Parasitol Res (2014) 113:941–952
DOI 10.1007/s00436-013-3726-3

ORIGINAL PAPER

Muscle system of *Diplodiscus subclavatus* (Trematoda: Paramphistomida) cercariae, pre-ovigerous, and ovigerous adults

Darya Y. Krupenko

Received: 24 September 2013 / Accepted: 27 November 2013 / Published online: 12 December 2013

Abstract The musculature of cercariae, pre-ovigerous, and ovigerous adults of *Diplodiscus subclavatus* was studied by means of TRITC-conjugated phalloidin staining of filamentous actin and confocal scanning laser microscopy. The body wall appears to include four muscle layers as follows: circular, outer longitudinal, diagonal, and inner longitudinal. Two layers of longitudinal muscle fibers are arranged in different modes due to the secondary transformed paramphistomid body construction. The organization of the acetabulum turned out to be more complex than ever described, with a radial layer, two layers of circular, two layers of meridional, an additional starry layer of muscle fibers, as well as a few separate muscle layers of the accessory sucker. Within the pharynx, I found a group of alar muscle fibers, never described before for any paramphistomids, and some morphological features which were not considered to be characteristic for *D. subclavatus* (namely—the middle semicircular layer and the transverse muscle fibers in the pre-sphincteric space). No significant reorganizations of the somatic musculature occur throughout the development from the cercaria to the ovigerous adult worm, so the metamorphosis goes in the manner of completion. The cercarial tail includes a layer of circular muscle fibers and a longitudinal muscle layer beneath. The latter consists of two medial longitudinal bundles of smooth muscle fibers and two lateral longitudinal bands of obliquely striated muscle fibers which are partially divided in halves.

D. Y. Krupenko (✉)
Department of Invertebrate Zoology, Faculty of Biology and Soil Science, Saint Petersburg State University, Universitetskaya emb. 7/9, St. Petersburg 199034, Russia
e-mail: midnightcrabb@gmail.com

Introduction

Diplodiscus subclavatus (Pallas, 1760) is an amphibian paramphistomid widespread in Europe. The morphological features of paramphistomid hermaphrodite generation show deep specialization, namely (1) the acetabulum (ventral sucker) is located on the posterior end of the body, in subterminal (and sometimes terminal) position, so that the body lacks distinct post-acetabular region; (2) the secondary excretory pore of adult worms (sensu Kuntz 1950) is located on the dorsal side of the body, whereas in the majority of trematodes, it is situated terminally on the posterior end, being its marker; (3) the oral sucker is absent and the extreme anterior part of the digestive system is the pharynx, which may have two posterior diverticula. These morphological peculiarities are obviously modifying the general trematode body construction and should hence affect the construction of muscle system. Furthermore, the muscular organs of paramphistomids have been of great value for systematics since the paper of K. E. Näsmark was published in 1937. However, Näsmark (1937) and his followers (Eduardo 1982; Sey 1983) described only the most significant muscle layers within the muscular organs, basing on the middle sagittal histological sections. The latest attempt to make full description of *D. subclavatus* musculature with no omissions of minor details dates even earlier (Fukui 1929). The modern methods of fluorescent staining and confocal microscopy have never been applied to the paramphistomid adult worms hitherto.

Materials and methods

Pre-ovigerous and ovigerous adult worms were recovered from the gut of *Rana temporaria* Linnaeus, 1758. Cercariae were collected upon their emission from *Planorbis planorbis* (Linnaeus, 1758). Both hosts originate from Leningrad

Oblast. All the material was fixed in 4 % solution of paraformaldehyde in phosphate-buffered saline (PBS). Ovigerous adults were flattened before fixing between microscope slides and cover slips. Specimens were washed in PBS with Triton-X100 (0.1 %) during 24 h before staining. Incubation in TRITC-labeled phalloidin solution (200 ng/ml) took another 24 h, followed by 2 h wash in PBS. Finally, the specimens were mounted in glycerol/PBS (9/1) and examined under the confocal scanning laser microscopes (CSLM) Leica TCS-SP5 and Leica TCS-SPE. All in all five cercariae, ten pre-ovigerous adult worms and five ovigerous adult worms were studied. ImageJ v. 1.46r software was used to process data from CSLM, to make snapshots, Z-stacks, and measurements. The reconstructions of optionally directed optical slices were made by using plugin “Volume Viewer” v. 1.31. The detailed description of muscle system is given for the pre-ovigerous adult worms, as it is supposed to be fully formed while bearing no signs of secondary modifications which may occur in some trematodes due to uterus overgrowth. This description is supplied with specifications on the cercarial and ovigerous adults' muscle system.

Results

Pre-ovigerous adults

Figure 1 shows the gross anatomy of the pre-ovigerous adults (Fig. 1a, b) and their acetabulum (Fig. 1c, d). Figure 1d additionally demonstrates the peculiarities of the paramphistomid body construction explained in the discussion.

Body wall

The body wall of pre-ovigerous adults includes four muscular layers as follows: circular, outer longitudinal, diagonal, and inner longitudinal (Figs. 1a and 2a, b). The circular muscle fibers form a dense layer with 0.5–1.5 μm spaces between adjacent fibers. The width of a single fiber can vary from 0.5 to 1.0 μm. The outer longitudinal fibers form a less regular layer; distances between the adjacent fibers vary from 2 to 5 μm; fibers are 1.5–2.0-μm thick. This layer is absent in the fore region of the body approximately to the posterior border of the pharynx (Fig. 2c, e, g, h). In the posterior half of the body, the outer longitudinal muscle fibers from the ventral surface partially turn to the side surfaces and then dorsally, so that they lie there obliquely (Fig. 2e, g, h). In the region posterior to the excretory pore, they partially substitute the diagonal muscle layer (Fig. 2h). The diagonal muscle fibers are more or less similar in thickness and density to the outer longitudinal ones. They are absent on the dorsal side of the body, in the region posterior to the excretory pore. Within the inner longitudinal muscle layer, fibers are rather thick (2–3 μm), forming sparsely arranged bundles (distance between adjacent bundles is commonly 4–7 μm, sometimes reaching 10 μm). The posterior ends of inner longitudinal muscle fibers turn ventrally in contrast to the outer longitudinal muscle fibers (Fig. 2a, f, g, h).

Musculature in the parenchyma

Numerous dorsoventral muscle fibers cross the parenchyma. On the ventral side of the body, they attach more medially than on the dorsal one. Two groups of thick longitudinal muscle fibers go through the parenchyma from the lateral surfaces of the pharynx, along the main collecting tubes, to the ventrolateral regions of the body wall, and to the fore border of the acetabulum (Fig. 2d).

Acetabulum

The acetabulum has a spacious cavity which opens ventrally (Fig. 1a, c). A small accessory sucker is located in the center of the cavity surface (Figs. 1a, c, d and 3a, g). There is a depression in the center of the outer surface opposite to the accessory sucker (Figs. 1c, d and 3d, g). The outer surface of the acetabulum is not entirely submerged into the parenchyma, but has a broad loose rim (Figs. 1a, c, d and 3g).

A layer of tangly arranged muscle fibers lies beneath the tegument lining the cavity (Fig. 3a, b, f, g). On the periphery of the cavity, these fibers form a grid with diamond-shaped meshes. In the central part of the cavity, most of these fibers form six wide bands which follow the chords of the acetabulum, so this layer was named “starry.” The bands give branches directed to the geometrical center of the acetabulum, i.e., to the accessory sucker. The group of these branches is labeled as the meridional muscle fibers of the accessory sucker, as they proceed to the center of its cavity (Fig. 3b). The border of the accessory sucker is lined by the semicircular muscle fibers, which are probably derived from the starry fibers as well (Fig. 3b, g).

