



Musculature of the sessile rotifer *Stephanoceros fimbriatus* (Rotifera: Gnesiotrocha: Collothecaceae) with details on larval metamorphosis and development of the infundibulum

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ABSTRACT

The sessile rotifer, *Stephanoceros fimbriatus*, is a member of the clade Collothecaceae (Monogononta: Gnesiotrocha) that includes species with indirect development and adults that lack a typical rotiferan corona. The short-lived larva undergoes a dramatic metamorphosis upon settlement, replacing the larval corona with the adult infundibulum, a highly unusual cup-shaped head that appears to develop from the anterior foregut. The infundibulum of *S. fimbriatus* bears five tentacles that function in the capture of small prey. We studied larval metamorphosis in this species and applied a fluorescent f-actin stain and confocal laser scanning microscopy to follow the development of the musculature from larva to adult. We determined that the larva contains a mostly orthogonal grid of somatic muscles, with 12 circular rings encompassing four thick pairs of longitudinal bands. Additional segmental muscles and a complex splanchnic component are also present. Interestingly, the larval infundibulum is an inverted pouch that itself is not muscular but does possess a thick muscular ring (infundibular muscle) at the anterior end of the larva, which will become the future base of the adult infundibulum. The infundibular muscle contributes to the muscle fibers that supply the five developing infundibular tentacles. During metamorphosis, the infundibular muscle slowly becomes reoriented from a slightly ventroanterior position to a terminal position at the top of the larva. The infundibulum then slowly everts through the presumed larval mouth, which has shifted to the top of the corona. The tentacles arise first, and contain a complex muscular network that is assumed to have a mostly supportive function since the tentacles themselves are rarely active. Despite this eversion and other dramatic changes to the larval body plan after metamorphosis – an elongating foot and thickening trunk – the muscle patterns of the adult remain extremely similar to the larva. The major exceptions to this otherwise conservative muscular body plan appear to include a repositioning of the somatic muscles as the infundibulum assumes the adult orientation, and the development of the tentacular muscular network.

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1. Introduction

Species of the sessile rotifer *Stephanoceros* have captured the attention of microscopists since their original description by Goldfuß in 1820. In fact, the appearance of these freshwater rotifers is so unusual that they even intrigued the famous English palaeontologist (and parish doctor and surgeon) Gideon Algernon Mantell (see Mantell, 1846), renowned for his discovery of the ichthyosaur, *Iguanodon*, and whose research was important in framing the debate on dinosaur evolution (Torrens, 2012). In a footnote to Chap-

ter XII in his 1846 monograph, *Thoughts on Animalcules; or, a Glimpse of the Invisible World Revealed by the Microscope*, Mantell writes, “Having seen the figures of the *Stephanoceros* in the splendid work of M. Ehrenberg, I was desirous of obtaining living specimens for examination. . .” He goes on to describe the animal: “The *Stephanoceros* appears under the microscope like a cylindrical vase, as clear as crystal, surmounted by a crown formed by the convergence of the points of five long arms, or tentacula, each fringed with about fifteen rows of delicate, short, verticillate cilia, which are in constant oscillation.” Such a depiction only hints at the unusual morphology of these species and flies in the face of conventional textbook descriptions of rotifers. In fact, species of *Stephanoceros* and many other rotifers of the superorder Gnesiotrocha appear so divergent that if it were not for the presence of the jaw-like trophi, one might question their affinities to the Rotifera: many

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gnesirotrochans are sessile, live within a secreted gelatinous tube, lack a corona, and produce larvae.

The Gnesiotrocha is a largely ill-defined clade (see de Beauchamp, 1965) within the Monogononta, which includes rotifers with only a single ovary, such as those in the more familiar clade Ploima (Wallace et al., 2015). The Gnesiotrocha contains a diversity of unusual species including solitary, colonial, and sessile forms distributed in two orders, Collotheceae and Flosculariaceae (Wallace et al., 2015). Unique to collotheid rotifers, all of which are sessile on submerged vegetation, is the infundibulum, a cup- or bowl-shaped head that develops precociously within the larval body prior to settlement. After settlement, the infundibulum emerges from the head and replaces the larval corona (Kutikova, 1995), to function largely as a passive trap for bacterio- and phytoplankton, although small ciliates are also captured (pers. obs.). Species of *Stephanoceros* differ from most other collotheids, as noted above, by the presence of five elongate tentacles that project off the infundibulum. The tentacles bear a series of stiff and elongate cilia called setae, the arrangement of which differs between the species and likely plays a role in prey capture (Koste, 1978; Meksuwan et al., 2013). Despite this unusual morphology, their is limited information on their internal anatomy outside of the earliest taxonomic descriptions (e.g., Vallentin, 1890; Montgomery, 1903; de Beauchamp, 1965). A recent exception is the study of the serotonergic nervous system of the larva and adult of *S. fimbriatus*, which revealed a relatively conservative organization (Hochberg and Hochberg, 2015). Herein, we extend these studies on the anatomy and development of *S. fimbriatus* through an examination of the species' musculature.

The muscular systems of rotifers have been explored in detail since their first descriptions in the 18th century, largely because of their transparent body walls. Based on some of the earliest works summarized by Hyman (1951), the rotifer muscular system consists mostly of small groupings of circular and longitudinal fibers that extend between regions of the integument (e.g., foot and trunk, trunk and corona) or the integument and viscera. Most muscles appear to function in locomotion and feeding, which are intimately linked behaviors because muscles insert on the ciliary rootlets of the corona, thereby controlling direction of movement and the creation of feeding currents (Clément and Wurdak, 1991). Thus far, the musculature of numerous species from across the phylum have been examined in detail and have provided valuable insights into feeding, locomotion, and phylogeny in species of Seisonidae (Leasi et al., 2012), Bdelloidea (Hochberg and Litvaitis, 2000; Leasi and Ricci, 2010), and Monogononta (Kutikova et al., 2001, 2004, 2006, 2016; Sørensen et al., 2003; Sørensen, 2005a,b; Santo et al., 2005; Hochberg and Ablak Gurbuz, 2007, 2008; Riemann et al., 2008, 2009; Hochberg and Lilley, 2010; Hochberg et al., 2010; Leasi et al., 2010; Wilts et al., 2009, 2010a, 2010b). Within Monogononta, the gnesiotrochan rotifers have received comparatively little attention and include only four studies on solitary planktonic species (*Testudinella patina*, Sørensen, 2005b; *Filinia longiseta*, Hochberg and Ablak Gurbuz, 2007; *Hexarthra mira*, Santo et al., 2005; *Hexarthra* sp., Hochberg and Ablak Gurbuz, 2008) and three studies on sessile rotifers (*Cupelopagis vorax*, Vasisht and Dawar, 1969; *Floscularia ringens*, Santo et al., 2005; *Sinantherina socialis*, Hochberg and Lilley, 2010). In total, these studies of seven gnesiotrochan rotifers revealed a wealth of muscle patterns from a clade of over 200 species (Wallace et al., 2015), suggesting that our knowledge of their muscular diversity is only in its infancy, especially considering their unusual morphologies and varied lifestyles.

