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Musculature arrangement and locomotion in notocotylid cercariae (Digenea: Notocotylidae) from mud snail *Ecrobia ventrosa* (Montagu, 1803)

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ABSTRACT

Cercariae of digenean family Notocotylidae are characterized by a set of morphological traits which make them easily distinguishable from any other. One of the key features is absence of ventral sucker. This affects basic ways of locomotion and attachment. To understand how these functions are fulfilled we studied musculature arrangement in cercariae of two species by means of fluorescent-phalloidin staining and confocal microscopy. We used *Cercaria Notocotylidae* sp. No. 11 and 12 Deblock, 1980 from mud snails *Ecrobia* (=*Hydrobia*) *ventrosa*. Information on gross morphology (especially body-tail junction) and basic behavioural patterns of these cercariae is also updated.

Major special features of musculature are associated with the ventral concavity: extreme development of dorsoventral muscle fibres and formation of annular arrangement of longitudinal muscle fibres on the ventral side. Additional body-wall and internal muscle bundles in the anterior region are also specific for notocotylid cercariae and seem to play important role in twisting movements during substratum testing. Musculature of dorsal adhesive pockets, oral sucker and tail is also described. These results are discussed in relation to observed locomotory patterns.

1. Introduction

Digeneans of family Notocotylidae are wide-spread parasites in marine and fresh-water ecosystems. They have dixenous life cycle with snails being first intermediate hosts and birds or mammals being definitive hosts [1,2]. Adults and cercariae of notocotylids are special in having no ventral sucker and pharynx. In most species adult worms possess ventral papillae or ridges, whereas the cercarial ventral surface is plain [2,3]. The function of ventral papillae is disputed, however it is unlikely that fixation on the host mucosa is provided by these structures [4]. Attachment in adult notocotylids is performed by the whole body which takes shape of a spoon, so that a structure named ventral concavity appears. The mechanism of negative pressure formation in this concavity is considered similar to that in a typical sucker, dorsoventral muscle fibres functionally replacing the radial musculature [4–7]. Cercariae are also capable of contracting so that the ventral concavity is prominent during swimming.

Notocotylid cercariae feature quite a complex behaviour that can result in ability to select a certain type of encystment substratum – like plant surface or mollusc shell [8]. Previously there were controversial accounts of the elements comprising this behaviour [9–11]. We were interested in describing actual movement types and understanding how they could be provided by musculature. The two notocotylid species studied here are *Cercaria Notocotylidae* sp. No. 11 and 12 Deblock, 1980, both utilizing mud snails *Ecrobia* (=*Hydrobia*) *ventrosa* (Montagu, 1803) as first intermediate hosts. We observed living cercariae and used TRITC-phalloidin labelling and confocal microscopy to describe musculature. We provide a single description because results did not differ for the two species.

2. Material and methods

The material was collected from the White Sea (Kandalaksha Gulf, Chupa Inlet, near Marine Biological Station of Saint Petersburg State University), Russia. Cercariae were obtained from naturally infected hosts *Ecrobia* (=*Hydrobia*) *ventrosa* by stimulating emission with the light. The two species, *C. Notocotylidae* sp. no. 11 and 12 Deblock, 1980 (Fig. 1a, b), differ slightly in size and clearly in the shape of the anterior part of main collecting ducts [12]. They belong to Monostomi and Imbricata morphotypes, respectively, following classification of M. Rothschild [13]. These features are easily recognized on temporary mounts of living cercariae.

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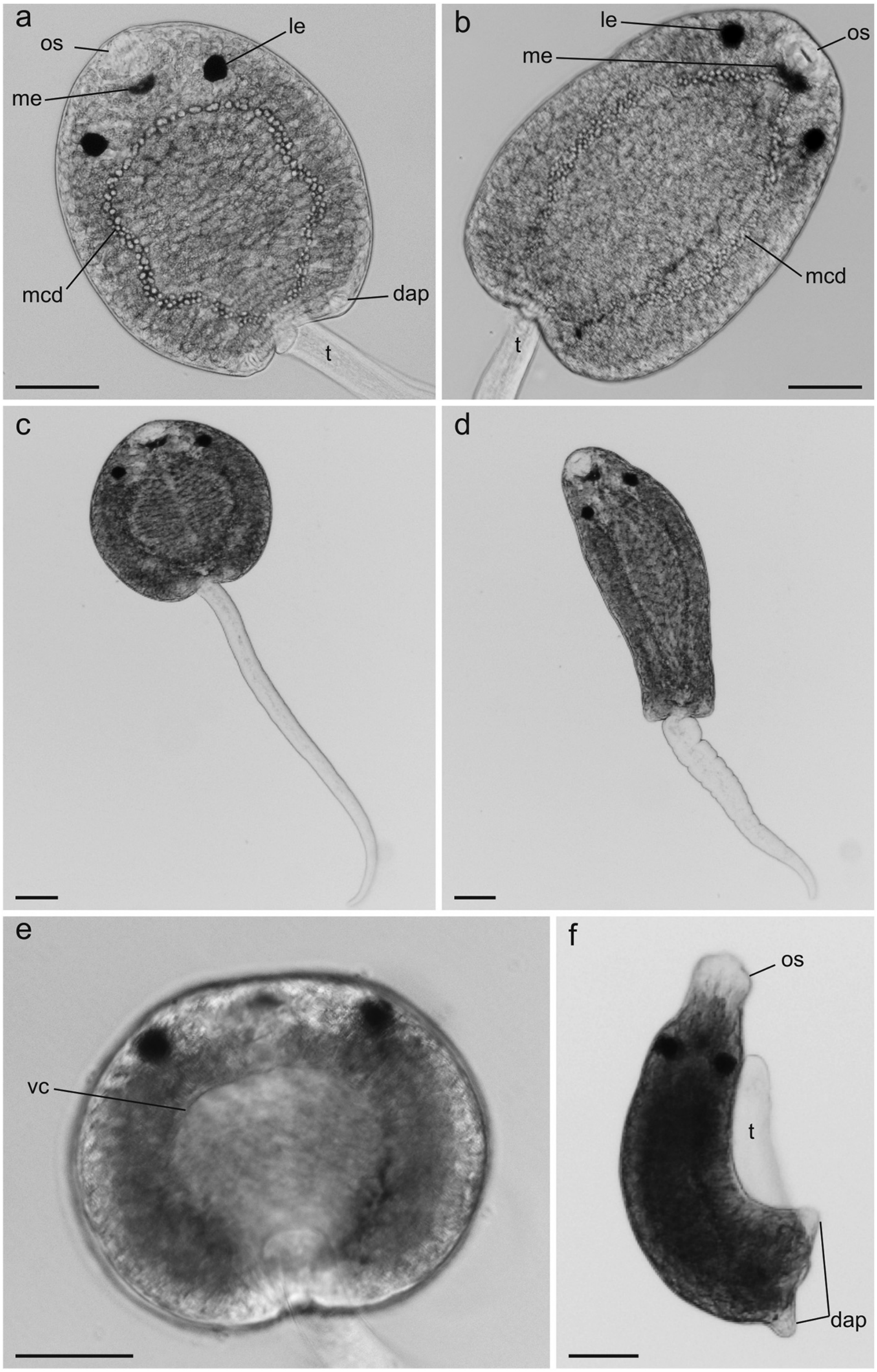