The circular muscle fibers are found beneath the starry layer. They occupy the periphery of the cavity and proceed to the loose rim of the outer surface (Fig. 3a, g). The outer meridional muscle layer lies between the circular muscle fibers and the tegument (Fig. 3c). This layer is not connected with the starry muscle layer of the cavity, and is found just on the loose rim of the acetabulum (Fig. 3g). More circular muscle fibers are found on the submerged part of the outer surface (Fig. 3d, g). As those are grouped into bands (unlike the circular muscle fibers of the cavity and the loose rim), they are considered to form a separate muscle layer—circular layer of the submerged outer surface. The inner meridional layer of muscle fibers lies beneath it (Fig. 3c, d, g). These fibers also form bands; they reach the loose rim of the acetabulum and finish on the border between the outer surface and the cavity. In the center of the outer surface, these meridional fibers are

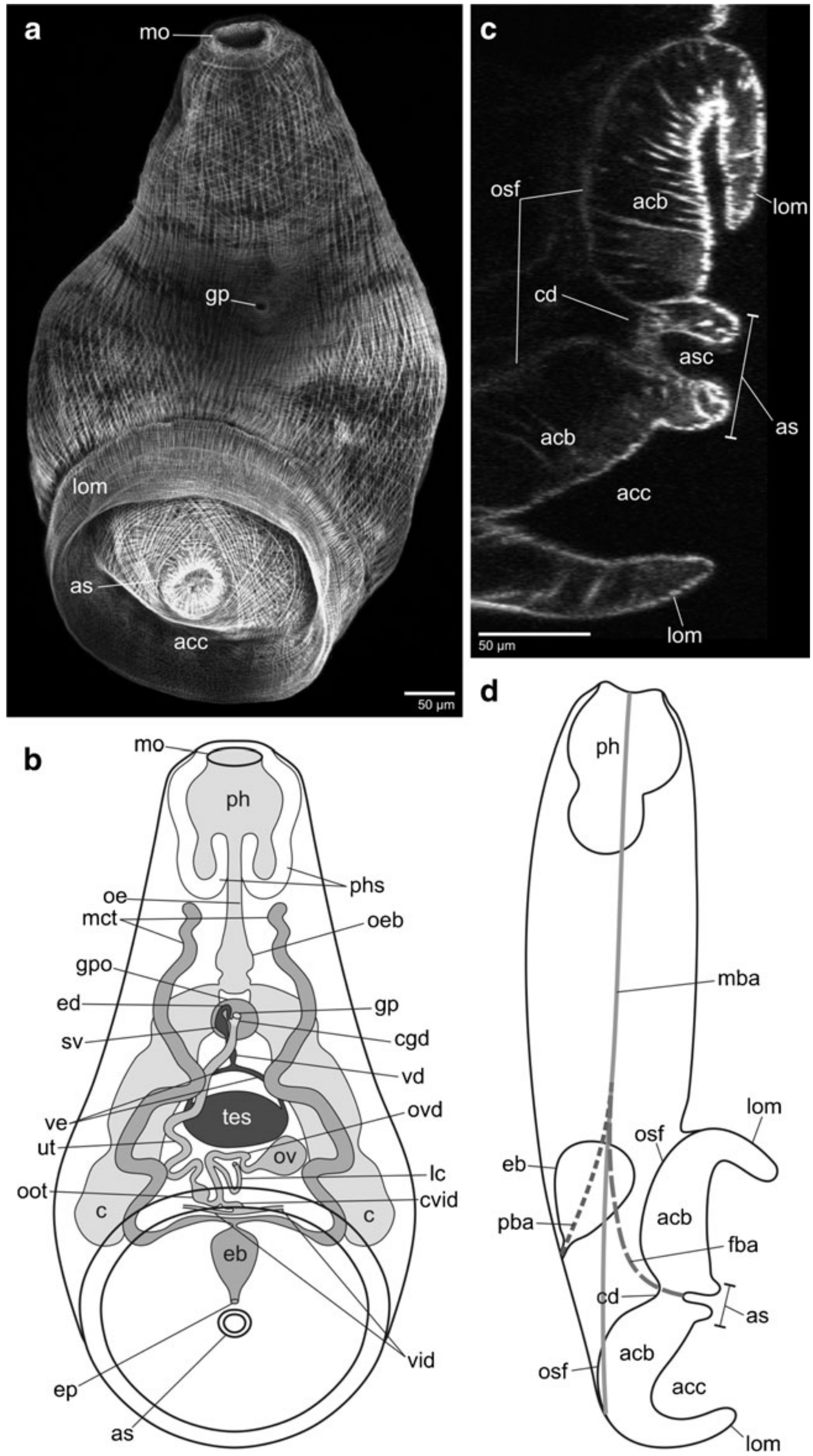


Fig. 1 Gross anatomy of *Diplodiscus subclavatus* pre-ovigerous adult. **a** Ventral view of the whole worm. **b** Scheme illustrating the location of main organs of digestive, excretory, and genital systems (except for the vitellaria and the distal parts of their ducts). **c** Reconstruction of the middle sagittal optical section through the acetabulum. **d** Scheme of the middle sagittal section through the whole worm (*continuous gray line* represents the morphological main body axis; *rarely spaced broken line* represents the deflection of the functional main body axis; *frequently spaced broken line* signifies the deflection of the primary main body axis). *acb* bulk of the acetabulum, *acc* cavity of the acetabulum, *as* accessory sucker, *asc* cavity of the accessory sucker, *c* caecum, *cd* central depression of the acetabulum, *cgd* common genital duct, *cvid* common duct of the vitellaria, *eb* excretory bladder, *ed* ejaculatory duct, *ep* excretory pore, *fba* functional main body axis, *gp* genital pore, *gpo* genital pouch, *lc* Laurer's canal, *lom* loose rim of the acetabulum, *mba* morphological main body axis, *mct* main collecting tubes, *mo* mouth opening, *oe* esophagus, *oeb* esophagus bulbus, *oot* ootype, *osf* outer surface of the acetabulum, *ov* ovary, *ovd* oviduct, *pba* primary main body axis, *ph* pharynx, *phs* primary pharyngeal sacs, *sv* seminal vesicle, *tes* testis, *ut* uterus, *vd* vas deferens, *ve* vasa efferentia, *vid* vitellaria ducts

attached to the bottom of the accessory sucker from the underside (Fig. 3d, g).

The bulk of the acetabulum is occupied by well-developed radial muscle fibers (Fig. 3c, g). The accessory sucker has its own radial fibers (Fig. 3e, g). Also, there is a ring of the oblique muscle fibers, which begin beneath the outer surface in one third of the radius form the center, and finish at the cavity wall close to its edge (Fig. 3g).