Herein, we provide the first detailed reconstructions of the muscular topography of *S. fimbriatus* in the context of its life history and behavior. We note that some early observations of the species' muscular system exist (Vallentin, 1890; Montgomery, 1903; de Beauchamp, 1965), but here, we provide additional details using

high-resolution microscopy, and more specifically, examine how metamorphosis affects muscle topography in an indirectly developing rotifer.

2. Materials and methods

Rotifers were collected between May and September 2009–2014. Three regular sites of collection were frequented: Ayers Pond in Hudson, NH (USA), sampled from the shoreline of Eayers Pond Road (GPS: 42° 42'30.37"N, 71° 25'08.58"W); Flint Pond in Tyngsborough, MA (USA), sampled from the shoreline off of Kendall Road (GPS: 42° 40'30.36"N, 71° 25'32.45"W), and Mascuppic Lake Dam in Tyngsborough, MA (USA), sampled from the shoreline off of Coburn Road (GPS: 42° 40'42.34"N, 71° 24'02.29"W). *Stephanoceros fimbriatus* showed a marked substrate preference for *Utricularia macrorhiza* LeConte, and so most collections focused on these plants. All plant materials were collected by hand and were placed in a plastic buckets full of pond water from the collection site. The buckets were then transported back to the University of Massachusetts for analysis.

Small pieces of plant material were placed into small glass bowls and examined with a dissection microscope (Zeiss Stemi 2000-C) to locate adults. When adults were found, they were removed from the bowls (along with a small piece of plant material) and placed into a small glass bowl (~5 ml) with filtered (64 µm) pond water. The adults were observed for several days and monitored for egg production and the eventual release of larvae. When larvae emerged, they were removed using micropipettes to separate bowls for subsequent examination and eventual photography and fixation. Feeding by the adult specimens was also examined during these times and, in many cases, was recorded with a Sony Handycam digital camera mounted on the Zeiss Stem 2000-C or a compound microscope (Zeiss A1 with DIC).

Adult rotifers were anaesthetized using a variety of chemicals. Not all chemicals worked all the time on either larvae or adults, and many animals exposed to a single anaesthetic displayed varying degrees of relaxation. The following anaesthetics were used: 10% EtOH, 1% MgCl₂ in filtered pond water, 0.05% bupivacaine in filtered pond water, menthol crystals, or lidocaine hydrochloride powder. These were added either drop-wise or grain-wise until the animals no longer contracted when prodded gently with a 000-insect pin. Larval specimens responded best to the drop-wise addition of 0.5% phenylephedrine (Neosynephrine).

Fifteen adults were prepared for filamentous actin staining after an initial fixation in 4% paraformaldehyde in 0.1 M phosphate buffer (PB) (pH 7.3) at room temperature for 1–24 h; larval specimens (n = 15) were placed in the same fixative at room temperature for a minimum of 1–3 h. The length of fixation did not affect the quality of the staining. Larvae and adults were then rinsed in 0.1 M PB at 4 °C overnight prior to being stained in Alexa-Fluor 488-phalloidin (Molecular Probes, Eugene, OR) for a minimum of one hour. Specimens were then rinsed in PBT (0.2% Triton X-100 in PB) for a minimum of one hour prior to being mounted on glass slides with Fluoromount G (Electron Microscopy Sciences, Hatfield, PA). The slides set for 48 h at –20 °C prior to viewing and imaging with an Olympus Fluoview FV300 confocal laser scanning microscope using Fluoview software.

All specimens were analyzed with an Olympus FV300 Confocal Laser Scanning Microscope at the University of Massachusetts Lowell. Confocal images were captured at 0.15–0.25 µm step intervals and processed as multi-TIF files with Fluoview software (Olympus) to generate z-stacks. The software package Velocity 5.0 (Visualization Module, PerkinElmer, 2008) was used to generate 3-D reconstructions of the TIF files and create both Quicktime (focus through) and QTVR videos. Adobe Photoshop CS5.1 was used to

