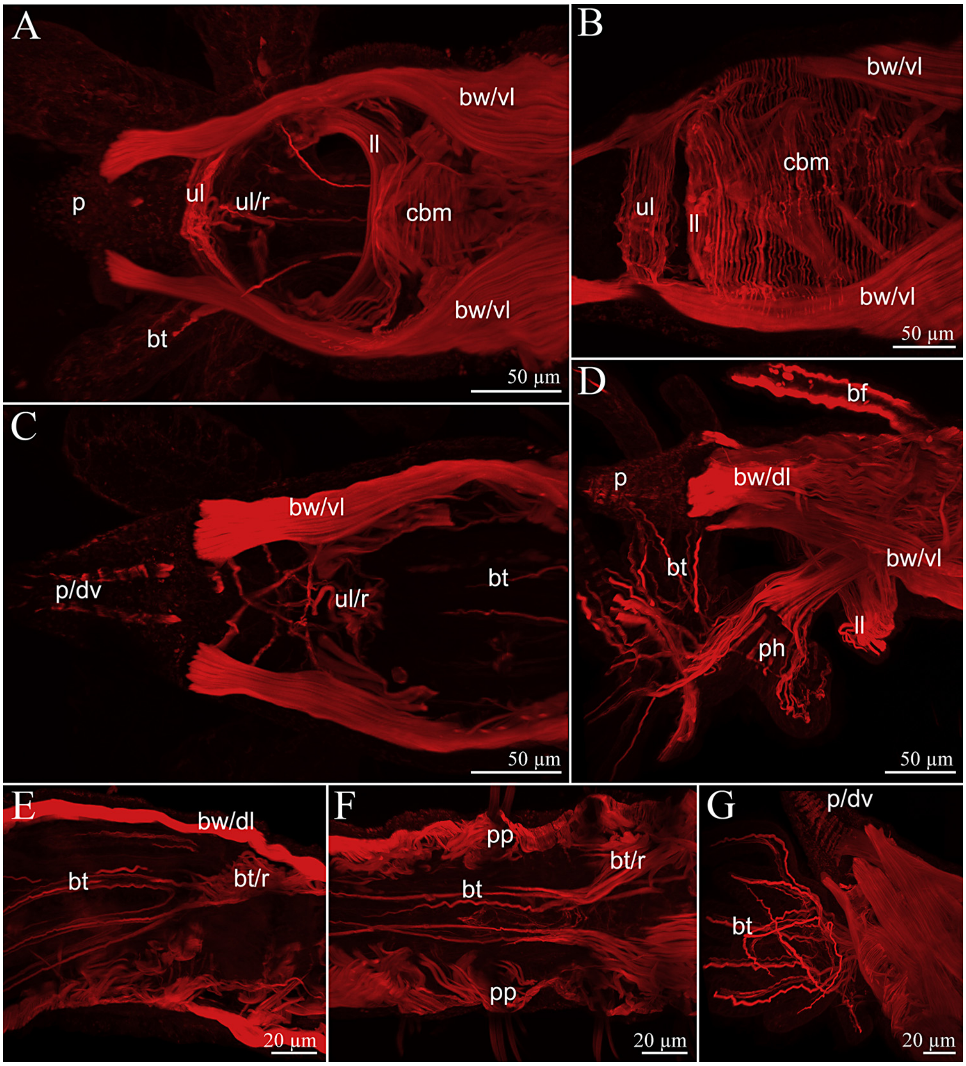

Fig. 5. CLSM micrographs showing phallacidin staining of musculature of anterior end, 2D projections. (A) Ventral view, buccal tentacles (bt) everted, mouth wide open; note upper lip (ul) with retractors (ul/r), lower lip (ll), and circumbuccal muscle fibres (cbm). (B) Ventral view, buccal tentacles inverted, mouth closed. (C) Dorsal view, dorsal longitudinal bands partly removed. (D) Lateral view, buccal tentacles (bt) and pharynx (ph) everted. (E) Lateral view, buccal tentacles (bt) inverted, left body wall removed to show tentacle attachment. (F) Dorsal view, buccal tentacles inverted, dorsal body wall removed to show tentacle attachment. (G) Dorso-lateral view with buccal tentacles (bt) everted. *Abbreviations*: bf/l, longitudinal muscle fibres of branchial filament; bt/r, buccal tentacles retractors; bw/dl, dorsal longitudinal bands; bw/vl, ventral longitudinal bands; cbm, circumbuccal muscles; ll, lower lip; p, prostomium; p/dv, prostomial dorso-ventral bundles; pp, parapodial complex; p/dv, prostomial dorso-ventral bundles; up, upper lip; up/r, upper lip retractor.

dorso-ventral bundles on the distal end of the prostomium have been described here for the first time. Their function should be the flattening of the prostomium dorso-ventrally. This motion is most likely used for creating a wedge shape in the prostomium that is used for propagating a crack during burrowing (Dorgan et al., 2006).

Rostral muscle fibres have been described in the prostomium of maldanids, dorvilleids and cirratulids (Filippova et al., 2005, 2006, 2010); in these families, they emerge from the dorsal longitudinal bands. We found similar muscle fibres in *C. pygodactylata* that also