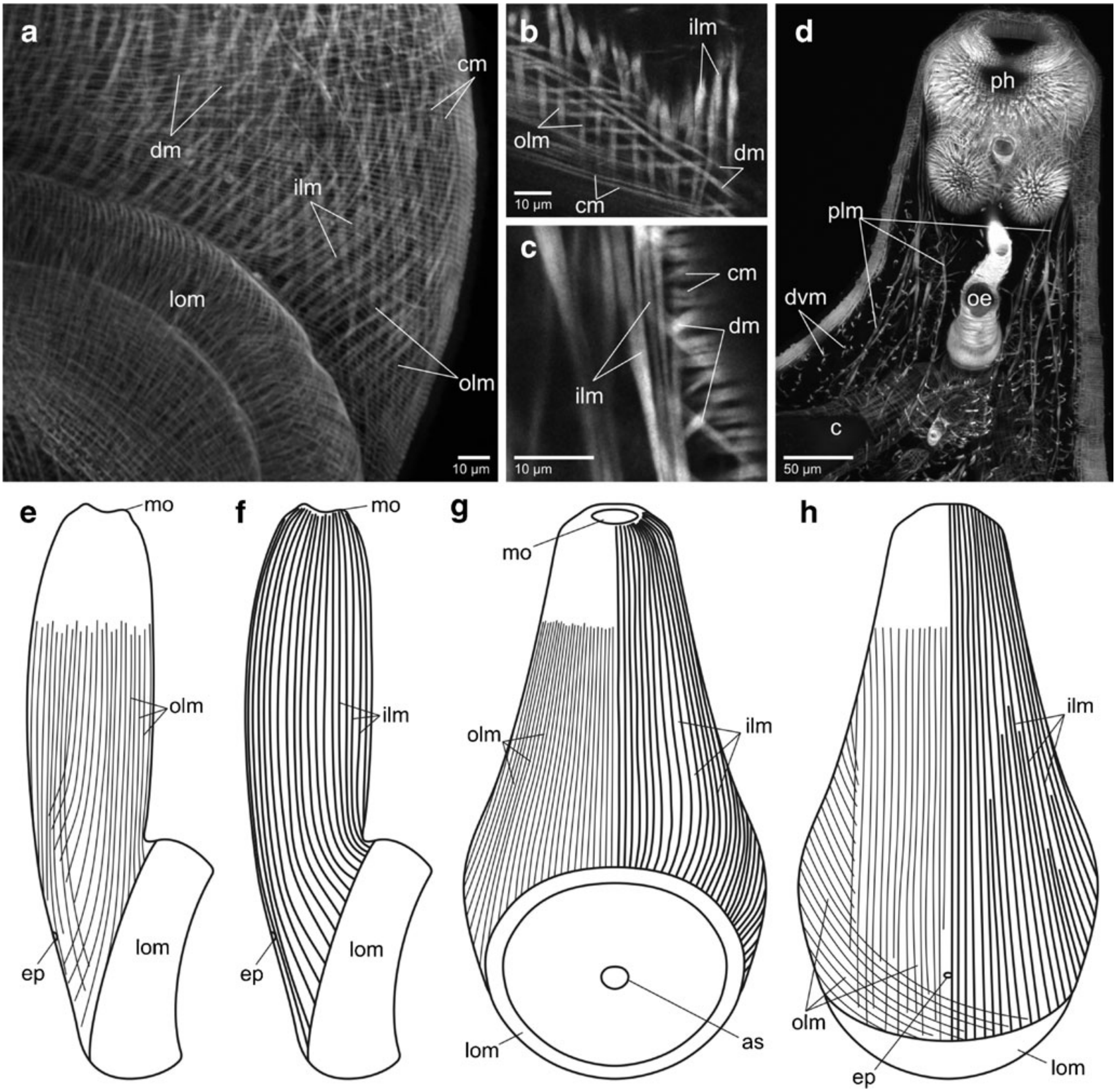


Fig. 2 Body wall musculature and muscle fibers in the parenchyma of *Diplodiscus subclavatus* pre-ovigerous adult. **a** Ventral view of the body wall region close to the acetabulum. **b** Oblique optical section of the body wall in the central part of the ventral surface. **c** Oblique optical section of the body wall close to the pharynx (ventral surface). **d** Musculature in the parenchyma. **e**, **f** Schemes illustrating the arrangement of outer (**e**) and inner (**f**) longitudinal muscle fibers on the side view. **g**–**h** Schemes illustrating the arrangement of the longitudinal muscle fibers on the ventral (**g**) and dorsal (**h**) surfaces of the body; *left half* of each scheme shows outer longitudinal layer, *right half* inner longitudinal layer. *as* accessory sucker, *c* caecum, *cm* circular muscle fibers, *dm* diagonal muscle fibers, *dvm* dorsoventral muscle fibers, *ep* excretory pore, *ilm* inner longitudinal muscle fibers, *lom* loose rim of the acetabulum, *mo* mouth opening, *oe* esophagus, *olm* outer longitudinal muscle fibers, *ph* pharynx, *plm* longitudinal muscle fibers in parenchyma

Digestive system

The mouth opening leading to the pharynx is located almost terminally on the fore end of the body, slightly dislodged to the ventral surface. In case of indrawn pharynx, a fold is formed around the mouth opening (Fig. 4d). Two primary pharyngeal sacs are located posteriorly and dorsally to the pharynx. They join together on the dorsal side; on the ventral side, the esophagus passes between them (Fig. 4e). The opening leading to the esophagus lies medially at the bottom of the pharynx ventral to the openings of the pharyngeal sac channels.