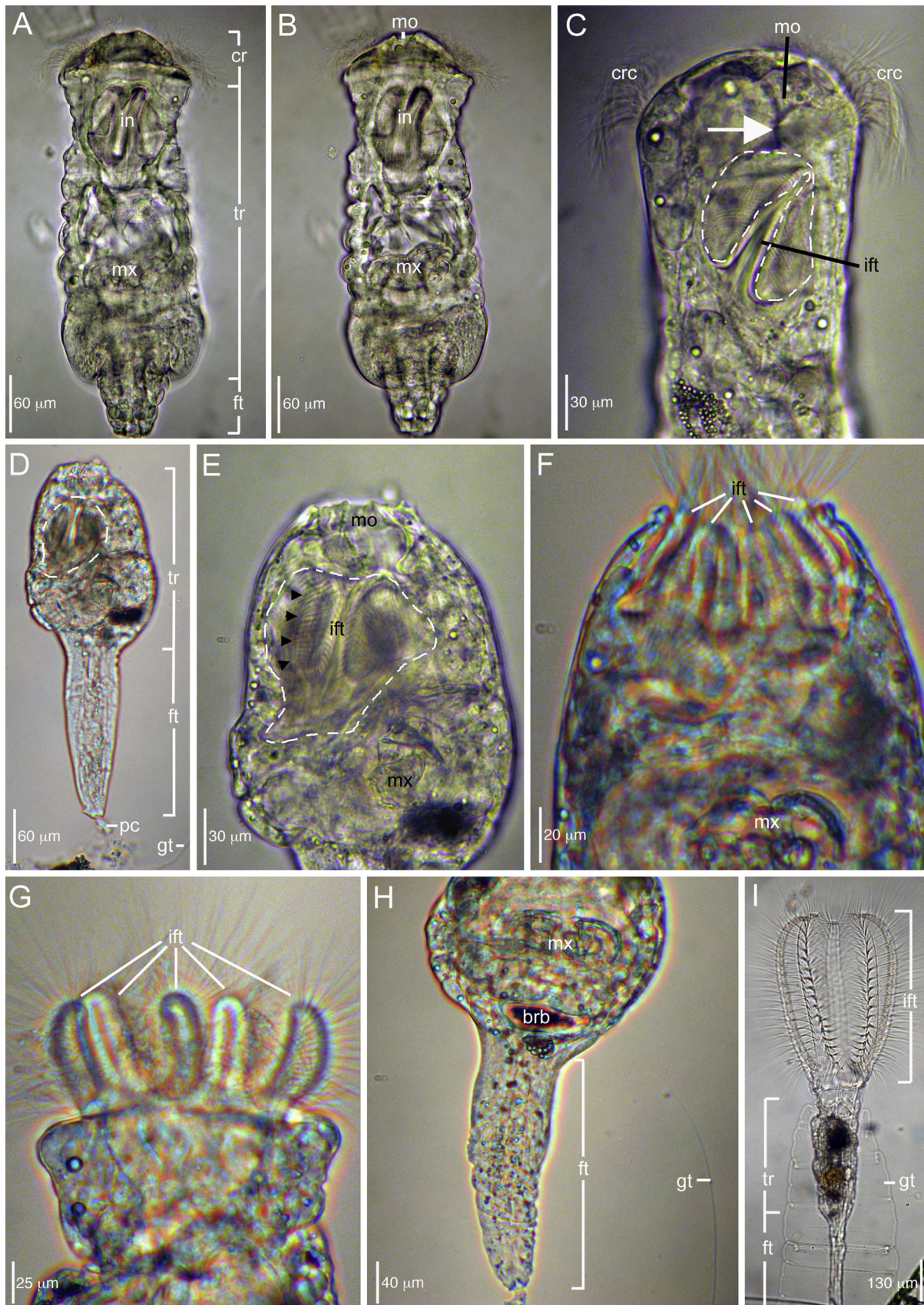


Fig. 1. Larval anatomy and metamorphosis of *Stephanoceros fimbriatus*: all images are of the same specimen except the adult. (A) Larva, dorsal view, approximately 1 h after eclosion. The corona (cr) is ciliated, the trunk (tr) contains the developing infundibulum (in) and mastax (mx), and the foot (ft) is very short. (B) Same larva as A but with ventral focus to reveal the apparent mouth (mo). (C) Closeup of larva, slightly lateral view, showing the coronal cilia (crc) and infundibulum. The infundibular cavity is outlined to show a focus on a tentacle (ift) and its setae. Note the presence of a potential foregut canal (arrow) between the mouth and infundibulum. (D) Larva in quiescent stage #1, with corona partially retracted. A gel tube (gt) has been secreted by the pedal glands, and pedal cilia (pc) can be observed protruding from the base of the foot.

crop and label all images. No alterations to the original images were made apart from conversion to greyscale. Original confocal files are available from the second author by request.

3. Results

3.1. Larval metamorphosis

Metamorphosis in *S. fimbriatus* has been documented previously (see Kutikova, 1995), but here, we provide additional observations that clarify the events surrounding the development of the infundibulum. We recorded the complete metamorphosis of a single larva of *S. fimbriatus* (Fig. 1A–I), although several specimens were documented at least partially. The time from hatching to settlement and complete metamorphosis was 1.5 h (minimum) to 4 h (maximum). Upon eclosion, larvae leave the protection of their mother's gel tube and swim for a period of time using their coronal cilia, occasionally pausing to touch a surface (e.g., plant, glass) with their corona. The corona appears as a ring of cilia at the anterior end, though its precise pattern was not documented. A small cavity on the ventral side at the anterior end is presumed to be the larval mouth and could only be visualized with DIC microscopy (Fig. 1C). The infundibulum was noted after ca. 30 mins (Fig. 1A). It appeared as an elongate cavity on the ventral side of the body below the corona (Fig. 1A–C). All five tentacles and their tentacular setae could be observed; the setae were observed to move while in the cavity. Eventually, a larva would stop swimming and attach itself to a surface (e.g., plant material, glass bowl, or glass slide) by way of a secretion from the terminal end of the foot (Fig. 1D). After attachment, the larva contracted and withdrew its corona into the body (Fig. 1D). The larva remained contracted and mostly quiescent for a period of at least 15 mins (=1st quiescent period). After this, the larva began to extend and contract rhythmically and the body swung gently from side to side; the protective gelatinous tube formed during this time (Fig. 1D,H), apparently being secreted from the pedal glands in the foot (see also Montgomery, 1903; de Beauchamp, 1965). Once a partial tube was constructed, the larva would withdraw into the tube and remain there in a contracted state for >50 mins. While this period was mostly a quiescent stage (=2nd quiescent stage), the larva would occasionally relax and extend, and during these times, the body was noticeably different (thinner foot, more robust trunk; Fig. 1D).

The second period of quiescence ended with the larva extending out of its tube. At this stage, the infundibulum could be seen just below the larval corona (Figs. 1 F,E and 2 D). The setae and the very tips of the infundibular tentacles were observed to emerge from what appeared to be the larval mouth (compare Fig. 1C, E, and F). The larval mouth appeared to shift and/or expand dorsally to allow for the complete emergence of the infundibulum. Eventually, the tentacles completely emerged from the mouth, and at this stage, the entire bowl-shaped infundibulum could be visualized at this stage (Fig. 1G). The tentacular setae remained relatively stationary during emergence. Once the process was complete, the animal could feed: setae occasionally flickered, but otherwise prey capture appeared to be solely dependent on a prey item (algal cell, ciliate) entering into the confines of the tentacles. Most small prey items appeared to be swept into the mouth by way of cilia in the anterior foregut.

3.2. Larval musculature

The musculature was present in a mostly orthogonal grid (Fig. 2 A–C). Circular muscles are described based on their structure (complete, incomplete) and their position relative to the anterior end (numbered consecutively from anterior to posterior). The longitudinal muscles are described based on their position in the body (ventral, ventrolateral, dorsal, dorsolateral) and whether or not they are complete (extend >50% of the body) or segmental (extend <50% body length). Several longitudinal muscles appeared as muscle blocks that consist of more than one muscle fiber. We noted that many such muscle blocks appeared to branch during their course through the body. The internal branches were short and often partially hidden by the larger external branches. These larger branches are described using alphanumeric subscripts. The first subscript denotes the position of the primary bifurcation relative to the center of the body (e.g., muscle A bifurcates into both medial (muscle A_m) and lateral (muscle A_l) branches); a second subscript denotes any secondary bifurcations anterior of the primary branch (e.g., muscle A_m bifurcates into muscle A_{m1} and muscle A_{m2}; the subscripts 1 and 2 denote positional information as well: 1 is medial, 2 is lateral).