Fig. 1. Microphotographs of whole-mounted living cercariae. a–b. Difference between *C. Notocotylidae* sp. no. 11 Deblock, 1980, morphotype Monostomi (a) and *C. Notocotylidae* sp. no. 12 Deblock, 1980, morphotype Imbricata (b). Note anterior region of fused main collecting ducts (*mcd*), oral sucker (*os*), lateral (*le*) and median (*me*) eyes, retracted dorsal adhesive pockets (*dap*) and tail (*t*). c–d. Cercaria moving between the microscopic slides: contracted (c) and elongated (d) body. e. Cercaria body contracted so that ventral concavity (*vc*) is prominent. f. Cercaria body elongated so that oral sucker (*os*) and dorsal adhesive pockets (*dap*) are prominent. Scale bars – 50 μm.

Observations on cercariae behaviour were conducted using stereomicroscope MBS-10 (LOMO) and compound microscope Leica DM 2500 (Leica Microsystems). Photographs and video of living cercariae were made with the camera Nikon DS Fi1 (for compound microscope) and Canon EOS 70D with adaptor MFU (LOMO) (for stereomicroscope).

Cercariae were fixed for 6–24 h in a 4% solution of paraformaldehyde in 0.01 M phosphate-buffered saline (PBS). Samples were washed in PBS and stored in PBS with 0.1% sodium azide (NaN_3). Before staining specimens were washed in PBS with Triton-X100 (0.1%) during 1–2 h. Incubation in TRITC-labelled phalloidin solution (200 ng/ml) took another 24 h, followed by 1–1.5 h wash in PBS. Finally the specimens were mounted in glycerol/PBS (9/1) and examined under the confocal scanning laser microscope (CSLM) Leica TCS-SP5.

ImageJ v. 1.46r software was used to process data from CSLM: to make snapshots and Z-stacks. The reconstructions of optionally directed optical slices were made using plug-in “Volume Viewer” v. 1.31. Schemes and plate setups were done with Corel Draw X3 and appropriate image modifications were made with Adobe Photoshop CS2.

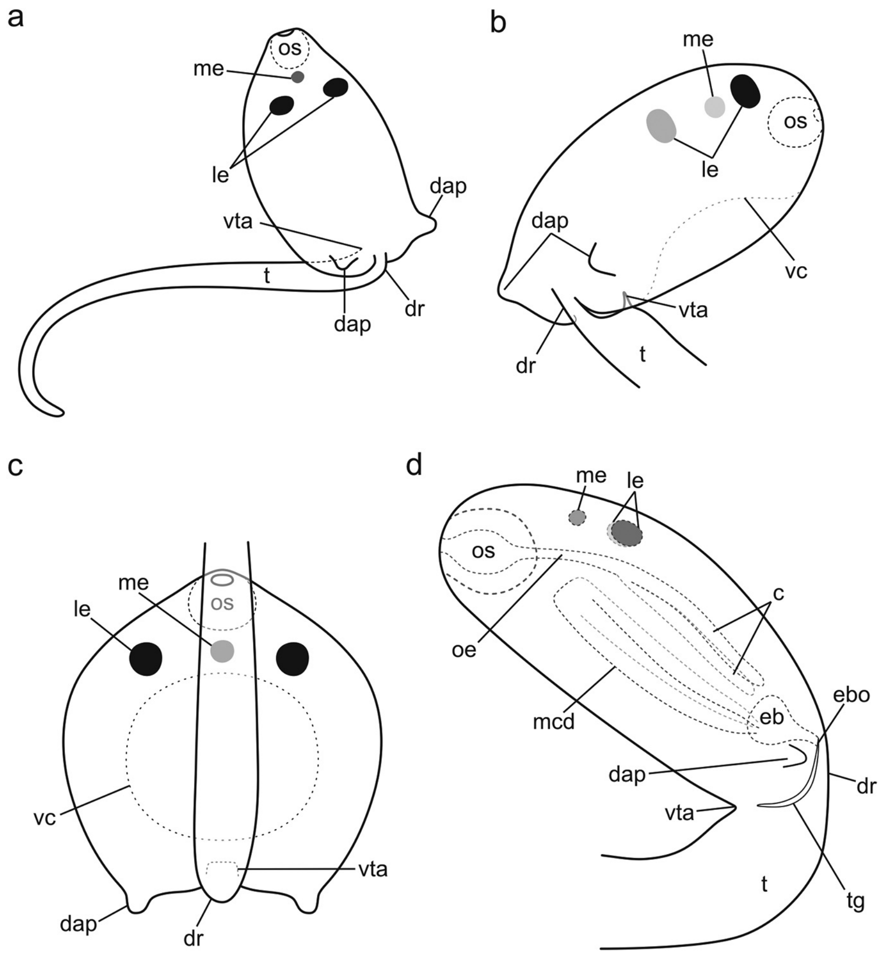

Fig. 2. Schemes showing gross-morphology of notocotylid cercariae in various natural positions. Oral sucker (*os*), lateral eyes (*le*), median eye (*me*), dorsal adhesive pockets (*dap*), tail (*t*), its dorsal ridge (*dr*) and ventral site of tail attachment (*vta*) are shown on each scheme. a. Dorsolateral view. b. Posterior-lateral view of the body. c. Ventral view of the body, base of the tail is bent, tail going along the body. On b and c the body is saucer-shaped contracted (swimming position), so that ventral concavity (*vc*) is prominent. d. Lateral view, main internal organs: oesophagus (*oe*), caecum branches (*c*), ring formed by main collecting ducts (*mcd*), excretory bladder (*eb*) and its opening (*ebo*). Groove on the side of the base of the tail (*tg*) is also shown.

3. Results

3.1. Gross morphology

Studied cercariae have appearance typical for Notocotylidae (Figs. 1, 2). The body is oval and flattened, without ventral sucker and with normally developed oral sucker. Two knobbles – dorsal adhesive pockets[1] – are placed dorsally on each side of the base of the tail. The base of the tail has a complex configuration with two attachment sites. On the ventral side the attachment zone of the tail is wide; on the dorsal side it forms a narrow ridge (Figs. 2b, c, 3). Thus base of the tail embraces medial part of the hind body from ventral and dorsal sides (Fig. 2b, d). Between the dorsal and ventral attachment sites grooves are present (Fig. 2d). The secondary (definitive) excretory pore[2] is located mid-dorsally beneath the anterior part of the ridge (Fig. 2d).