start from longitudinal bands, and therefore we chose the same name for them. The difference in cossurid rostral muscle fibres is that they emerge from both dorsal and ventral bands, and ventral rostral muscle fibres are much thicker than dorsal rostral muscle fibres. Additionally, rostral muscle fibres are rather short and do not extend to the distal part of the prostomium where the dorso-ventral bundles are located. Transverse bundles coming from rostral muscle fibres in some projections can give the appearance of crossing elements, but their homology with the crossing muscle fibres of other polychaetes is doubtful. Both crossing muscles and rostral muscles are present in the prostomium of *Dorvillea kastjani* Tzetlin, 1980 (Filippova et al., 2006).

The circumbuccal complex is a rather conservative structure organised similarly in different polychaete families (Filippova et al., 2006, 2010). It consists of an external layer of transverse fibres and an inner layer of longitudinal or oblique muscle fibres. In *C. pygodactylata*, it generally follows the same pattern. Therefore, only part of the circumbuccal complex, including the lower lip, was described. In the present study, we also described the thinner upper lip and its retractor muscle fibres.

4.4. The buccal tentacles

The buccal tentacles of Cossuridae are considered a unique structure for polychaetes (Rouse and Pleijel, 2001). The present study has shown that they represent a transformed dorsal part of the axial non-muscular pharynx, as described for many sedentary families. Similar structures occur independently in some opheliids (Tzetlin and Zhadan, 2009). On the other hand by their position the buccal tentacles resemble the dorsal pharynx that