Splanchnic muscles were present in circular, transverse, and longitudinal orientations. There were a total of 12 somatic circular muscles from corona to trunk (cm1–12, Figs. 2 C and 4). Most circular muscles were robust in appearance and appeared to consist of more than one muscle fiber, with the exception of cm1, which was extremely thin (~1 µm; other cms ~2–4 µm wide). Circular muscles 1–4 and 6 were all complete circular muscles (Figs. 2 C and 4); cm5 was incomplete on the dorsal side of the larva; and cms 7–12 were ventrally incomplete (Figs. 2 C and 4). On the ventral side of the larval head, cm2 appeared to bend posteriorly around the larval mouth and come into contact with cm3 (Fig. 1A,D).

Most somatic longitudinal muscles were present as blocks of multiple muscle fibers, but the proximity of the fibers to one another made distinguishing their numbers difficult. For the purposes of this description, muscles are described from posterior to anterior; most longitudinal muscle origins were in the posterior half of the body and their contractions, based on behavioral observations, led to postural changes in the anterior body half where muscles are inserted.

Four pairs of somatic longitudinal muscles originated in the foot region: two ventral muscles (vm), two ventrolateral muscles (vl), two dorsolateral muscles (dl), and two dorsal muscles (dm) (Figs. 2 and 4). The ventral muscles were thicker at the base of the foot and thinned out considerably as they extended toward the anterior end (Fig. 2A). At approximately 50% body length, the vm divided into a medial branch (vm_m) and lateral branch (vm_l). Approximately 20 µm anterior of the first division, the medial branch further subdivided into a medial branch (vm_{m1}) and lateral branch (vm_{m2}). The medial branch inserted on or very close to cm3 around the larval mouth (see Figs. 2 D and 4 B). The lateral branch inserted on the ventrolateral-most aspect of the infundibular muscle (im, Figs. 2 A,E and 4 B). Vm_l bent toward the lateral body wall and branched into a medial muscle (vm_{l1}) that inserted deep within the body (see Figs. 2 D and 4 B) and a lateral muscle (vm_{l2}) that inserted in the corona (Fig. 2D,E). The ventrolateral muscles (vl) were close to the ventral muscles in the foot region and often appeared indistinguishable from them; at approximately

The infundibular cavity is outlined. (E) Specimen in quiescent stage #2. Closeup of the anterior end showing the terminal position of the mouth and presence of mobile setae (arrowheads) on the tentacles within the infundibular cavity (dashed outline). (F–G) Several minutes later, with a focus on the emergence of the tentacles (ift). (H) Focus on posterior end displaying the gel tube outline (gt), elongating foot (ft), and the presence of a birefringent crystal (potential food source: Wallace, 1993) in the developing gut. (I) Adult specimen, approximately four days old.

Source: Wallace, 1993) in the developing gut. (I) Adult specimen, approximately four days old.