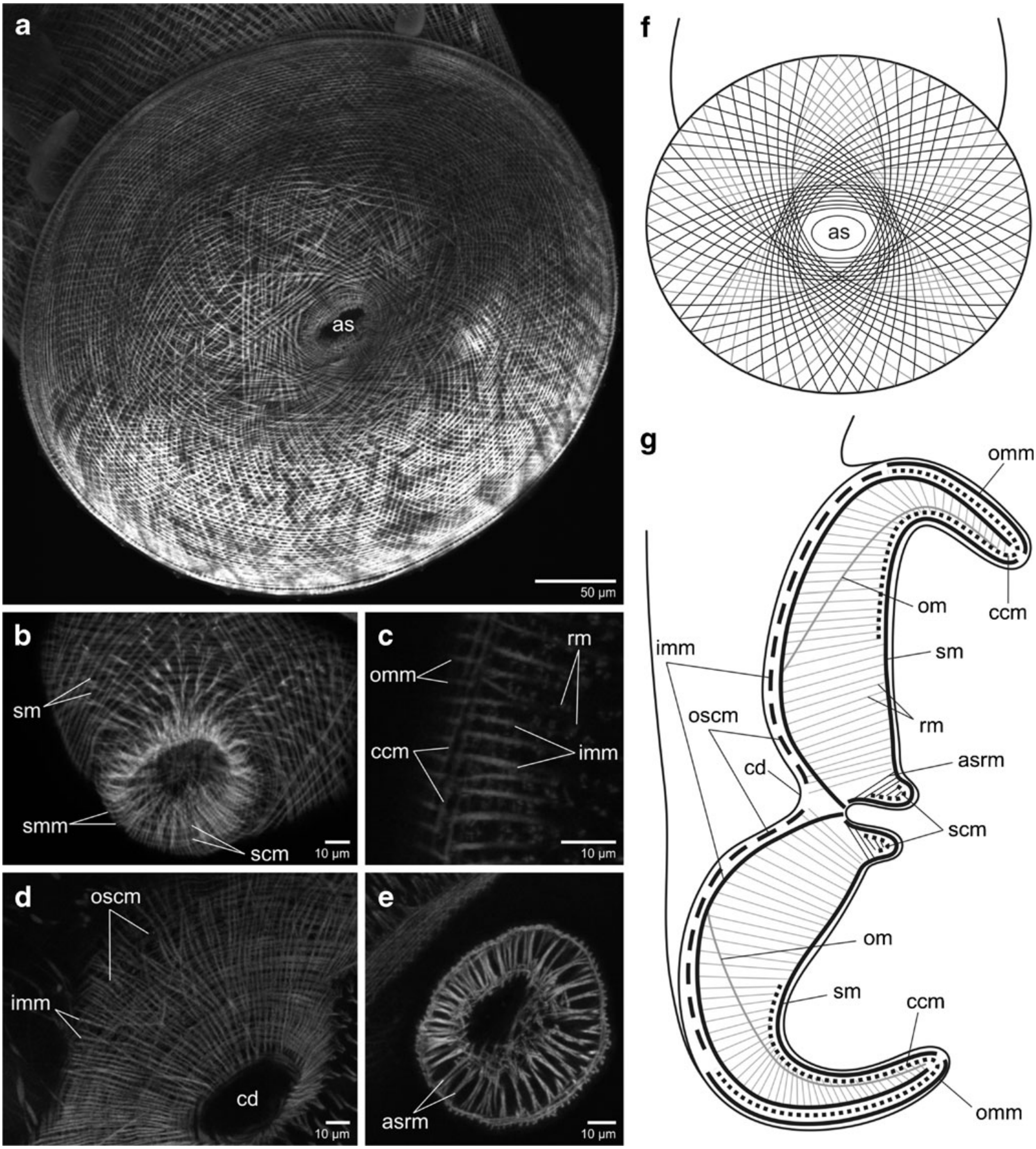


Fig. 3 The acetabulum of *D. subclavatus* pre-ovigerous adult. **a** General view of the spread acetabulum–cavity wall. **b** Surface of the accessory sucker. **c** Oblique optical section of the loose rim of the acetabulum. **d** Muscle fibers on the submerged part of outer surface of the acetabulum. **e** Optical section of the accessory sucker. **f** Scheme illustrating the arrangement of the main groups of the muscle fibers of the starry layer (main six band are shown in *black lines*). **g** Scheme of the sagittal section of the acetabulum showing interrelationship of the muscle layers. *as* accessory sucker, *asrm* radial muscles of the accessory sucker, *ccm* circular muscle fibers of the cavity and loose rim, *cd* central depression on the outer surface, *imm* inner meridional muscle fibers of the outer surface, *om* oblique muscle fibers, *omm* outer meridional muscle fibers of the loose rim, *oscm* circular muscle fibers of the submerged part of the outer surface, *rm* radial muscle fibers of the acetabulum, *scm* semicircular muscle fibers of the accessory sucker, *sm* starry muscle fibers, *smm* meridional muscle fibers of the accessory sucker

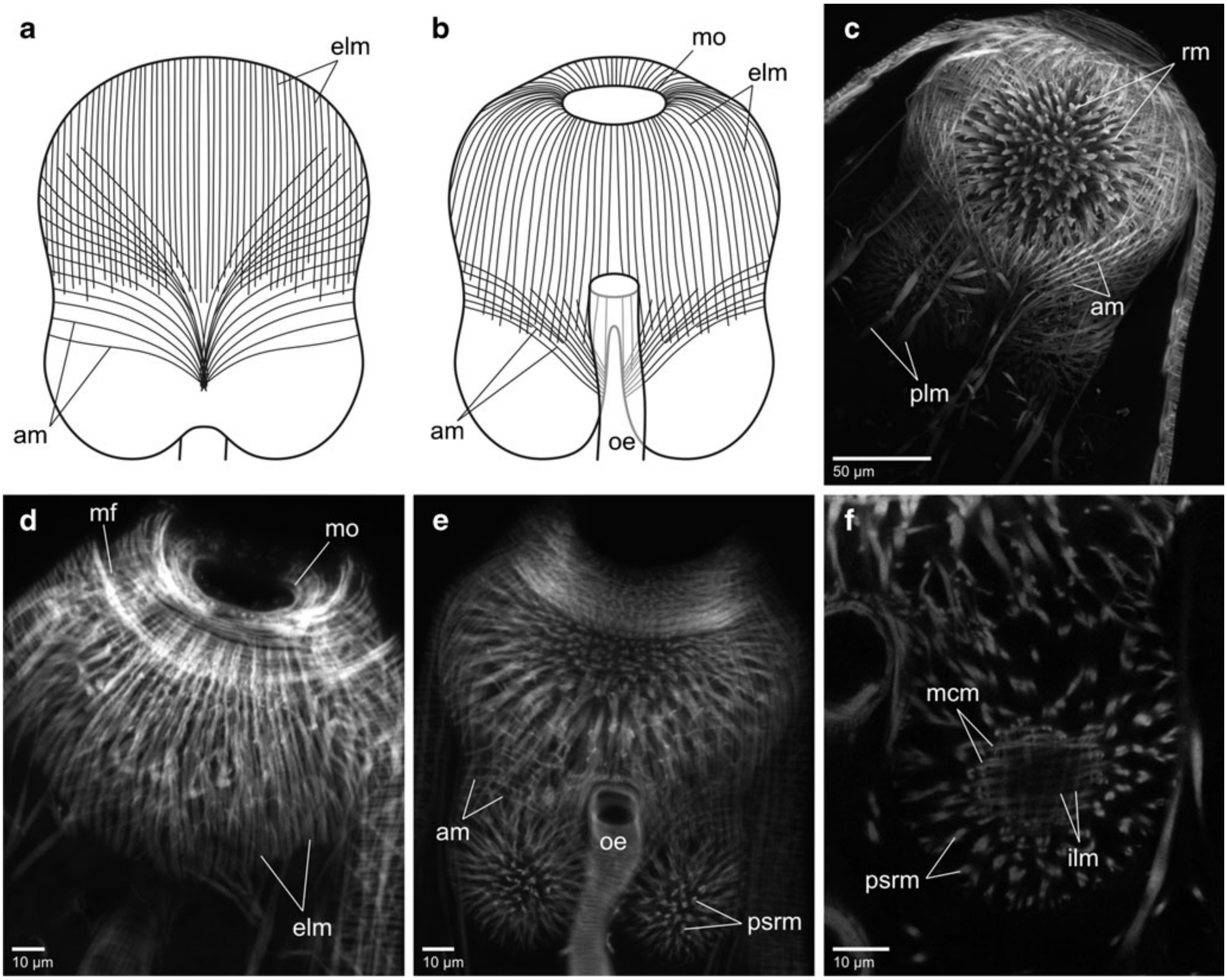


Fig. 4 Digestive system of *D. subclavatus* pre-ovigerous adult. **a**, **b** Schemes illustrating the arrangement of the muscle fibers in pharynx on the outer dorsal (**a**) and ventral (**b**) surfaces. **c** Dorsal view of the pharynx outer surface. **d**, **e** Ventral view of the pharynx outer surface. **f** Dorsal surface of the primary pharyngeal sac cavity. *am* alar muscle fibers, *elm* exterior longitudinal muscle fibers of the pharynx, *ilm* interior longitudinal muscle fibers of the pharynx, *mcm* middle layer of the semicircular muscle fibers, *mf* fold around the mouth opening, *mo* mouth opening, *psrm* radial muscle fibers of the primary pharyngeal sac, *oe* esophagus, *plm* longitudinal muscle fibers in parenchyma, *rm* radial muscle fibers of the pharynx

The outmost muscle layer of the pharynx is the exterior longitudinal layer made of closely arranged well-developed muscle fibers forming small bundles. These exist only in the main part of the pharynx and somewhat get into the bases of the primary pharyngeal sacs (Fig. 4a, b, d). Separate sparsely arranged thin muscle fibers form an alar muscle layer (Fig. 4a, b, c, e). They radiate anteriolaterally from the median region of the pharyngeal sacs' outer surface. The alar muscle layer is better developed on the dorsal side of the pharynx.