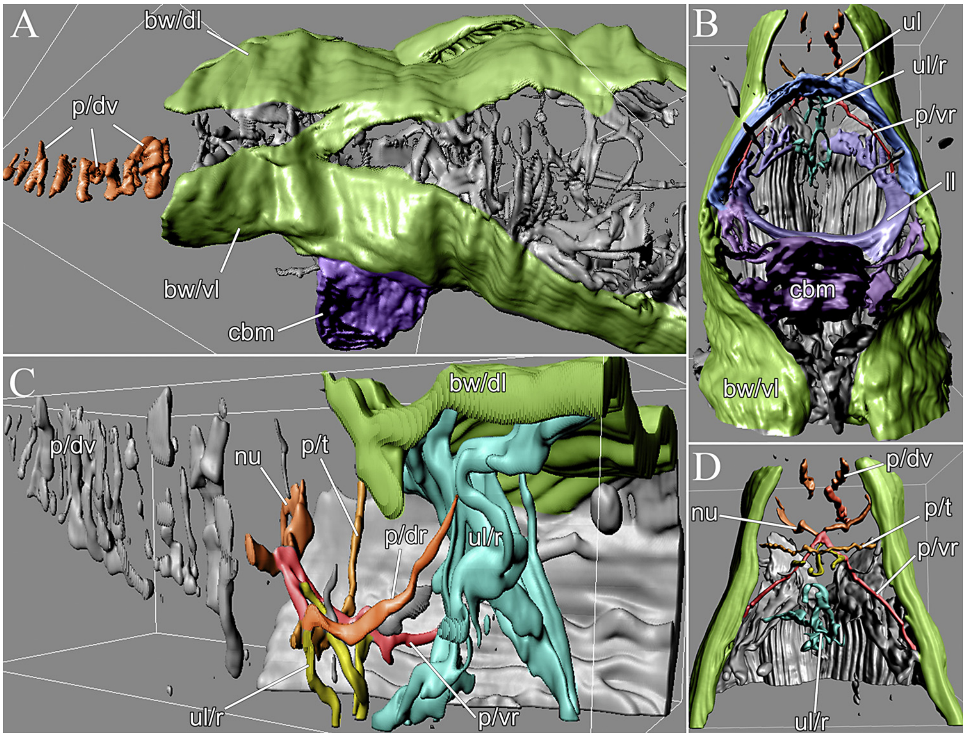

Fig. 6. Musculature of anterior end, 3D reconstructions. (A) Lateral view, showing (coloured) dorsal (bw/dl) and ventral longitudinal bands (bw/vl), prostomial dorso-ventral bundles (p/dv), and circumbuccal muscle fibres (cbm). (B) Ventral view, showing (coloured) ventral longitudinal bands (bw/dl), circumbuccal muscle fibres (cbm), and prostomium musculature. (C) Lateral view, ventral and left lateral body walls removed to show prostomium musculature and upper lip retractors (up/r); only the basal part of the left ventral rostral muscle (p/vr) is shown. (D) Dorsal view, dorsal body wall removed to show prostomium musculature and upper lip retractors (up/r). *Abbreviations*: bt/r, tentacles retractors; bw/dl, dorsal longitudinal bands; bw/vl, ventral longitudinal bands; ll, lower lip; nu, retractors of nuchal organs; p/dv, prostomial dorso-ventral bundles; p/dr, dorsal rostral muscle fibres of prostomium; p/t, transverse muscle fibres of prostomium; p/vr, ventral rostral muscle fibres of prostomium; up, upper lip. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

evolved independently in Clitellata, *Hrabeiella periglandulata* Pižl and Chalupský, 1984 and *Capitella teleta* Blake, Grassle, Eckelbarger, 2009, (Purschke, 2003; Boyle and Seaver, 2009). In the present paper, we show for the first time that the tentacles of cossurids are paired structures, their number is always even, and their musculature is rooted symmetrically to the right and left dorsal longitudinal bands by thick retractor muscles. The muscles of the tentacles include only longitudinal fibres, which means that their variety of movements will be limited. It can be supposed that food gathering occurs mostly with the help of the long cilia covering the tentacles. Dorgan et al. (2006) supposed that cossurids feed below the sediment surface, or very near to or even at the sediment-water interface, in cracks they have created. According to our observations in the White Sea, cossurids inhabit the upper 5–10 cm of soft mud. They most likely do not need to reach the sediment surface for feeding, as was suggested by Tzetlin (1994), but rather expand their tentacles into cracks in the sediment. It is still unclear how protraction of buccal tentacles occurs. There is no coelomic cavity either in the tentacles or in the thoracal region of the body (Tzetlin, 1994), so creating extra hydrostatic pressure by contracting the anterior muscles is unlikely to be the underlying mechanism.