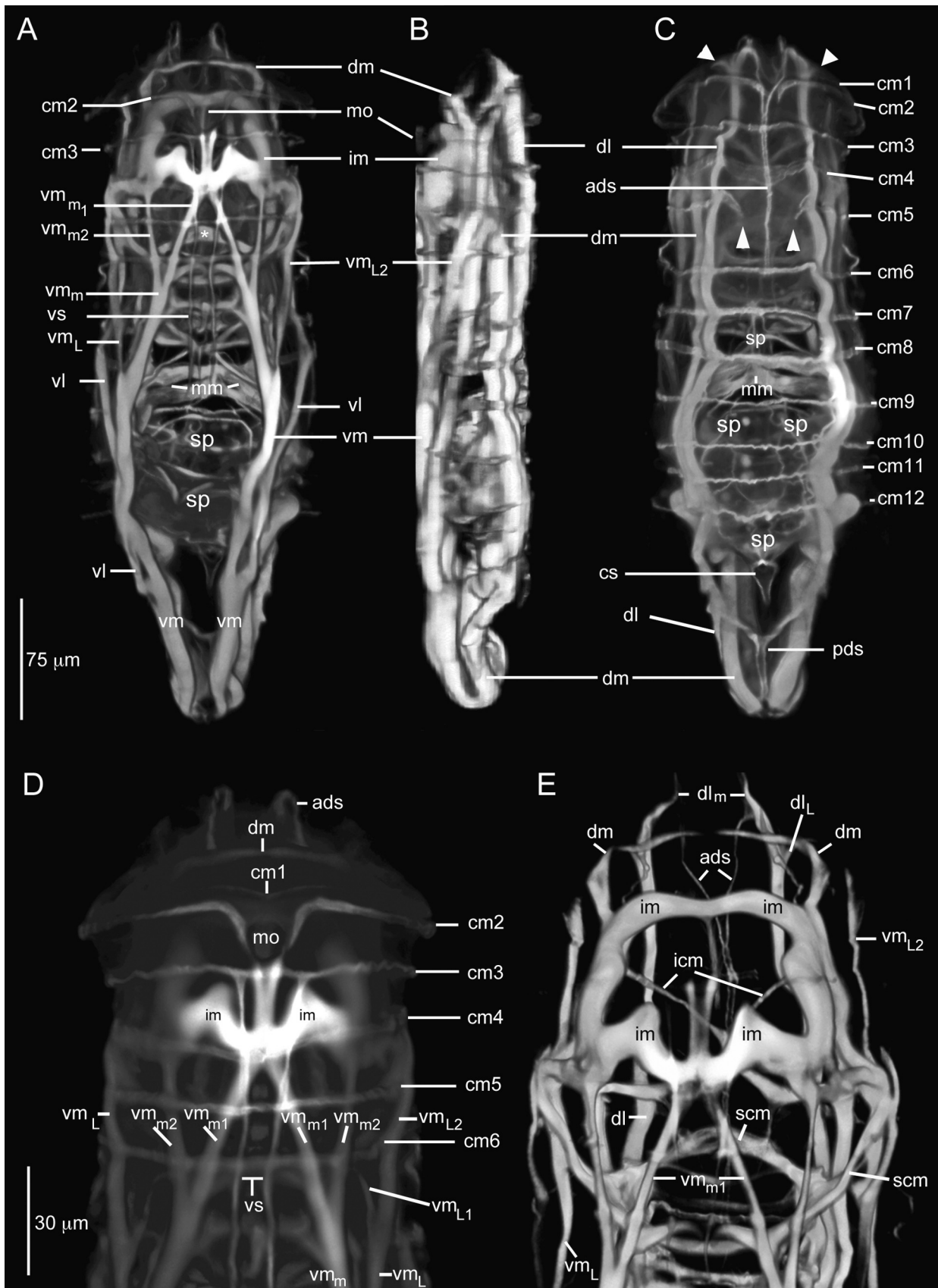


Fig. 2. Velocity-rendered Z-projections of the muscles in the larva of *Stephanoceros fimbriatus*, specimen approximately 1 h after eclosion. (A) Dorsal view. (B) Lateral view. (C) Ventral view. (D) Closeup of anterior end with a focus on the ventrum, revealing a portion of the infundibular muscle (im). (E) The anterior end with a focus through the somatic circular muscles to reveal the trapezoidal shape of the infundibular muscle. Abbreviations: ads, anterior dorsal segmental muscle; cm1–12, somatic circular muscles from anterior to posterior; cs, cloacal sphincter; dl, dorsolateral longitudinal muscle; dl_L, lateral branch of the dl; dl_m, medial branch of the dl; dm, dorsal longitudinal muscle; icm, dorsal cross-over muscles of the infundibulum; im, infundibular muscle; mm, mastax musculature; mo, approximate position of mouth;

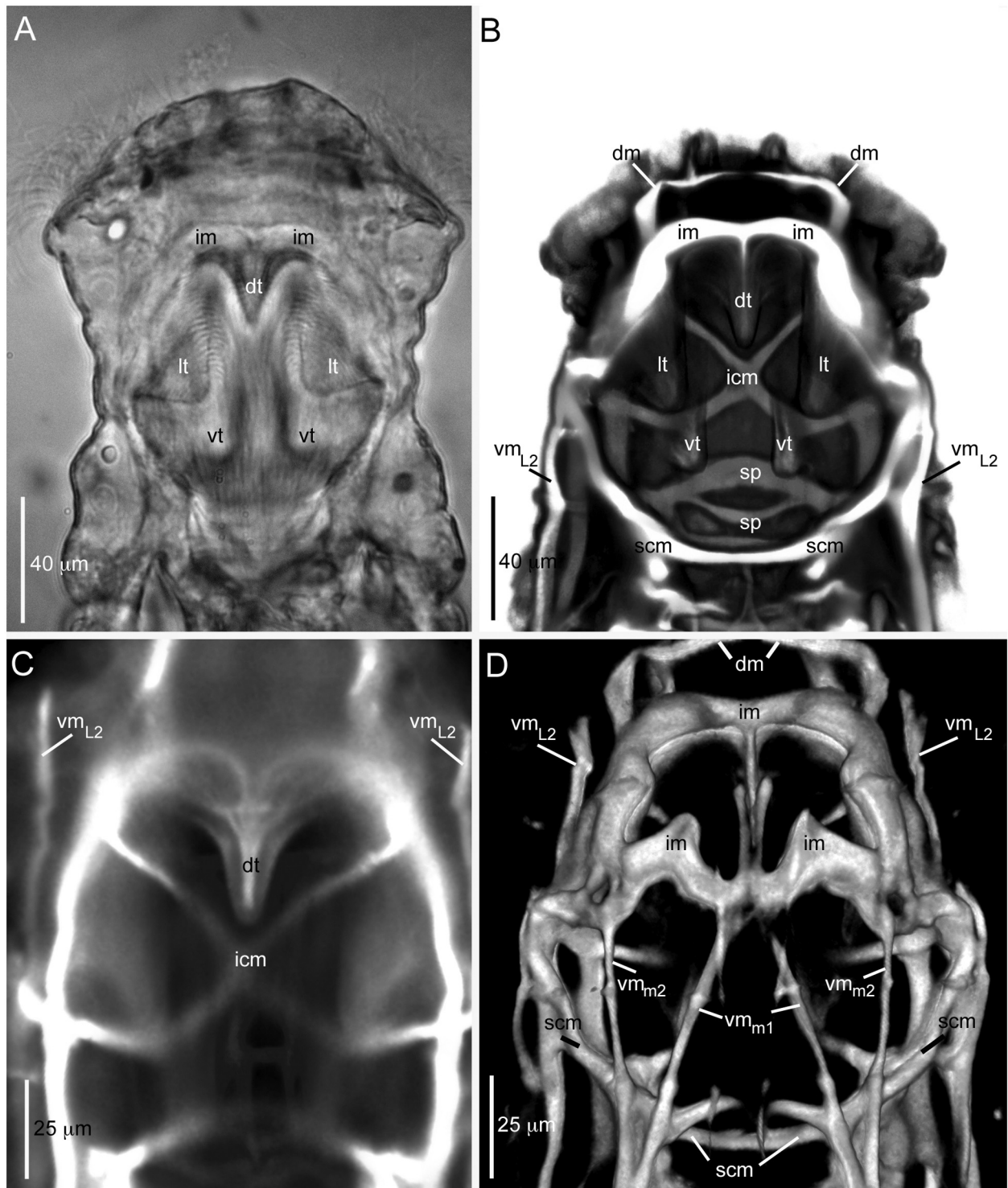


Fig. 3. Anterior end of a 1 h old larva of *Stephanoceros fimbriatus*. (A) DIC photograph of a fixed specimen revealing each of the five developing infundibular tentacles and the infundibular muscle (im). (B) Velocity-rendered z-projection of the musculature, medial view to reveal the developing infundibular tentacles. Note the presence of a light fluorescence signal at the base (anterior end) of the dorsal tentacle (dt), which indicates early development of the muscular network within the body of the tentacle. (C) Confocal z-projection of the infundibular region, with a focus on the dorsal tentacle and the cross-over muscle (icm) on the dorsal side of the infundibulum. (D) Velocity-rendered z-projection with a focus on the infundibular muscle and the surrounding somatic and splanchnic components. Abbreviations: dm, dorsal longitudinal muscle; dt, dorsal tentacle; icm, dorsal cross-over muscles of the infundibulum; im, infundibular muscle; lt, lateral tentacles; scm, semicircular muscle of the infundibulum; sp, splanchnic muscles; vm, ventral longitudinal muscle; vm_L, first lateral branch of the vm; vm_{L#}, subsequent branches of the vm_L; vm_m, first medial branch of the vm; vm_{m#}, subsequent branches of the vm; vs, ventral segmental muscle; vt, ventral tentacles.

pds, posterior dorsal segmental muscle; sp, splanchnic musculature; vl, ventrolateral longitudinal muscle; vm, ventral longitudinal muscle; vm_L, first lateral branch of the vm; vm_{L#}, subsequent branches of the vm_L; vm_m, first medial branch of the vm; vm_{m#}, subsequent branches of the vm; vs, ventral segmental muscle.