The most superficial muscle layer beneath the tegument of the pharyngeal cavity is the interior circular layer (Fig. 5a, d). It is found only in the main cavity of the pharynx and absent in the primary pharyngeal sacs. The next one is the interior longitudinal layer (Fig. 5a, b, d). The layer traditionally called "middle circular" one lies beneath it and appears to consist of dorsal and ventral groups of semicircular muscle fibers (Fig. 5a, d). All these three layers are more or less similar in density and fibers' thickness. The interior longitudinal and the middle semicircular muscle layers proceed to the primary pharyngeal sacs (Fig. 4f). In the anterior part of the pharynx, the muscle fibers of the middle semicircular layer are very thick and closely arranged, forming the anterior sphincter (Fig. 5a, b, d). Some sparsely packed transverse muscle fibers are found in the pre-sphincteric space (Fig. 5b, c, d). The posterior sphincter (around the opening leading to esophagus) is rather weak. The bulk of the pharynx and of the primary pharyngeal sacs is occupied by thick bundles of radial muscle fibers (Figs. 4c, f and 5a, b). The finer oblique muscle fibers also exist in the main part of the pharynx (Fig. 5a, b, d).

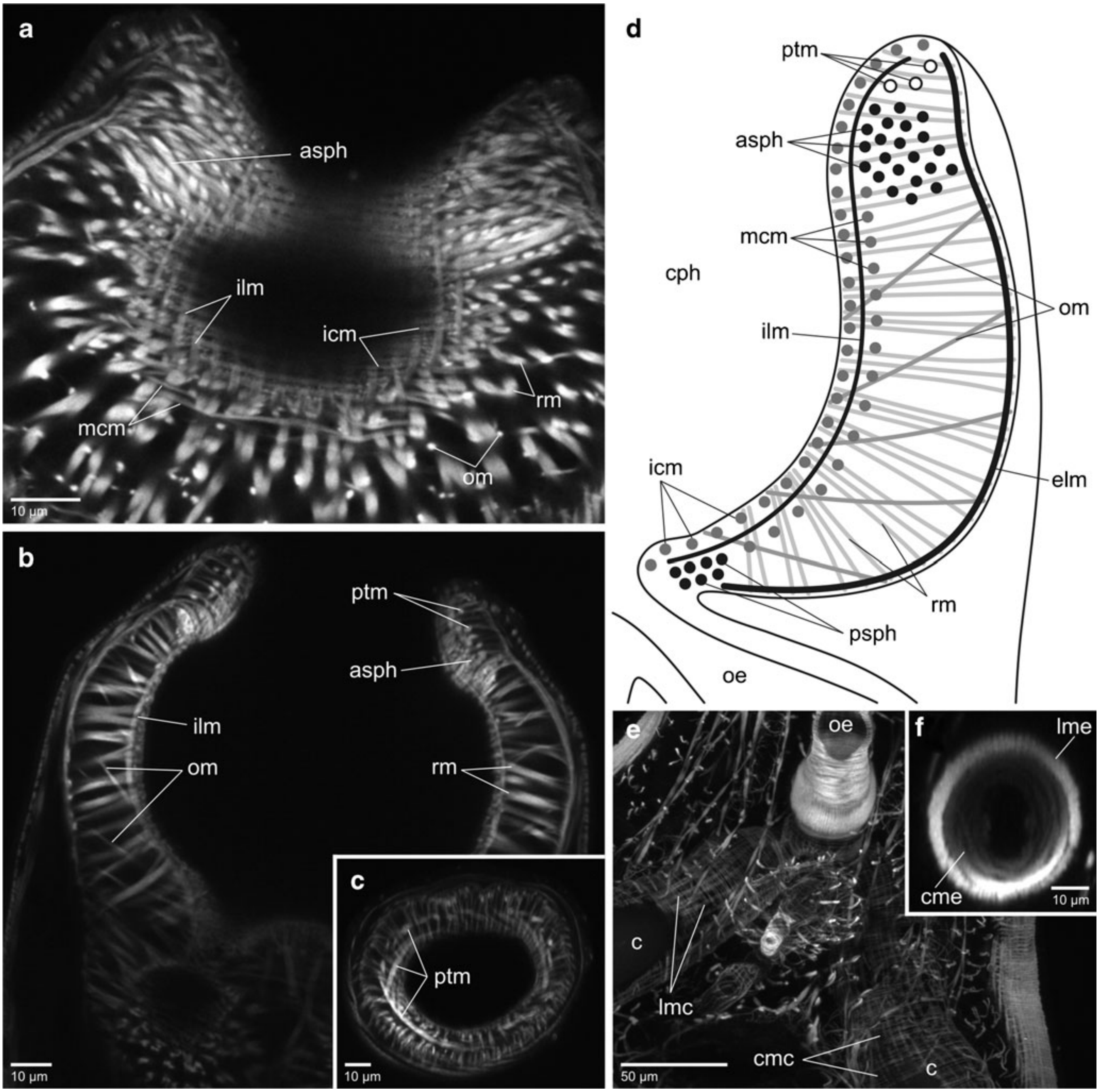
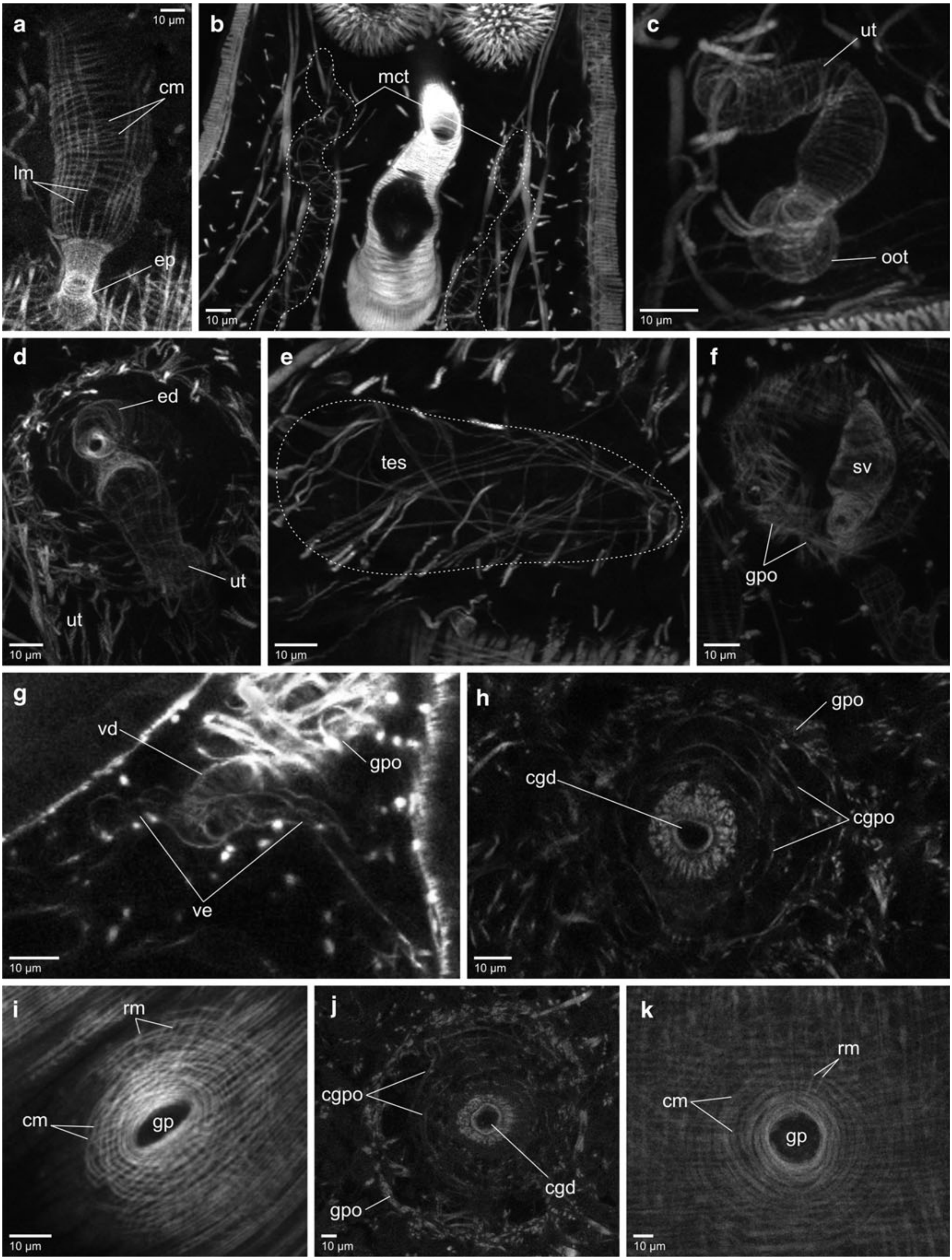