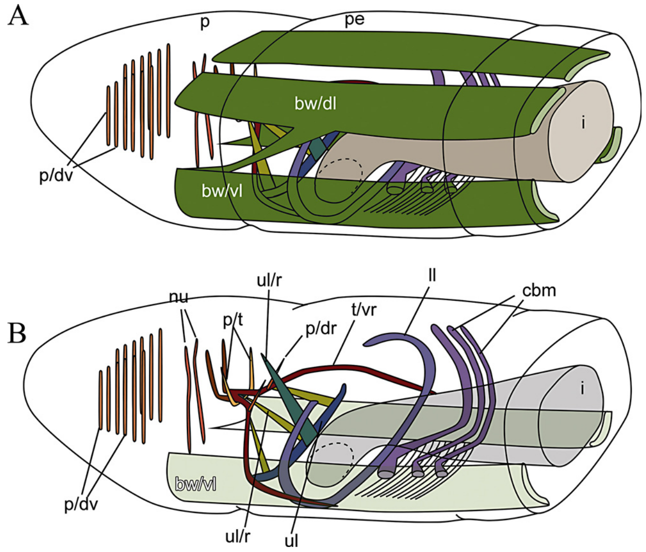

Fig. 7. Anterior end, lateral view, schematic reconstructions. (A) Dorsal and ventral longitudinal bands, prostomial dorso-ventral bundles (p/dv) and digestive tract (i) are observed. (B) Dorsal longitudinal bands are removed, ventral longitudinal bands and digestive tract are transparent to show prostomium musculature and circumbuccal complex (cbm). *Abbreviations*: bw/dl, dorsal longitudinal bands; bw/vl, ventral longitudinal bands; ll, lower lip; nu, retractors of nuchal organs; p, prostomium; pe, peristomium; p/dr, dorsal rostral muscles of prostomium; p/vr, ventral rostral muscles of prostomium; ul, upper lip; ul/r, upper lip retractors.

4.5. The branchial filament

The branchial filament contains only longitudinal fibres, which contradicts the suggestion of Fournier and Petersen (1991) that it also possess circular muscle fibres. Muscle fibres of the branchial filament do not join to the body musculature. This suggests that the branchial filament is capable of only very restricted movements, which is confirmed by our observations of live worms. Usually, branchial filaments are directed along the body of the worm close to its dorsum and dragged after it. It is surprising that branchial filaments with such weak musculature never completely autotomise. We suggest that the mechanism of autotomy requires special circular musculature in the basal part of the structure. Our unpublished data show that the branchial filament is innervated from segmental