20% body length, the vl bent laterally and extended along the lateral body wall, eventually bending medially to insert at the base of the infundibular muscle (Fig. 4B).

A single pair of segmental muscles (vs) was present on the ventral side. The muscles originated around mid-body length and extended anteriorly toward the corona, eventually inserting on or very close to cm3 (Figs. 2 and 4 B).

The dorsal longitudinal muscles (dm) had very thick origins at the base of the foot and extended anteriorly along the sides of the body (Figs. 2 C and 4 A). They took a deep position in the body, often beneath the dorsolateral longitudinal muscles, making their course difficult to follow in some specimens. At the anterior end, each dm bent medially and ventrally, ultimately inserting close to the midline just anterior of the larval mouth (Figs. 2 and 4): the insertions of each muscle were sufficiently close enough to each other to appear as a single transverse unit (Figs. 2 A,D and 4). The dm also sent a small branch to the infundibular muscle, which was ventral to the dm (not shown). The dorsolateral longitudinal muscles (dl) originated close to the base of the foot on the dorsolateral body wall, just anterior of the dorsal longitudinal muscles (Figs. 2 C and 4). The dl extended anteriorly along the lateral margin of the body, prior to bending medially toward the corona. In the coronal region, the dl bifurcated into a short ventral branch (dl_v) that inserted on the underlying dorsal longitudinal muscle (not shown); the medial branch (dl_m) extended anteriorly and appeared to insert terminally on the head (Figs. 3 B and 4).

Two pairs of dorsal segmental muscles were present. The posterior pair, pds, originated close to the midline of the foot (Figs. 2 C and 4). The muscles extended forward approximately 20–30 μ m and then bent toward the lateral body wall. The muscles remained in close contact with the dorsal and dorsolateral muscles and could not be followed to their site of insertion, which was a position in the anterior half of the body. The anterior pair, ads, originated close to 50% body length, but whether they originated from the dorsal body wall or somewhere deeper in the body could not be determined. Both muscles remained in close contact with each other along the dorsal midline until the coronal region, where each bent laterally, forming a Y-shape at the anterior end (Figs. 2 C and 4 A). Each muscle extended to the tip (overlapping the dl) and then arced ventrally, eventually extending back toward the infundibulum on the ventral side of the body where they inserted (Fig. 4B).

The splanchnic component was extremely complex and difficult to identify with accuracy. There appeared to be four distinct sets of splanchnic muscles: (1) a splanchnic component of the posterior gut region, which had very thin muscles posterior of the mastax; (2) the mastax musculature; (3) a series of thicker muscles in unusual orientations anterior of the mastax; and (4) the infundibulum musculature, which consisted of a series of complex interconnected muscles (im, icm, scm) separate from the anterior splanchnic component (Figs. 2–4).

The splanchnic musculature of the digestive tract consisted of a meshwork of thin and thick circular and longitudinal fibers. Several of the muscles followed circuitous routes and did not appear to adhere to a strictly circular or longitudinal orientation. Several of the muscles anterior of the mastax were robust in appearance (Figs. 2 A, 3 B and 4), while those posterior of the mastax were thin and delicate (Figs. 2 A,C and 4). A tear-shaped cloacal sphincter muscle was present at the terminus of the intestine (Figs. 2 C and 4 A). The mastax musculature was present around mid-body length on the dorsal side (Figs. 2 C and 4 A). The mastax musculature consisted of numerous small fibers that encircled the uncinat trophi.

The musculature of the infundibulum consisted of a series of muscles that were interconnected and mostly separate from the remaining splanchnic component. The most prominent muscle was the infundibular muscle (im). This muscle surrounded the base of the developing infundibulum, which in the larval stage

is an inverted cavity containing tentacles that are posteriorly directed (Figs. 2–4). This muscle appeared to be a thick, trapezoidal-shaped block of several thin muscle fibers that received insertions from longitudinal muscles posterior and dorsal of the developing infundibulum (described above). The musculature of the im contributes to the structure of all five tentacles, though this is not easily visualized due to a weak fluorescence signal (dt, lt, vt; Fig. 3C). The entire im was often tilted so that its posterior border was against the ventral body wall and its anterior border was bent towards the terminal end (Fig. 3B). A large semicircular muscle (scm) extended from the anteroventral borders of the im (Fig. 3B); we could not determine if the im was the origin of the muscle, or if the scm was an extension of another muscles (perhaps the icm). A pair of robust strap-shaped muscles (icm) was present on the dorsal side of the im; these muscles crossed at the midline on the dorsal side (Figs. 3 B,C and 4 A) and looped around the dorsolateral muscle were it inserted on the im.

3.3. Adult musculature

The muscle patterns of the adult females were similar to those of the larva. Both the circular muscles and the longitudinal muscles maintained their general morphology and position, despite the elongation of the foot and the eversion of the infundibulum. However, muscle patterns were not identical, and in some cases, we assumed ontogenetic homology between larva and adult by relying on similarities in muscle origins and/or insertions instead of muscle position only, since body shape changed dramatically after metamorphosis (compare Figs. 4 and 6). Below, we briefly describe the adult muscle plan, with a focus on the anterior end.

Twelve circular muscles were present in the adult trunk (Fig. 5). In many cases, the circular muscles appeared to consist of more than one muscle fiber (not shown). Circular muscles 1–6 (cm1–6) were complete and cms7–12 were ventrally incomplete. Despite the elongation of the body, the mastax maintained its relative position around cm9, similar to the larva. There were no circular muscles in the foot region.