3.2. Typical movements

After leaving the mollusc cercariae swim with their body contracted and tail actively moving. The tail is not extended along the main body axis but rather its base is bent to the ventral side (like in Fig. 2c). During continuous swimming the tail whips getting the appearance close to figure of eight pattern. Meanwhile the body of the cercariae stays saucer-shaped contracted with hollow ventral surface, so that the ventral concavity is very prominent (Figs. 1e, 2b, c). Swimming is interrupted by periods when cercaria, facing substratum with its dorsal side, extends dorsal adhesive pockets and uses them to attach to the substratum. In this position cercaria usually twists its fore part and touches the substratum with the oral sucker (Suppl. 1, 2).

As cercaria gets older similar but incomplete movements may take place. Attached with its dorsal adhesive pockets, dorsal side down, cercaria rises its body only slightly and does not twist it to bring the oral sucker in contact with the substratum (Suppl. 3). Cercaria may also perform bending movements without attaching at all.

Before encystment the body becomes roundish, facing the substratum with its ventral surface. Thin primary cyst is formed very quickly. Right before it cercaria performs peristaltic movements (Suppl. 4). Later while building inner cyst layers cercaria may bend left and right and sometimes revolve about its dorsoventral axis. The tail is detached from the body upon the primary cyst formation and either remains "glued" to the cyst or swims freely in the water column. In both cases it continues rapid movements for quite a while.

If the cercaria is placed between the slides for microscopic examination it usually extends and contracts at the same spot, but may revolve and crawl slowly. During these movements shape of cercaria changes drastically from circular to elongated, with length changing twofold (Fig. 1c, d).

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[1] These organs were called differently: "locomotory pockets" by M. Rothschild [13], "locomotory appendages" by Z. Ždárská [37], "posterior-lateral glands" by V. Southgate [24]. We decided to use term "dorsal adhesive pockets" by G. Rees [16], as the dorsal position of these organs appears to be functionally important in course of cercariae behaviour.

[2] Opening of excretory bladder according to Kuntz [38].

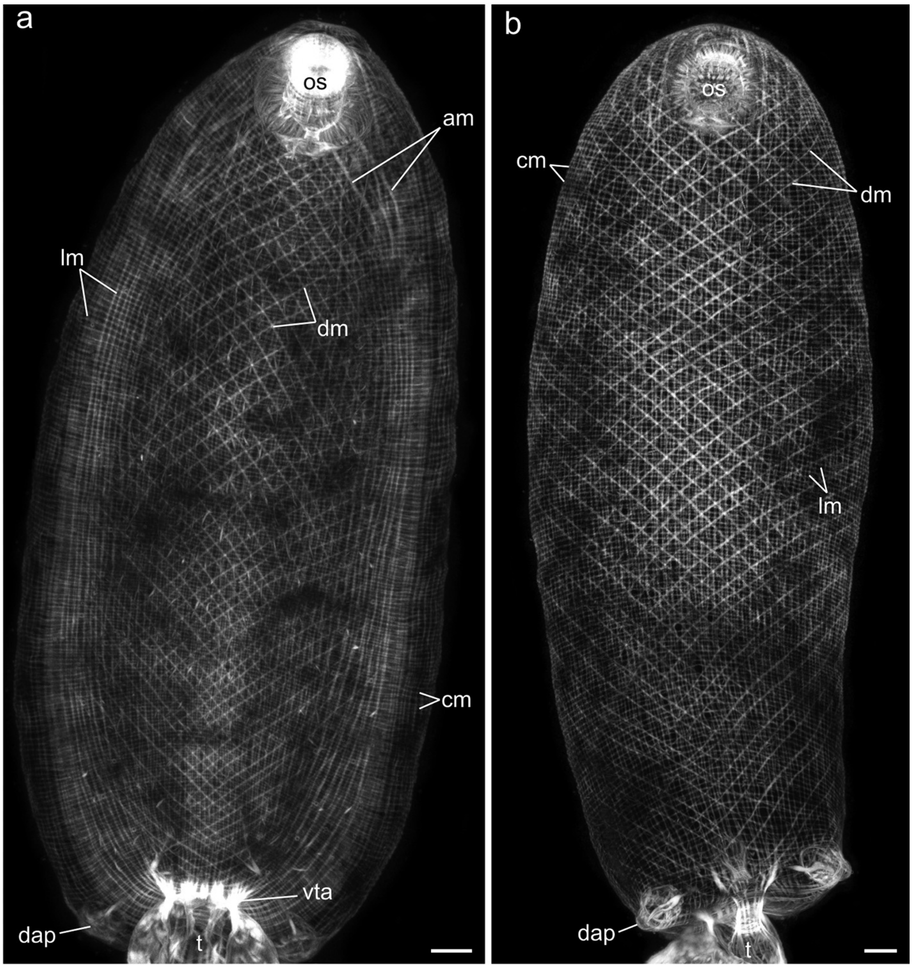

Fig. 3. a–b. Body-wall musculature (TRITC-phalloidin labelling), ventral (a) and dorsal (b) view, with circular (*cm*), longitudinal (*lm*), diagonal (*dm*) muscle fibres, additional muscle bundles in the anterior region (*am*) and dense short bundles at the ventral site of tail attachment (*vta*). Oral sucker (*os*), tail (*t*) and dorsal adhesive pockets (*dap*) are also visible. Scale bars – 10 μm.

3.3. Musculature arrangement

The body-wall musculature comprises outer circular, intermediate longitudinal and inner diagonal muscle fibres (Fig. 3). Circular fibres are arranged compactly and regularly. On the ventral side at the base of the tail they are bent to pass around it anteriorly (Fig. 4a). Longitudinal muscle fibres of the dorsal side form quite a uniform layer (Fig. 3b). On the ventral side within the layer of longitudinal fibres three sets can be defined: ventromedial, ventrolateral, and lateral (*vmlm*, *vllm* and *llm* in Figs. 4a, 5). The longitudinal fibres of the ventromedial set are compactly arranged; they cover an area from the oral sucker to the ventral site of the tail attachment. The ventrolateral longitudinal muscle fibres form an annular pattern. Their anterior ends lie at the lateral sides of the oral sucker. Near the posterior end of the body they turn medially and join together posteriorly to the ventral site of the tail attachment. Thus ventrolateral fibres actually lie in a U-shaped manner and outline the ventral concavity. There are two subsets within ventrolateral set differing by thickness of fibres (Figs. 4a, 5). The lateral sets of the longitudinal muscle fibres proceed to the posterior border of the body and extend slightly to the dorsal side (Fig. 4b). The circular and longitudinal muscle fibres of the body wall extend into the knobbles of the dorsal adhesive pockets (Fig. 4c).