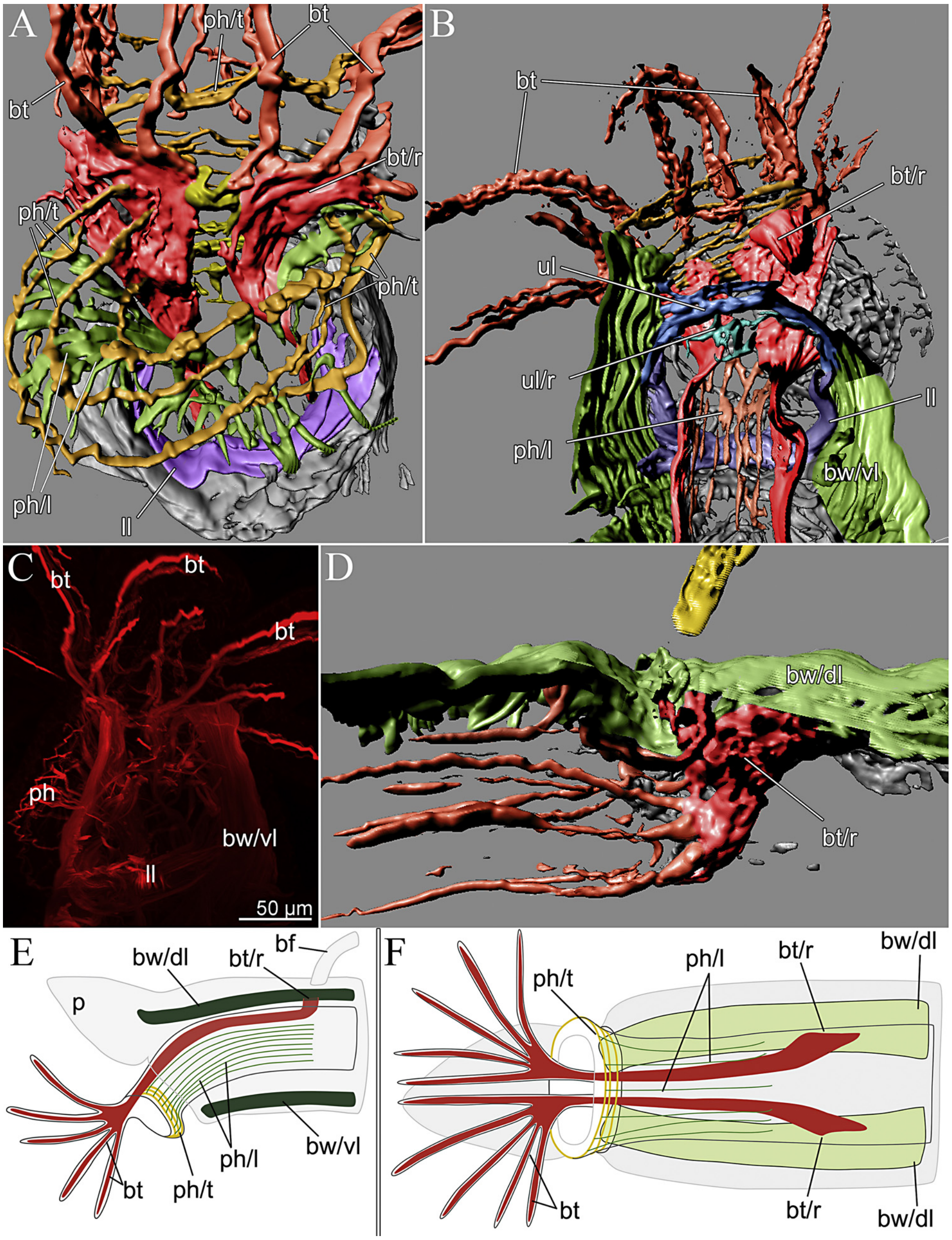

Fig. 8. Pharynx and buccal tentacles. A, B, D – 3D reconstructions, C – CLSM micrographs, phallacidin staining, 2D projection. (A) Anterior-ventral view, pharynx (ph) and tentacles (bt) everted. (B) The same specimen, dorsal view, dorsal body wall removed to show upper and lower lips, pharynx and tentacles musculature. (C) Same specimen, ventral view. (D) Lateral view, tentacles inverted, left body wall removed to show attachment of tentacles to dorsal longitudinal band. (E), (F) Schemes showing muscle fibres of pharynx, buccal tentacles (bt), retractors of buccal tentacles (bt/r) and their attachment to dorsal longitudinal bands (bw/dl); circumbuccal complex is not shown. (E) Lateral view. (F) ventral view, ventral body wall is omitted. *Abbreviations*: bf, branchial filament; bt, buccal tentacles; bt/r, buccal tentacles retractors; bw/dl, dorsal longitudinal bands; bw/vl, ventral longitudinal bands; ll, lower lip; nu, muscles of nuchal organs; p, prostomium; ph/l, pharyngeal longitudinal muscle fibres; ph/t, pharyngeal transverse muscle fibres; ul, upper lip; ul/r, upper lip retractors.

nerves of the second chaetiger, so it cannot be homologous to the palps of other polychaetes that innervated through nerve roots emanating from dorsal and ventral roots of circumesophageal connectives and their comissures (Orrhage and Muller, 2005). This finding likely indicates the autapomorphy of cossurids. It is interesting to note that some Paraonidae that form a sister group with Cossuridae according to molecular data also have unpaired median antenna on the posterior border of the prostomium, but its homology with cossurid branchial filaments cannot be suggested due to different location and innervating (Orrhage and Muller, 2005).