Fig. 5 Digestive system of *D. subclavatus* pre-ovigerous adult (continuation). **a** Frontal optical section of the ventral wall of the pharynx showing the arrangement of muscle fibers close to the cavity surface. **b** Frontal optical section of the pharynx. **c** Reconstructed transverse section of the pharynx. **d** Scheme illustrating the arrangement of the muscle fibers on the middle sagittal section of the ventral wall of the pharynx. **e** Esophagus and caecum. **f** Reconstructed transverse section of the esophagus bulbus. *asph* anterior sphincter, *c* caecum, *cmc* circular muscle fibers of the caecum, *cme* circular muscle fibers of the esophagus, *cph* pharynx cavity, *elm* exterior longitudinal muscle layer of the pharynx, *icm* interior circular muscle layer of the pharynx, *ilm* interior longitudinal muscle layer of the pharynx, *lmc* longitudinal muscle fibers of the caecum, *lme* longitudinal muscle fibers of the esophagus, *mcm* middle semicircular muscle layer of the pharynx, *oe* esophagus, *om* oblique muscle fibers, *psph* posterior sphincter, *ptm* transverse muscle fibers in the pre-sphincteric space, *rm* radial muscle fibers of the pharynx

The wall of esophagus contains well-developed outer longitudinal and inner circular muscle fibers. In the last quarter of esophagus (within the esophageal bulb), these layers become extremely thick: longitudinal up to 4 μm, circular up to 10 μm (Fig. 5e, f). The cecal wall contains outer longitudinal and inner circular muscle layers as well; however, these are much thinner and more sparsely arranged than in esophagus. The longitudinal muscle fibers form rare minor ribbon-like bunches; the circular fibers do not form bands and lie closer to each other (Fig. 5e). Between the esophagus and the cecal branches, the circular muscle fibers form sphincters.


a
10 μm
cm
lm
ep
b
mct
10 μm
c
ut
oot
10 μm
d
ed
ut
ut
10 μm
e
tes
10 μm
f
sv
gpo
10 μm
g
vd
gpo
ve
10 μm
h
gpo
cgd
cgpo
10 μm
i
rm
cm
gp
10 μm
j
cgpo
cgd
gpo
10 μm
k
rm
cm
gp
10 μm

◀ **Fig. 6** Excretory and reproductive system of *D. subclavatus* adults. **a** Excretory bladder. **b** Main collecting tubes (outlined with *broken line*). **c**–**i** Muscle fibers within the reproductive system of pre-ovigerous adults. **c** Uterus and ootype. **d** Frontal optical section through the genital pouch. **e** Testis (outlined with *broken line*). **f** Seminal vesicle lying within the genital pouch. **g** Vasa efferentia and vas deferens. **h** Frontal optical section through the genital pouch. **i** The body wall around the genital pore. **j**–**k** Muscle fibers within the reproductive system of ovigerous adults. **j** Frontal optical section through the genital pouch. **k** The body wall around the genital pore. *cgd* common genital duct, *cgpo* muscular concentric circles within the distal part of the genital pouch, *cm* circular muscle fibers, *ed* ejaculatory duct, *ep* excretory pore, *gp* genital pore, *gpo* outer wall of the genital pouch, *lm* longitudinal muscle fibers, *mct* main collecting tubes, *oot* ootype, *rm* radial muscle fibers, *sv* seminal vesicle, *tes* testis, *vd* vas deferens, *ve* vasa efferentia, *ut* uterus

Excretory bladder

The wall of the excretory bladder is supported by longitudinal and circular muscle fibers. The latter forms a sphincter of the excretory pore (Fig. 6a). The main collecting tubes bear diffuse sparse muscle fibers (Fig. 6b).

Reproductive system

The ovary, as well as the vitellaria, seems to have no developed muscle elements within its wall. However, the walls of the vitelline ducts contain loose diffuse muscle fibers. The walls of oviduct and Laurer's canal contain thin but closely arranged circular muscle fibers. The walls of the ootype and the uterus are more muscular, containing inner circular and outer longitudinal muscle fibers (Fig. 6c, d).

The outer surface of medially located testis is supported by the layer of diffuse muscle fibers (Fig. 6e). The walls of vasa efferentia have loose, diffusely organized longitudinal muscle fibers (Fig. 6g). Their distal common part—vas deferens—also contains circular muscle fibers (Fig. 6g). The seminal vesicle displays a compact but irregular arrangement of dense circular muscle fibers (Fig. 6f); outer longitudinal muscle fibers are few. Within the ejaculatory duct, circular muscle fibers are less dense and arranged in a more regular way (Fig. 6d). The common genital duct is rather short and very muscular with inner circular and outer longitudinal muscle layers (Fig. 6h). The latter is up to 6-μm thick. The circular muscle layer passes on to the body wall as far as 15 μm from the genital pore, lying above all the muscle layers of the body wall (Fig. 6i). The distal ends of longitudinal muscle fibers are partially attached to the body wall around the genital pore, and partially proceed radially in the body wall beneath the circular muscles of the common genital duct (Fig. 6i).

The seminal vesicle, the ejaculatory duct, and the distal part of the uterus lie inside the genital pouch, which has its own muscular wall of numerous and dense irregular muscle fibers (Fig. 6f, g). Moreover, the distal part of the genital pouch bulk (around the common genital duct) is subdivided by the muscular concentric circles (Fig. 6d, h).

Ovigerous adults

No structural differences between the muscle system of pre-ovigerous and ovigerous adults of *D. subclavatus* were observed. However, it should be noted that the proportions of different organs change in the course of growth. Thus, the diameter of esophagus, the width of pharynx, acetabulum, and the accessory sucker increase slower than the diameter of genital pouch and the size of the whole worm.

Cercariae

The trunk

The main characteristics of the cercarial somatic musculature do not differ from those of the pre-ovigerous adults. Their body wall contains the same four muscle layers (circular, outer longitudinal, diagonal, and inner longitudinal) (Fig. 7a, b). The dorsoventral muscles and bunches of longitudinal muscle fibers in the parenchyma are present, too. The pharynx of cercaria resembles the one of the pre-ovigerous adult, but I could not discern some of its muscle layers (the alar and the inner longitudinal ones). As the other parts of the digestive system are underdeveloped, the muscle layers of the esophagus and gut are hardly seen. The reproductive system of cercariae, being even further from completely developed state, is barely muscular as well.

The acetabulum functions already at the stage of cercariae, so the radial muscle layer, the starry muscle layer, and the circular muscle layer of the cavity and the loose rim can be found within it. Yet I could not recognize the inner meridional and circular muscle layers of the outer surface.

The tail

The tail of the cercariae is attached dorsally to the excretory pore region. Within it, two muscle layers can be recognized. Transverse muscle fibers lie beneath the tegument (Fig. 7d, e).