The sets of diagonal muscle fibres of the dorsal side and of the ventral side do not overlap. The diagonal fibres of the ventral set occupy the medial region – the bottom of the ventral concavity, and do not overpass the ventrolateral set of longitudinal muscle fibres (Figs. 3a, 4a, 5). The posterior ends of diagonal muscle fibres within the dorsal set reach the lateral surfaces only in the fore half of the body. In the hind part of the body the lattice of dorsal diagonal muscle fibres is rarefied (Fig. 3b).

Two pairs of additional muscle bundles are present within body wall in the anterior region: they proceed from the oral sucker posterior-laterally (*am* in Figs. 3a, 5). These bundles lie between the layers of longitudinal and diagonal muscle fibres. Near the ventral site of the tail attachment several short and dense muscle bundles are present (*vta* in Figs. 3a, 4a, 5). Between the dorsal adhesive pockets and beneath the base of the tail there is a transverse muscle band lying within the body-wall layer of longitudinal muscle fibres (*ptm* in Figs. 4a, b, 5)

Internal musculature includes two major sets of fibres (dorsoventral and oblique) and three smaller groups; all the components are shown in Fig. 6. Dorsoventral muscle fibres are numerous. The dorsal ends of these fibres are clearly inclined laterally which is least prominent in the medial area and most – in the lateral areas (Fig. 7a). In the anterior region the dorsoventral fibres are inclined in yet another direction: the dorsal ends lie more anteriorly than ventral (Fig. 7a, b).

A large group of oblique longitudinal muscle fibres is present along the whole body (*om* in Figs. 6, 7a, b). Although these fibres also connect dorsal and ventral sides of the animal, they form a group distinct from dorsoventral fibres. The difference is in their inclination: the dorsal ends lie much more posteriorly and slightly more laterally than the ventral ones.

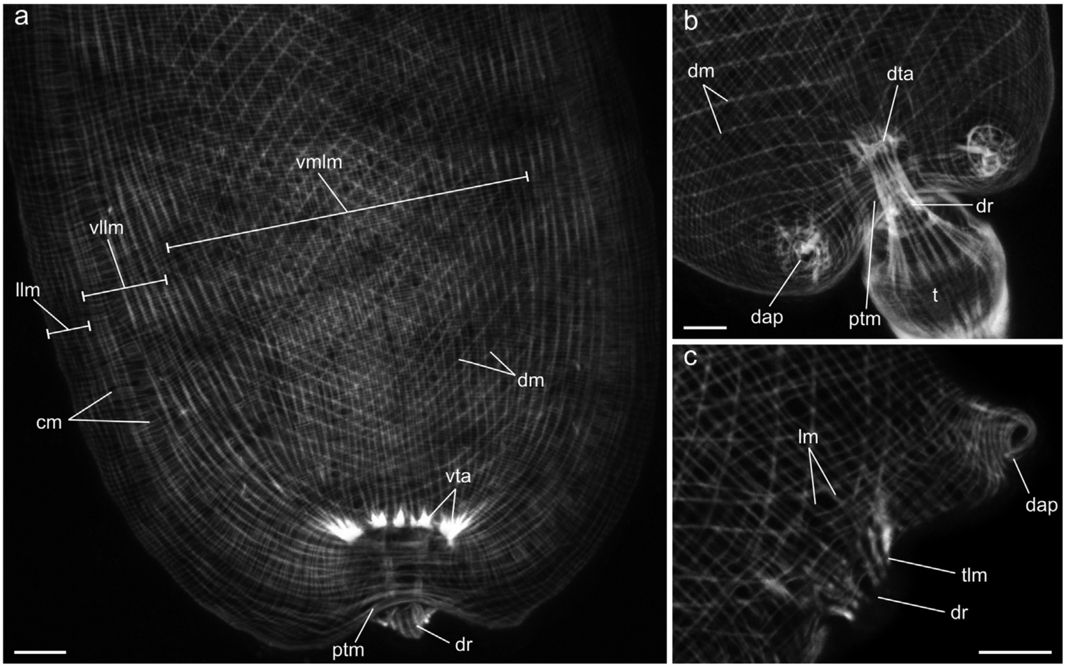

Fig. 4. Body-wall (TRITC-phalloidin labelling). a. Posterior half of the body, reconstruction of ventral body-wall musculature; base of the tail apart from the dorsal ridge (*dr*) is removed. Note circular (*cm*) and diagonal (*dm*) muscle fibres, ventromedial (*vmlm*), ventrolateral (*vllm*) and lateral (*llm*) longitudinal muscle fibres, posterior transverse muscle band (*ptm*) and dense short bundles at the ventral site of tail attachment (*vta*). b. Posterior part of the body and base of the tail, dorsal view; note whole dorsal ridge (*dr*) of the tail (*t*) and dorsal site of tail attachment (*dta*). Dorsal adhesive pockets (*dap*) retracted. Diagonal muscle fibres (*dm*) and posterior transverse muscle band (*ptm*) are also visible. c. Several tangential optical slices at the dorsal site of tail attachment; note interdigitating body-wall longitudinal muscle fibres (*lm*) and tail longitudinal musculature (*tlm*) at the anterior of dorsal ridge (*dr*). See also body-wall musculature at the surface of protracted dorsal adhesive pockets (*dap*). Scale bars – 10 μm.

Three additional compact groups of internal musculature are: (1) several paired bundles running from the anterior end of the body, along the lateral surfaces of the oral sucker, to the ventral body wall (*alm* in Figs. 6, 7c, d); (2) eight pairs of bow-shaped muscle bundles starting from the anterior and lateral surfaces of the oral sucker, running beneath the body wall dorsolaterally, and attaching to the dorsal body wall (*bsm* in Figs. 6, 7c–e); (3) the retractors of dorsal adhesive pockets running ventromedially and attaching to the body wall anteriorly to the tail attachment site (*rdp* in Fig. 7f–h). The retractors of the dorsal adhesive pockets run from the tip of each pocket inwards. The pockets are delimited from the parenchyma by an irregular net of muscle fibres (*inm* in Fig. 7f–h).

The shape of the oral sucker varies from roundish to bottlelike depending on its physiological state. The loose rim[3] is rather narrow. The outmost muscle layer of the oral sucker comprises two parts. Most of the surface is covered by longitudinal (meridional) muscle fibres (*mm* in Fig. 7d, i). However there is a clear border with no musculature delineating meridional layer from the posterior part of the sucker where three pairs of muscular bands surround the oesophagus (*pmb* in Figs. 7i, 8a). The loose rim of the sucker has prominent circular musculature forming a sphincter (*sph* in Fig. 7d). The buccal cavity has a lining of circular and longitudinal muscle fibres (*bcm* and *blm* in Fig. 7k). The bulk of the sucker comprises numerous radial muscle bundles and two sets of oblique muscle fibres which pass dorsoventrally through the sucker (*rm* and *oms* in Figs. 7j, k, 8b).

Inner circular and outer longitudinal muscle fibres are present in the oesophagus, and the same layers are seen in the caecum branches though lying much more sparsely (Fig. 7b).