The major somatic longitudinal muscles consisted of a pair of ventral, ventrolateral, dorsal, and dorsolateral muscle blocks, each consisting of multiple fibers, and some of which branched in a similar pattern to those of the larva. All muscles originated in the foot and maintained close contact with each other until the trunk, where they spread out and often followed the contour of the body. Importantly, the position of the anterior insertions of some longitudinal muscles changed relative to what was observed in the larva because of the eversion of the infundibulum during metamorphosis. After metamorphosis, the infundibular muscle (im) was positioned perpendicular to the anterior-posterior axis (Fig. 5), whereas in the larva, the im was mostly parallel to the a-p axis (see Fig. 2A,B). The ventral and ventrolateral longitudinal muscles (vm_{m1}, vm_{m2}, vl) maintained their insertions on the im: vm_{m1} inserted at the midline on the im (in between the two ventral tentacles), while vm_{m2} inserted on the im at the base of each ventral tentacle (Figs. 5 and 6). Curiously, vm_{L1} and vm_{L2} were not observed or could not be distinguished from the underlying splanchnic muscles. The dorsal longitudinal muscles (dm) followed the contour of the body and appeared to insert deep in the anterior third of the body, just posterior of the im (Fig. 6; as opposed to anterior of the im in the larva, see Fig. 4). The dorsolateral muscles (dl) appeared to insert on cm1; these muscles may branch prior to cm1, but this could not be confirmed in all specimens.

The segmental muscles of the larva (vs, ads, pds) all appeared to be present in the adult, but their insertions could not always be determined with accuracy since all muscles were displaced due to the extreme width of the adult trunk. It was also difficult to distinguish these muscles from the underlying splanchnic component.

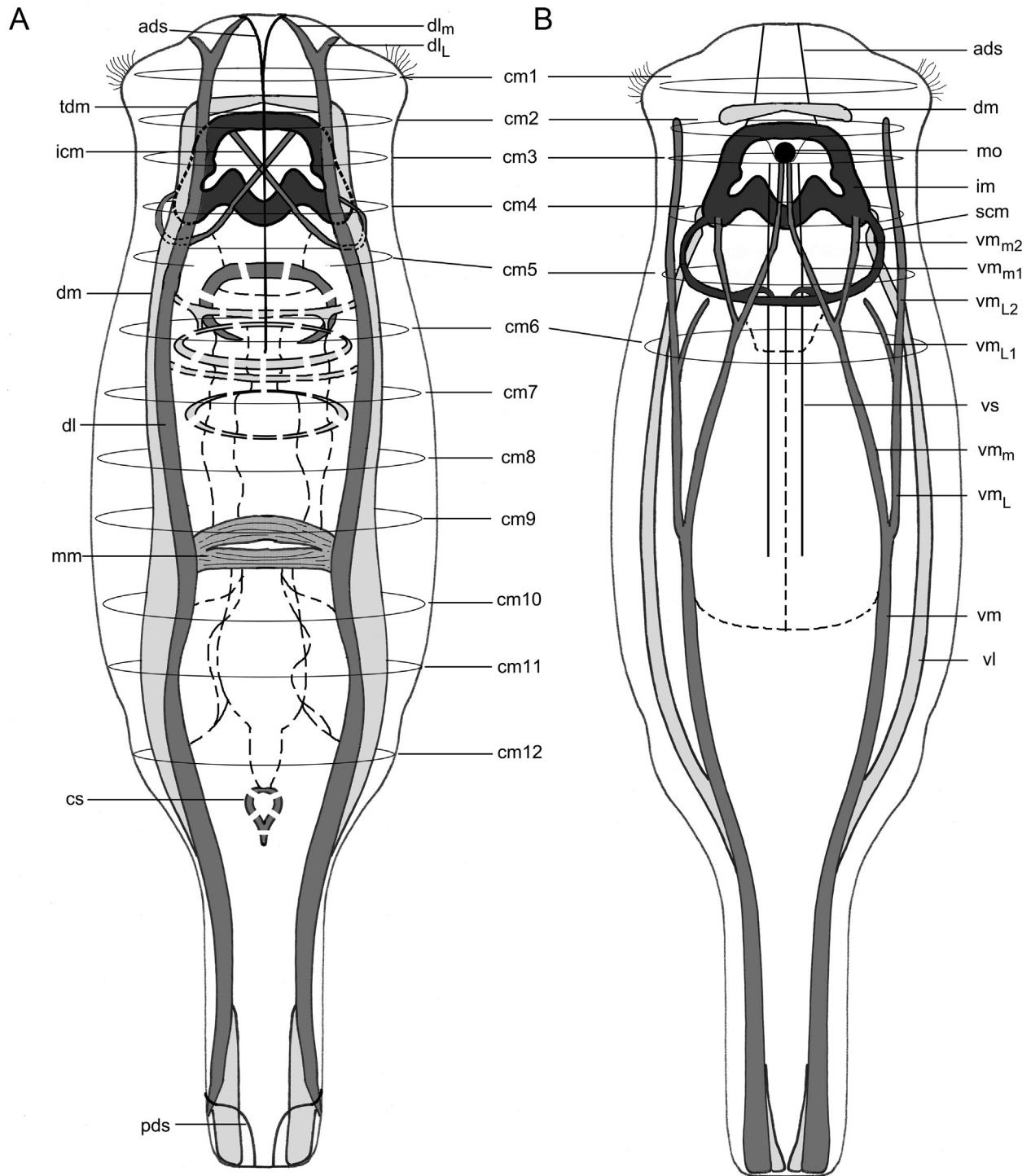


Fig. 4. Schematics of the muscle patterns in the larva of *Stephanoceros fimbriatus*. Splanchnic muscles not directly affiliated with the infundibulum are represented by dashed lines; not all splanchnic muscles are shown. Potentially homologous muscles of the larva and adult (Fig. 6) have identical grey scale coding. (A) Muscles on the dorsal and dorsolateral sides of the body. (B) Muscles on the ventral and ventrolateral sides of the body. Abbreviations: ads, anterior dorsal segmental muscle; cm1–12, somatic circular muscles from anterior to posterior; cs, cloacal sphincter; dl, dorsolateral longitudinal muscle; dl_L, lateral branch of the dl; dl_m, medial branch of the dl; dm, dorsal longitudinal muscle; icm, dorsal cross-over muscles of the infundibulum; im, infundibular muscle; mm, mastax musculature; mo, mouth; pds, posterior dorsal segmental muscle; scm, semicircular muscles of the infundibulum; sp, splanchnic muscles; vl, ventrolateral longitudinal muscle; vm, ventral longitudinal muscle; vm_L, first lateral branch of the vm; vm_{L#}, subsequent branches of the vm_L; vm_m, first medial branch of the vm; vm_{m#}, subsequent branches of the vm; vs, ventral segmental muscle.