The longitudinal muscle fibers are differentiated into few groups, though the layer is nearly continuous. Two minor bands of common smooth muscle fibers go along the dorsal and ventral surfaces (Fig. 7c, d). The lateral surfaces are occupied by thick bands of the obliquely striated muscle fibers (Fig. 7c, e). Here, the muscle processes appear U-shaped in transverse section (Fig. 7h). Near the tail basis, two lateral striated bands join together (Fig. 7g, j). Yet for the most of the tail length, a longitudinal furrow lies in the middle of each band (Fig. 7h, k). Thus, the four functional striated muscle bands are actually formed. Closer to the tail's tip the furrow disappears (Fig. 7i, l). Then wide spaces appear between the

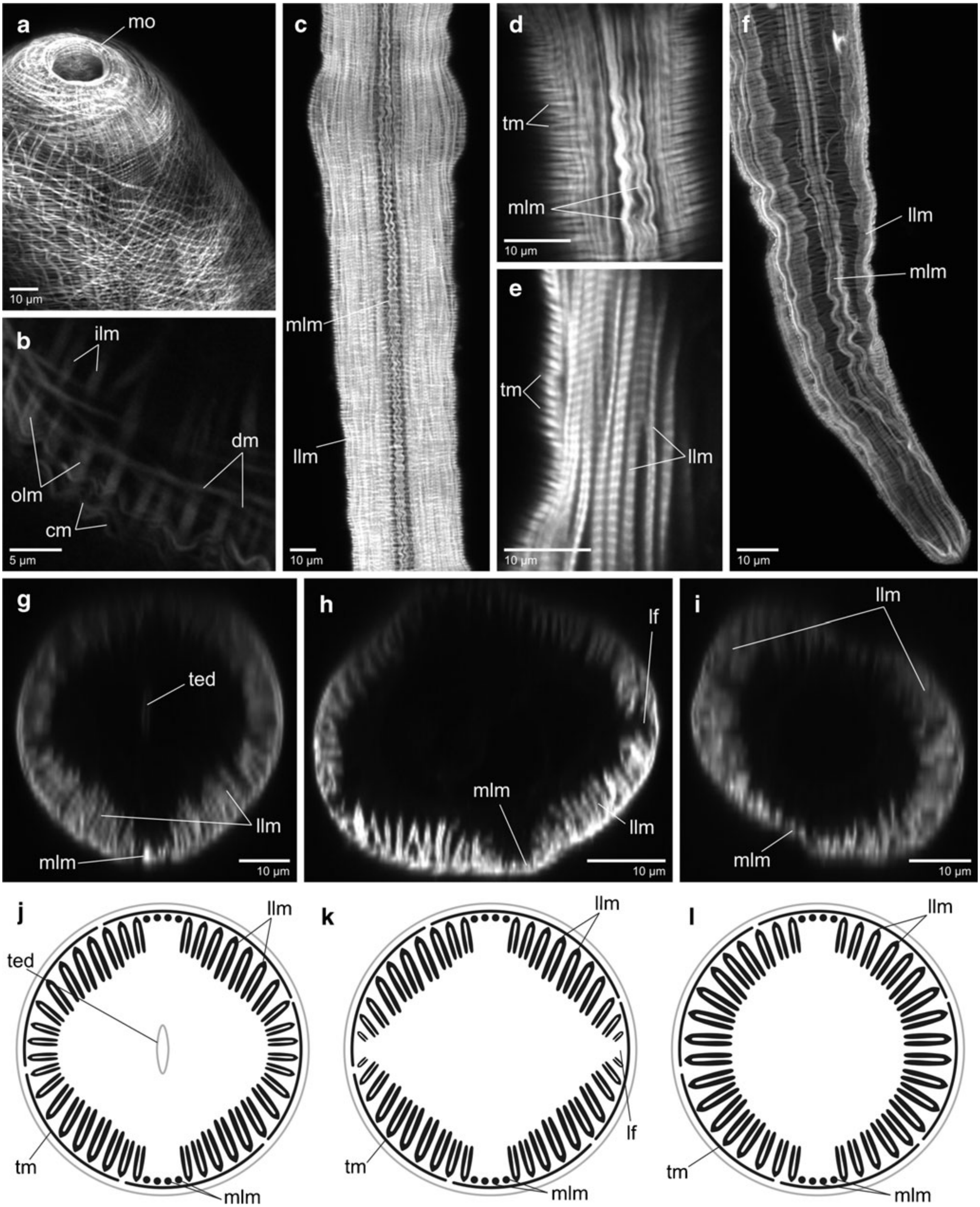


Fig. 7 Cercariae of *D. subclavatus*. **a** Ventrolateral view of the anterior region of cercaria. **b** Oblique optical section of the cercaria body wall. **c**–**l** Tail of the cercaria. **c** Dorsal view. **d**–**e** Frontal optical section of the tail wall. **f** Tail's tip. **g**–**i** Reconstructed transverse sections of the tail: basis (**g**), middle (**h**), and the region close to the tip (**i**). **j**–**l** Schemes of the transverse sections of the tail: basis (**j**), middle (**k**), and the region close to the tip (**l**). *cm* circular muscle fibers, *dm* diagonal muscle fibers, *ilm* inner longitudinal muscle fibers, *lf* lateral furrow, *llm* lateral longitudinal muscle bands, *mlm* medial longitudinal muscle bands, *mo* mouth opening, *olm* outer longitudinal muscle fibers, *ted* tail excretory duct, *tm* transverse muscle fibers

lateral, dorsal, and ventral bands, and the fibers of the lateral bands become smooth (Fig. 7f). On the very tip of the tail, all the bands are drawn together again, forming the continuous layer (Fig. 7f).

Discussion

The general pattern of the body wall musculature organization supposed to be typical for the hermaphrodite generation of the trematodes includes three muscle layers as follows: circular, longitudinal, and diagonal (Ginetsinskaya 1968; Stewart et al. 2003; Halton and Maule 2004; Šebelová et al. 2004). Within the Paramphistomida, it is usually supplemented by the extra layer of inner longitudinal muscle fibers, forming larger bundles (Fukui 1929; Yastrebova et al. 2000). However, no attempts to compare the layout of inner and outer longitudinal muscle fibers were made. As it was shown for *D. subclavatus*, those are arranged in different ways. This may be a consequence of the body plan modification and an alteration of the main body axis. The secondary excretory pore (sensu Kuntz 1950) appearing at the place of the cercarial tail detachment marks the most posterior region of the adult and metacercarial body of trematodes. In paramphistomids, the secondary excretory pore is placed dorsally, far from the morphological posterior end of the body (the standpoint of Pearson 1992, who considered paramphistomids to have tertiary excretory pore seems to be fallacious; however, it is the subject for a separate discussion). So the posterior part of the primary main body axis is deflected from the morphological (actual) main body axis (Fig. 1d). A functional main body axis reflects the manner of movement of the worm; it goes through the pharynx and the center of the acetabulum. Thus, the posterior part of the functional main body axis if deflected from the morphological main body axis as well (Fig. 1d). My results show that the outer longitudinal muscle fibers turn dorsally in the posterior part of the body, which may mean that they most likely follow the primary main body axis. The inner longitudinal muscle fibers evidently follow the functional main body axis. It seems to be obvious that the inner longitudinal muscle layer is an evolutionary acquisition of paramphistomids, and it is no wonder that it follows the functional body axis. I suppose the customary outer longitudinal muscle layer to be more conservative, so it can be adhered rather to the primary main body axis. Hence, to provide the contraction along the functional main body axis, new inner longitudinal muscle layer might have formed. It may be due to the lack of the oral sucker and the consequent reorganization of the whole region that the outer longitudinal muscle layer is absent on the fore-end of the body.