4.6. *Musculature of internal organs*

The intestine of cossurids is not differentiated along its length and contains only thin circular fibres. Nothing similar to the muscular stomach of other polychaetes was found.

Cossurids have, despite their small size, developed a blood circulatory system. The dorsal blood vessel has a strong muscular wall in the anterior part of the body, which we called a heart. Its wall is composed of longitudinal muscle fibres with regular striations, in addition to transverse muscular septations observed inside the

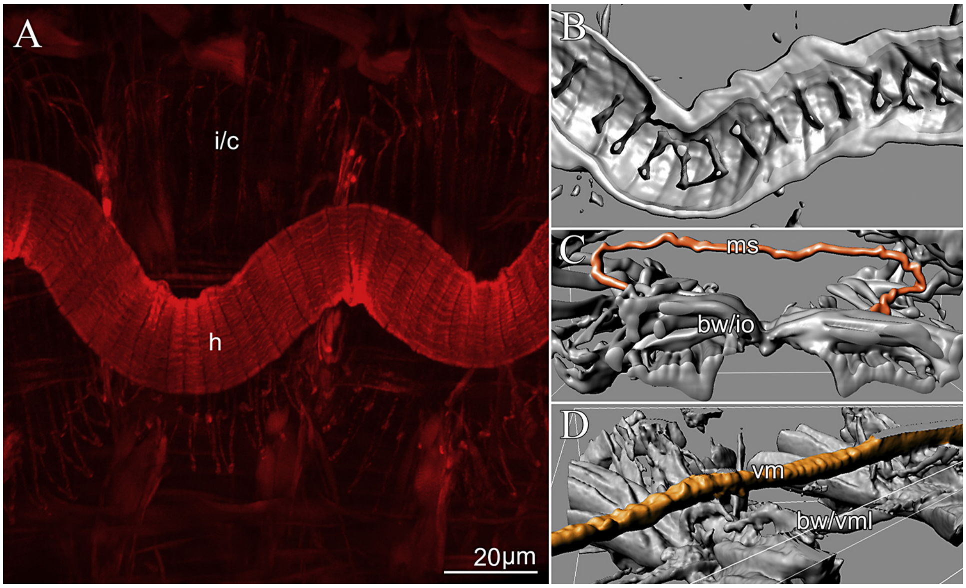

Fig. 9. Internal organs. A – CLSM micrographs, phallacidin staining, 2D projection, B–D – 3D reconstructions. (A) Body cavity, dorsal view, showing heart (h) with outer longitudinal muscles forming short rings, and intestinal circular fibres (ic). (B) Heart with one wall removed to show internal muscular septae. (C) Posterior view of thoracal segment, showing the semi-circular muscle of dissepiment (ms). (D) Body cavity, anterior-lateral view, most part of body wall and intestine removed to show ventral muscle of mesenterium (vm). *Abbreviations*: bw/io, intersegmental oblique bundles; bw/vml, medial longitudinal ventral bundle.

structure. Such a structure has not been described in other polychaete groups and needs further investigation.

5. Conclusion

The musculature of *C. pygodactylata* has some elements that are homologous with other polychaetes (e.g., body wall, most parapodial muscles) and several unique features, mostly at the anterior end. The existence of these unique muscular features is partially due to the very peculiar morphology of cossurids. Another reason is that the muscular system has been studied with CLSM for only a few families of polychaetes. In the last few years, there has been significant progress in confocal microscopy and 3D computer reconstruction, which has allowed researchers to describe polychaete musculature in greater detail and with improved accuracy. It will be very interesting to use CLSM based investigations to study the musculature of groups that, according to molecular analysis, are closely related to cossurids – namely the Paraonidae and Fauveliopsidae.

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