The tail has its own musculature with quite special arrangement (Fig. 9). The outmost muscular layer of the tail is composed of circular fibres (Fig. 9b, c, h). Beneath there is a layer of powerful longitudinal musculature. It is connected to the body-wall musculature at the dorsal and ventral sites of tail attachment. On the dorsal side the longitudinal muscle fibres of the tail interdigitate with the longitudinal fibres of the body (Figs. 3b, c, 9d, e). On the ventral side tail longitudinal musculature is connected to several short and dense muscle bundles of the body (*vta* in Figs. 3a, 9d, e, f).

The longitudinal musculature of the tail forms two compact bundles of smooth muscle fibres medially – one on the dorsal and another on the ventral side (*vmlm* and *dmlm* in Fig. 9a, c, g, h), and four lateral striated muscle bands. Gaps between the striated bands of lateral pairs are visible only near the base of the tail (Fig. 9d, f). Contractile filaments of the striated longitudinal muscle fibres are lying within the muscular processes in a U-shaped manner on a cross-section (Fig. 9g). The striation of lateral longitudinal muscle bands is lost near the tail tip (Fig. 9a).

In addition a pair of dorsoventral muscle bands is present near the base of the tail (Fig. 9i).

4. Discussion

4.1. Gross morphology

Notocotylid cercariae are easily distinguished from any other. They belong to Monostome type in M. Lühe classification [14] characterized by presence of only oral sucker. Other features are three eye spots, pigmented body with numerous cystogenous glands, dorsal adhesive pockets and excretory formula $2[(3+3+3)+(3+3+3)]=36$ [3]. Gross morphology of notocotylid cercariae was described before in many papers [9,11,15–17] and appears to be very uniform. However there are controversial reports on some details.

Dorsal position of adhesive pockets which we emphasize here was previously stated only in few works (e.g. [16,3]). In many other studies cercariae were drawn flattened, apparently due to cover slide pressure. In these cases it is only clear that adhesive pockets are located posterior-laterally, but their shift to the dorsal side is not visible. Specific position of adhesive pockets is important for interpretation of certain locomotory patterns (see below – Section 4.3).

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[3] By "loose rim" we mean a part of oral sucker outer surface which lies right beneath the outer tegument and is not covered with the body-wall musculature.

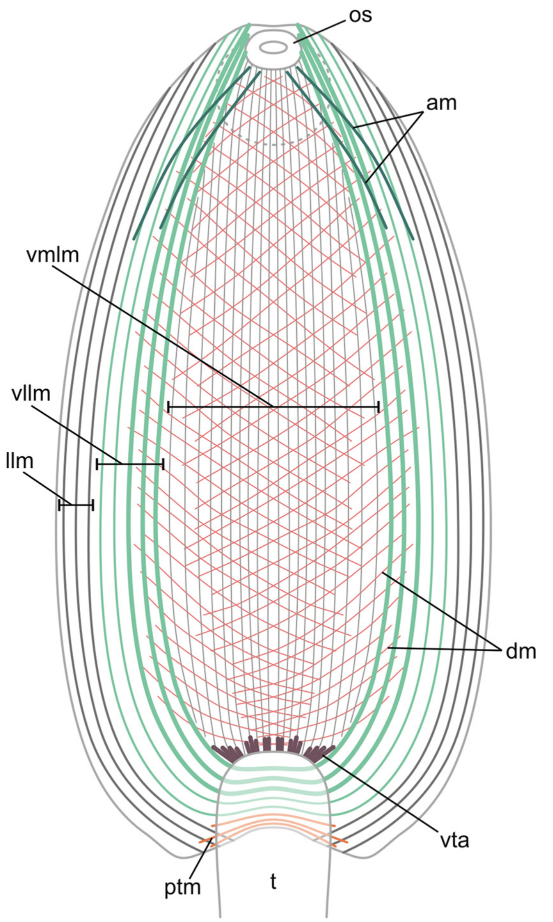

Fig. 5. Scheme showing the arrangement of muscle fibres within body wall, ventral side. Ventromedial (*vmlm*), ventrolateral (*vllm*) and lateral (*llm*) sets of longitudinal muscle fibres, diagonal muscle fibres (*dm*), additional muscle bundles in the anterior region (*am*), posterior transverse muscle band (*ptm*) and dense short bundles at the ventral site of tail attachment (*vta*). Other abbreviations: oral sucker (*os*), tail (*t*). Circular muscle fibres are not shown.

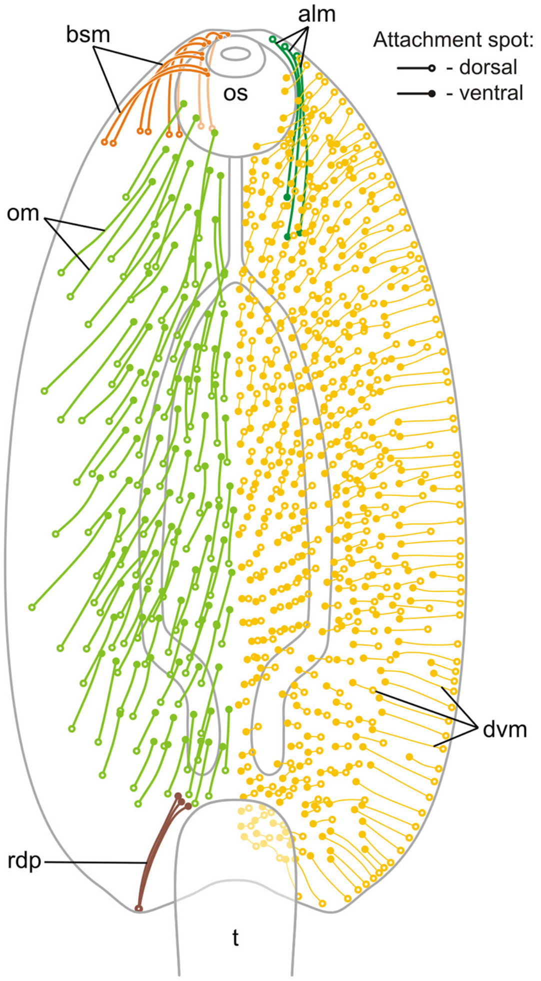

Fig. 6. Scheme showing the arrangement of internal musculature, ventral view. Dorsoventral muscle fibres (*dvm*) and longitudinal bundles in the anterior region (*alm*) are shown only at the right side; oblique longitudinal muscle fibres (*om*), bow-shaped muscle bundles (*bsm*) and retractors of dorsal adhesive pockets (*rdp*) are shown only at the left side. Other abbreviations: oral sucker (*os*), tail (*t*).