The number of muscle layers within body wall of *D. subclavatus* and their relative thickness do not differ in cercariae, pre-ovigerous, and ovigerous adult worms. That might be typical for the trematodes with "smooth" type morphogenesis of hermaphroditic generation (in terms of Galaktionov and Dobrovolskij 2003). I should note that the cercariae have four muscle layers as well, contrary to the data of Tolstenkov et al. (2012), who described only three common muscle layers.

The other important point to discuss is the structure of muscular organs, including the acetabulum, the pharynx, and the terminal genitalium. Paramphistomida represent the unique case when the musculature is taken into account for the needs of systematics due to the paper of Näsmark published in 1937. So the data on the paramphistomids musculature outnumber the information on all the other trematodes. However, when classifying the muscular organs, Näsmark (1937) himself and his followers used only the middle sagittal histological sections (Eduardo 1982; Sey 1983, 1987; Jones 1988), which confines the reliability of their descriptions. Speaking about the acetabulum, those muscle layers which Näsmark considered minor and unimportant for classification are omitted in his and subsequent schemes and descriptions. So the ultimate fullest description of *D. subclavatus* acetabulum was given by Fukui in 1929. The most significant muscle layers within it never mentioned by the other researchers are the longitudinal (they probably correspond to the outer meridional muscle layer in present description) and two sets of oblique muscle fibers (those should correspond to the starry layer in present description). Two sets of circular muscle fibers at the margin (internal and external) by Fukui (1929) are equivalent to the dorsal and ventral exterior and interior circular groups by Näsmark and his followers. I consider them to be the parts of the continuous circular layer (at least for this very species). A number of paramphistomids are known to have the "median exterior circular series" (Eduardo 1982), which corresponds to the circular muscle layer on the submerged part of the outer surface of the acetabulum in *D. subclavatus*.

The pharynx of *D. subclavatus* was described by Sey (1983) and referred to the type Subclavatus (together with *Dermatemytrema trifoliata*). The special characteristics of this type were the absence of middle circular layer and the absence of circular muscle fibers in pre-sphincteric space. I showed that both these muscle groups are present (except for the detail that the muscle fibers of the middle layer appear to be semicircular and ones in the pre-sphincteric space—transverse), which makes the pharynx of *D. subclavatus* similar to Ferrum-equinum type (held by Sey 1983 for *Opisthodiscus diplodiscoides*).

The organization of tail musculature was studied for quite an amount of different cercariae by different methods including TEM, CSLM, and traditional histological sections (Pearson 1961; Rees 1971, 1975; Chapman 1973; Nuttman 1974; Malkova 1989). Among few sufficiently detailed and comparable descriptions, the one of Nuttman (1974) made for *Schistosoma mansoni* is the most similar to my results on

D. subclavatus. The longitudinal muscle fibers within the tail of *S. mansoni* are organized into six bundles as follows: two medial (dorsal and ventral) of a smooth type and four lateral that are obliquely striated. The longitudinal muscle fibers within the tail of *D. subclavatus* are less clearly divided into bundles, especially in the anterior and posterior regions. However, the longitudinal furrow divides the lateral striated bands in two in the most functionally significant middle part of the tail. So the tail of *D. subclavatus* represents the intermediate morphological condition between clearly divided muscle bands of *S. mansoni* and undifferentiated layer of longitudinal muscle fibers found in *Parorchis acanthus* (Rees 1971), *Maritrema subdolum*, and *Microphallus claviformes* (Malkova 1989). Still, the comparison of different types of tail organization is to be carried out in the future.

Acknowledgments I am grateful to Dr. Andrej A. Dobrovolskij for his helpful advices and support during this study and to Anna G. Gonchar and Alexei A. Mirolyubov for improvement of the English version of the manuscript. The study was carried out using the equipment of Chromas Resource Research Center and Educational Resource Center of Microscopy and Microanalysis (Saint Petersburg State University). The project was funded by Saint Petersburg State University (grant number 1.37.80.2011).

References

Chapman HD (1973) The functional organization and fine structure of the tail musculature of the cercariae of *Cryptocotyle lingua* and *Himasthla secunda*. Parasitol 66:487–497

Eduardo SL (1982) The taxonomy of the family Paramphistomidae Fischoeder, 1901 with special reference to the morphology of species occurring in ruminants. I. General considerations. Syst Parasitol 4:7–57

Fukui T (1929) Studies on Japanese amphistomatous parasites, with revision of the group. Jpn J of Zool, Transl Abstr 2:219–351

Galaktionov KV, Dobrovolskij AA (2003) Biology and evolution of trematodes. An essay on the biology, morphology, life cycles, transmission, and evolution of digenetic trematodes. Kluwer Academic Publ, London

Ginetsinskaya TA (1968) Trematodes, Their Life Cycles. Biology and Evolution, Nauka, Leningrad, USSR (in Russian)

Halton DW, Maule AG (2004) Flatworm nerve-muscle: structural and functional analysis. Can J Zool 82:316–333

Jones A (1988) Amphistome acetabular types: an amendment to the duplicitestorum acetabular type of Näsmark (1937). Syst Parasitol 11:215–217

Kuntz RE (1950) Embryonic development of the excretory system in fork-tailed cercariae of the schistosomes and in a blunt-tailed brachylaemid cercaria. Trans Am Microsc Soc 69:1–20

Malkova II (1989) Tail ultrastructure in microphallid cercariae (Trematoda, Microphallidae). Parazitologiya 23:159–165 (in Russian)

Näsmark KE (1937) A revision of the trematode family Paramphistomatidae. Zool Bidrag Fr Uppsala 16:301–565

Nuttman CJ (1974) The fine structure and organization of the tail musculature of the cercaria of *Schistosoma mansoni*. Parasitol 68:147–154

Pearson JC (1961) Observations on the morphology and life cycle of *Neodiplostomum intermedium* (Trematoda: Diplostomatidae). Parasitol 51:133–172

Pearson JC (1992) On the position of the Digenean family Heronimidae: an inquiry into a cladistic classification of the Digenea. Syst Parasitol 21:81–166

Rees FG (1971) Locomotion of the cercaria of *Parorchis acanthus*, Nicoll, and the ultrastructure of the tail. Parasitol 62:489–503

Rees FG (1975) The arrangement and the ultrastructure of the musculature, nerves, and epidermis in the tail of the cercaria of *Cryptocotyle lingua* (Creplin) from *Littorina littorea* (L.). Proc R Soc Lond B Biol Sci 190(1099):165–186

Šebelová Š, Stewart MT, Mousley A, Fried B, Marks NJ, Halton DW (2004) The musculature and associated innervation of adult and intramolluscan stages of *Echinostoma caproni* (Trematoda) visualized by confocal microscopy. Parasitol Res 93:196–206

Sey O (1983) Reconstruction of the systematics of the family Diplodiscidae Skrjabin, 1949 (Trematoda: Paramphistomata). Parasitol Hung 16:63–89

Sey O (1987) Typifying and classifying of the muscular organs of amphistomes (Trematoda: Amphistomida). Parasitol Hung 20:45–63

Stewart MT, Marks NJ, Halton DW (2003) Neuroactive substances and associated major muscle systems in *Bucephaloides gracilescens* (Trematoda: Digenea) metacercaria and adult. Parasitol Res 91:12–21

Tolstenkov OO, Akimova LN, Terenina NB, Gustafsson MKS (2012) The neuromuscular system in continuously swimming cercaria from Belarus. II Echinostomata, Gymnocephala, and Amphistomata. Parasitol Res 111:2301–2309

Yastrebova IV, Yastrebov MV, Freze VI (2000) Body wall musculature of some rheophilic representatives of Trematoda (Plathelminthes). Tr Inst Parazitol (Trans of the Inst of Parasitol) 42:348–361 (in Russian)