Usually descriptions of notocotylid cercariae omit the details on the position of definitive excretory pore, tail excretory duct and body-tail junction. Two sites of tail attachment were previously described by M. Horsfall [15], and here we confirm her data, and supplement it with new schemes and optical slices. Some authors propose that the tail excretory duct is linked to the excretory bladder, so that wastes go out through the primary pores in the tail [10,17,18]. However several authors [15,19,20] and especially R. Kuntz [21] give credible description of these organs loosing connection during development. The tail excretory duct gets reduced and is completely absent in fully formed cercariae. The excretory atrium is formed between dorsal and ventral sites of tail attachment, opening laterally.[4] We also observed lateral grooves on the sides of the base of the tail between dorsal and ventral attachment sites that probably lead to the excretory atrium, though we could not see it clearly.

4.2. Ventral concavity

Somatic musculature in Digenea typically comprises three main muscle layers of the body wall (circular, longitudinal and diagonal) and dorsoventral musculature [5,6,22]. Notocotylids share these features. However, the absence of ventral sucker greatly affects not only their gross morphology, but also major ways of locomotion and attachment, and, subsequently, musculature. Specifically, in notocotylids there is no additional body-wall and internal musculature connected with the ventral sucker, very common in digeneans with standard body construction [23]. Instead, ventral concavity – the alternative suction structure – is formed, and the somatic musculature is transformed to maintain it.

It is known that presence of ventral concavity in notocotylid adults correlates with striking development of dorsoventral musculature [4–6]. Supposedly these muscle fibres act like radial musculature of the suckers [7]. In studied cercariae dorsoventral muscle fibres are also extremely prominent. Their arrangement and particularly inclination probably serves to keep saucer-shape of the body. Massive group of oblique internal muscle fibres seems to serve the same purpose, but might represent a specialized derivation from dorsoventral musculature.

B. MacKinnon [4] noted that lateral edges of the worm are probably important for the attachment of adult notocotylids to the host caecum epithelium by forming marginal seal. Even at the cercaria stage we found muscular structure able to support this seal – the ventrolateral longitudinal fibres forming annular arrangement. Another point is separation of dorsal and ventral sets of diagonal musculature – they do not overlap with the border of the concavity, and thus do not hinder the formation of this border.

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[4] These openings were called "tertiary excretory pores" by R. Kuntz [21].

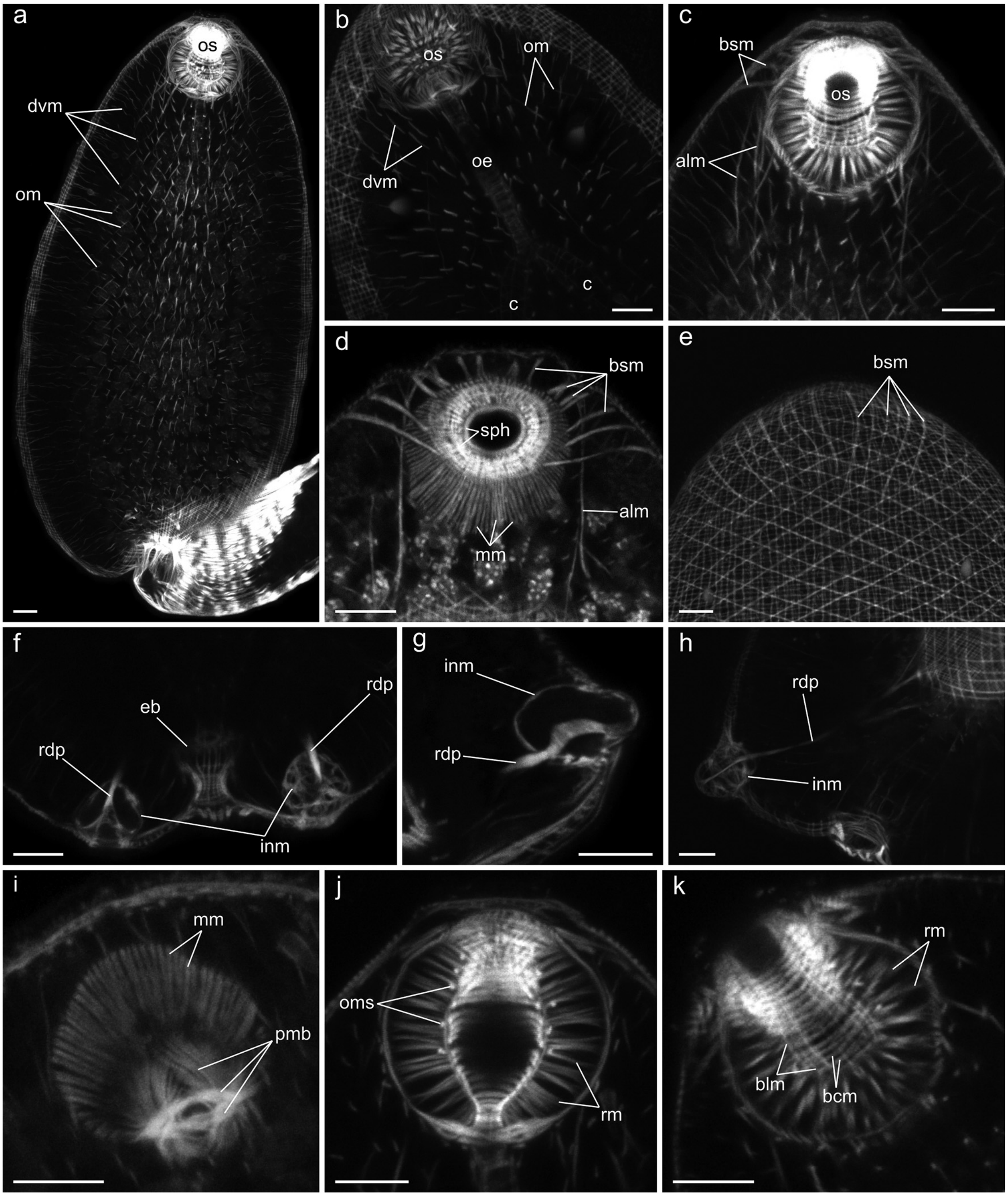

Fig. 7. Internal musculature, oral sucker (*os*) and dorsal adhesive pockets (TRITC-phalloidin labelling). a. Stack of several frontal optical slices through whole body; dorsoventral (*dvm*) and oblique longitudinal (*om*) muscle fibres are visible. b–d. Stacks of several frontal optical slices through anterior region with internal musculature. On c and d bow-shaped muscle bundles (*bsm*) and longitudinal bundles in the anterior region (*alm*) are visible. On b dorsoventral (*dvm*) and oblique longitudinal (*om*) muscle fibres of the body, and also circular and longitudinal musculature of oesophagus (*oe*) and caecum branches (*c*). On d note the sphincter of the oral sucker opening (*sph*) and meridional muscle fibres (*mm*) of the oral sucker. e. Body-wall musculature at the dorsal side, anterior region, with bow-shaped muscle bundles (*bsm*) visible through it. f–h. Musculature of dorsal adhesive pockets, retracted (f) and protracted (g, h). Retractors of pockets (*rdp*) and irregular net of muscle fibres (*inm*) confining pockets from the parenchyma are visible. See also circular and longitudinal muscle fibres of excretory bladder (*eb*) on f. i. Posterior-dorsal view of oral sucker; meridional muscle fibres (*mm*) and paired muscular bands surrounding the oesophagus (*pmb*) are visible. j. Frontal optical slice through oral sucker, radial (*rm*) and oblique (*oms*) muscle fibres of the sucker are visible. k. Frontal optical slice through oral sucker, circular (*bcm*) and longitudinal (*blm*) muscle fibres of the buccal cavity, as well as radial muscle fibres (*rm*) are visible. Scale bars – 10 μm.

In digeneans possessing ventral sucker formation of ventral concavity is also possible, though it occupies not the whole ventral surface but only the preacetabular region.[5] In this case there is similar inclination of dorsoventral fibres and annular construction of anteriolateral muscle fibres, U-shaped muscle set and ventrolateral longitudinal fibres of preacetabular region [23]. Thus we see how in digeneans with different body construction similar elements are required to form a ventral concavity, although they may have different origins.

The role of the ventral concavity in attachment of notocotylid cercariae actually remains uncertain. Although it could potentially be used to attach to the substratum before the primary cyst is secreted, we didn't

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[5] Preacetabular region refers to part of the digenean body anterior to the ventral sucker.

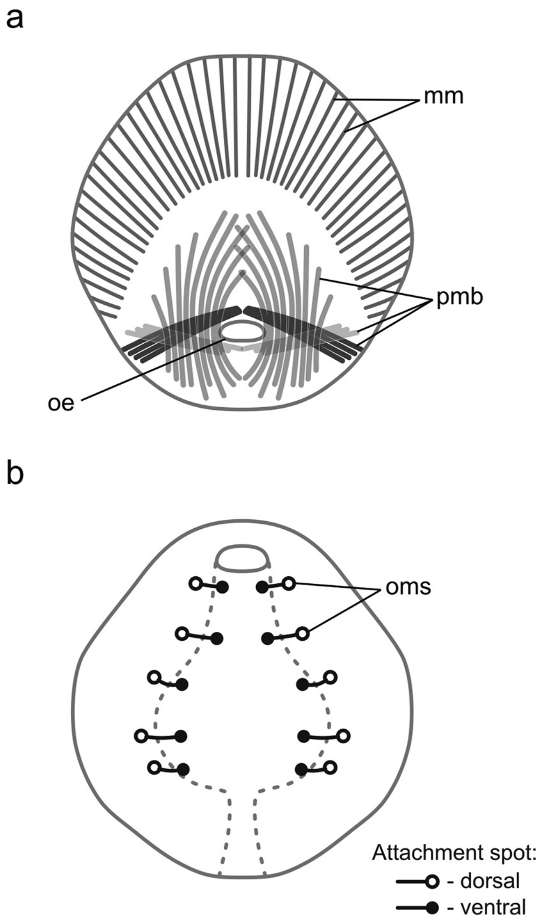

Fig. 8. a. Scheme showing the arrangement of superficial musculature of the oral sucker, posterior-dorsal view; meridional muscle fibres (*mm*) and paired muscular bands (*pmb*) surrounding the opening leading to oesophagus (*oe*) are shown. b. Scheme showing the arrangement of oblique muscle fibres of the sucker (*oms*), frontal plane.

manage to observe this. What we have observed is the consistent saucer-shape of the body with prominent ventral concavity while swimming (discussed in Section 4.4).

4.3. Substratum testing: roles of oral sucker and dorsal adhesive pockets

According to our data swimming of notocotylid cercariae is intermitted by periods of contacting the substratum, supposedly to test its appropriateness for encystment. In these moments tail stops active beating, and the main actions are performed by oral sucker and dorsal adhesive pockets. However previously their function was interpreted in a different way. The most questionable is contribution of dorsal adhesive pockets to the locomotion on the substrate proposed by W. Cort [9], G. Rees [16], L. Filimonova [3], B. Werding [18] and some other authors. First, in our observations the locomotion on the substratum was not present in natural conditions: crawling was evident only for the cercariae placed between the microscopic slides. Second, dorsal position of the adhesive pockets makes them unfavourable for locomotion on the ventral side. Our observations agree with those of J. Dönges [11] and show that these organs are used only for brief attachment while testing substratum.

V. Southgate [24] described secretory granules in the tegument of dorsal adhesive pockets and also found longitudinal, circular and transverse muscle fibres within them. Our data reconstruct the musculature of these organs in full and also allow discussing how they operate. The protraction of dorsal adhesive pockets is usually accompanied by body elongation, and must be a result of general contraction of circular muscle fibres of the body wall and of irregular net of muscle fibres delineating the pockets from the parenchyma. The drawing of these pockets back is provided by their retractors.

The attachment by means of dorsal adhesive pockets is followed by body twist and contact of the oral sucker with the substratum. These movements obviously involve action of both body-wall and internal additional muscle bundles in the anterior region. Oral sucker functions normally, providing temporary attachment, and has all the typical muscular elements, of which most important are radial muscle fibres and circular musculature of the sphincter. These structures were reported to be present within the suckers of various digeneans [25–27]. However paired muscular bands in the posterior part have not been described before.

It is remarkable that twisting movements of notocotylid cercariae during substrate testing have a lot in common with the behaviour of *Transversotrema patialensis* (Transversotrematidae[6]) cercariae attaching to fish host surface. These have two dorsal "arm processes" on the base of the tail used for primary adhesion; as one of them detaches, the cercaria rolls over to face the fish with the ventral surface, and attaches with the ventral sucker [28]. However such movements in *T. patialensis* are launched only by contact with fish surface, not any other (e.g. container glass), unlike in notocotylids. Specificity of this reaction is explained by presence of mammiform receptors at the distal tips of "arm processes".

4.4. Tail musculature and locomotion in water column

Rees [16] and Stunkard [29] described outer circular and inner longitudinal muscle fibres within the tail of notocotylid cercariae. Dönges [11] mentioned cross-striation of the tail longitudinal musculature in cercariae *Notocotylus ralli*. The use of CSLM allowed us to find the differentiation of longitudinal muscle fibres into two medial groups and four lateral striated bands. Such arrangement really resembles one previously observed in *Schistosoma mansoni* and several other cercariae [30,31]. Tail musculature arrangement in *Diplodiscus subclavatus* is most similar to what we found here for notocotylids: both lack clear separation of lateral longitudinal muscle bands [32].

Short dense muscle bundles at the ventral site of tail attachment while contracted keep the body bent towards tail. This must play an important role during locomotion in water column. The similar position while swimming was described previously for cercariae from various taxa (*Himasthla secunda*, *H. elongata*, *Cryptocotyle lingua*, *Maritrema subdolum*, *Renicola* sp.) and also notocotylid *Paramonostomum anatis* [33–35]. The specific feature in notocotylid cercariae is that dorsoventral flexure is not in the middle of the body, but at the body-tail junction. The most convincing explanation for this pose was given by V. Prokofiev [34,35]. He pointed out that the cercariae move directionally with its posterior-dorsal part of the body forward. Basing on the fact that detached tail cannot perform directed progressive movement Prokofiev concludes that the body works as a stabilizer for tail during swimming. The saucer-shape of the body is pretty important as the convex dorsal side provides minimal water resistance. Also even smallest changes of body shape greatly affect the whole system, so the body actually serves as a rudder [35].

5. Conclusion

Cercariae of digeneans follow different strategies depending on the general life-cycle composition. Cercariae of family Notocotylidae encyst

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[6] This family is very distant from Notocotylidae.

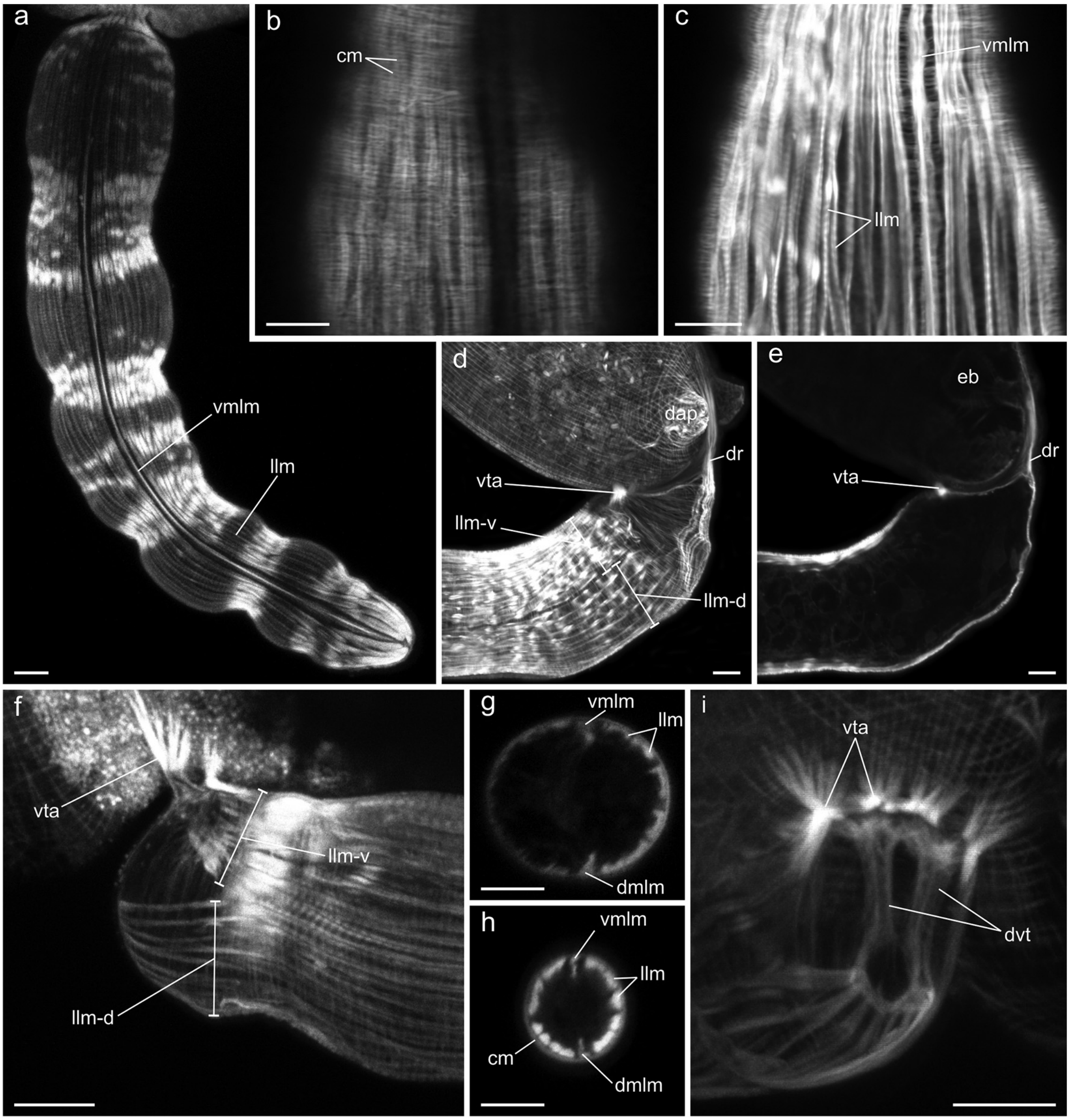

Fig. 9. Tail musculature (TRITC-phalloidin labelling). a. Ventral view of the tail, ventral medial longitudinal muscle bundle (*vmlm*) and lateral longitudinal muscle bands (*llm*) are visible. b. Frontal optical slice through the layer of circular muscle fibres (*cm*). c. Next optical slice through the layer of longitudinal musculature; ventral medial bundle (*vmlm*) of smooth muscle fibres and lateral bands (*llm*) of striated muscle fibres are visible. d. Lateral view of the base of the tail; ventral site of tail attachment (*vta*), dorsal ridge of the tail (*dr*) and dorsal adhesive pockets (*dap*) are visible; also note the origin of dorsal (*llm-d*) and ventral (*llm-v*) lateral longitudinal muscle bands of the tail. e. Midsaggital slice from the same stack; ventral site of tail attachment (*vta*) and dorsal ridge of the tail (*dr*); position of excretory bladder (*eb*) is also visible. f. Lateral view of the base of the tail; note again the origin of dorsal (*llm-d*) and ventral (*llm-v*) lateral longitudinal muscle bands of the tail, and their position relative to dense short bundles at the ventral site of tail attachment (*vta*). g–h. Reconstructions of transverse slices through the tail at midpart (g) and near tip (h); circular fibres (*cm*), dorsal (*dmlm*) and ventral (*vmlm*) medial longitudinal bundles and lateral longitudinal bands (*llm*) are visible. i. Oblique optical section at the base of the tail, pair of dorsoventral muscle bands of the tail (*dvt*) is visible, as well as their position relative to dense short bundles at the ventral site of tail attachment (*vta*). Scale bars – 10 μm.

in the open and the definitive host gets infected consuming cysts by chance. In some species cercariae were observed to prefer certain substrata for encystment [8,11,36], and this probably increases their chance of successful transmission. Complex movements preceding the encystment must be important for choosing substratum, and in present paper we described musculature providing these movements. However this behaviour cannot be interpreted completely through existing data. What would be helpful for resolving this problem is investigation on sensory structures of notocotylid cercariae, never done before, and also detailed experimental study on their behaviour.

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