One muscle pair in the adults, presumably the ventral segmental muscles (vs) inserted close to cm3, which was similar to the condition of some muscles (vs) observed in the larva (Fig. 4). Neither the ads or pds could be followed along their course.

The splanchnic musculature was extremely complex in the adult and appeared to be of a similar orientation as the larva; however, many adults also carried developing eggs that also stained with phalloidin, making it difficult to distinguish developing larval muscles from the adult splanchnic component.

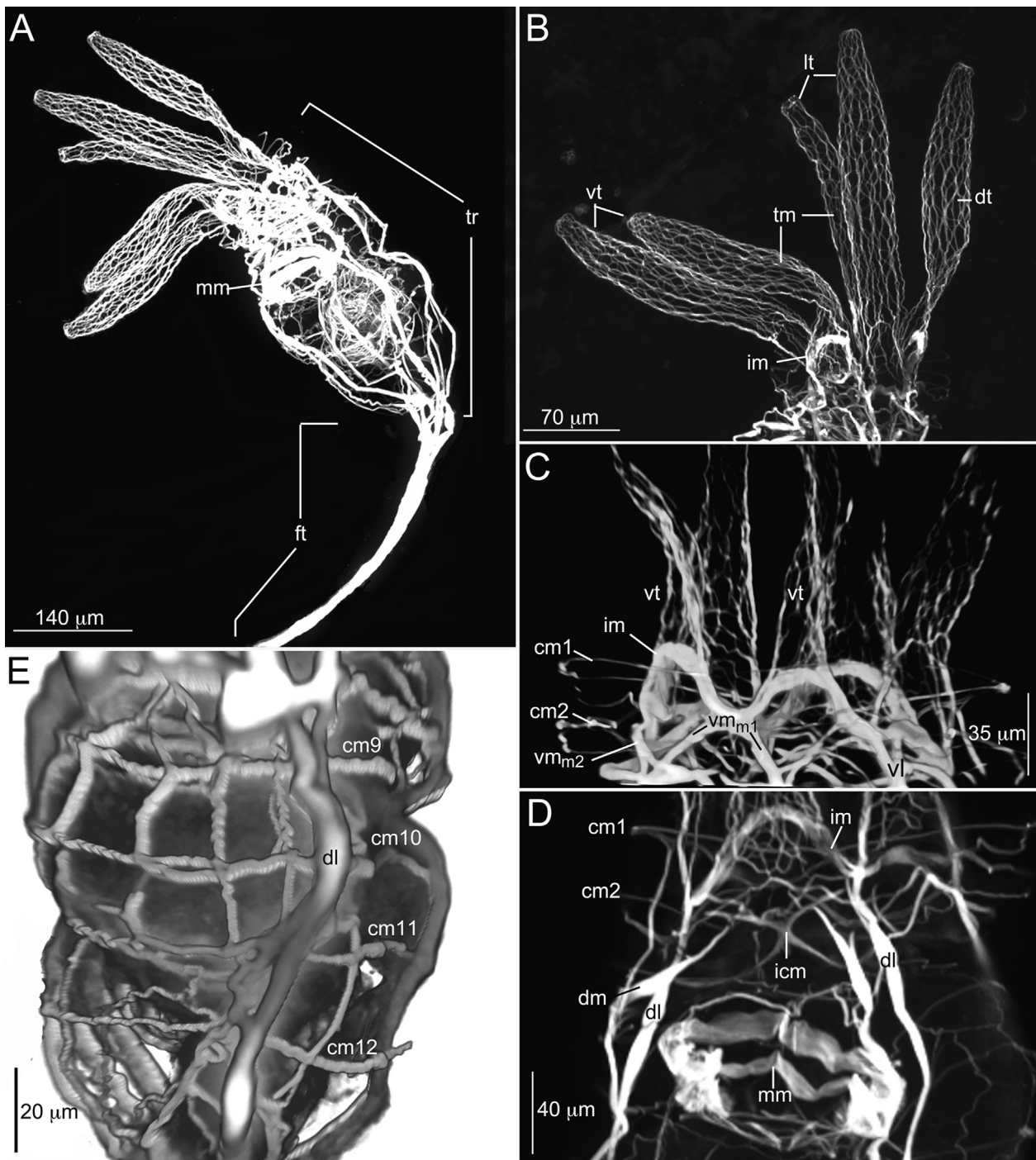


Fig. 5. Z-projections of the musculature of the adult female of *Stephanoceros fimbriatus*. (A) Confocal z-projection of an adult, approximately 5 days post metamorphosis. The two major body regions, foot (ft) and trunk (tr), correspond to those in Fig. 11. The mastax musculature (mm) has a very strong fluorescent signal. (B) Confocal z-projection of the infundibular tentacles to show the network of muscles (tm) that arise from the infundibular muscle (im). All five tentacles are in view: the single dorsal tentacle (dt), paired lateral tentacles (lt) and paired ventral tentacles (vt). (C) Velocity-rendered z-projection of the infundibular muscle and the network of tentacular muscles that appear to arise from the im. (D) Confocal z-projection of the mastax musculature (mm) and some somatic and splanchnic components in the anterior trunk. (E) Velocity-rendered z-projection of the muscles in the lower third of the dorsal trunk. Abbreviations: cm1–12, somatic circular muscles from anterior to posterior; dl, dorsolateral longitudinal muscle; ft, foot; icm, dorsal cross-over muscles of the infundibulum; im, infundibular muscle; mm, mastax musculature; tm, tentacular muscles; tr, trunk; vl, ventrolateral longitudinal muscle; vm, ventral longitudinal muscle; vm_{m1}, first medial branch of the vm; vm_{m#}, subsequent branches of the vm.

The infundibular muscle was the most prominent muscle at the anterior end, similar to what was observed in the larva (Figs. 5 A–C and 6). The im was oriented perpendicular to the long axis and appeared as a very thick circular muscle, in between cms1 and 3, depending on the state of contraction of the adult. The im had an undulating outline, forming both concave (posterior) dips and con-

vex (anterior) ridges (relative to the long axis). The concave dips in the im appeared to correspond to where the somatic longitudinal muscles inserted on the im. The convex ridges appeared to correspond to where the musculature of the infundibular tentacles arose. Most of the musculature of the tentacles originated in the im, but some thin muscle fibers may also have origins in the somatic lon-

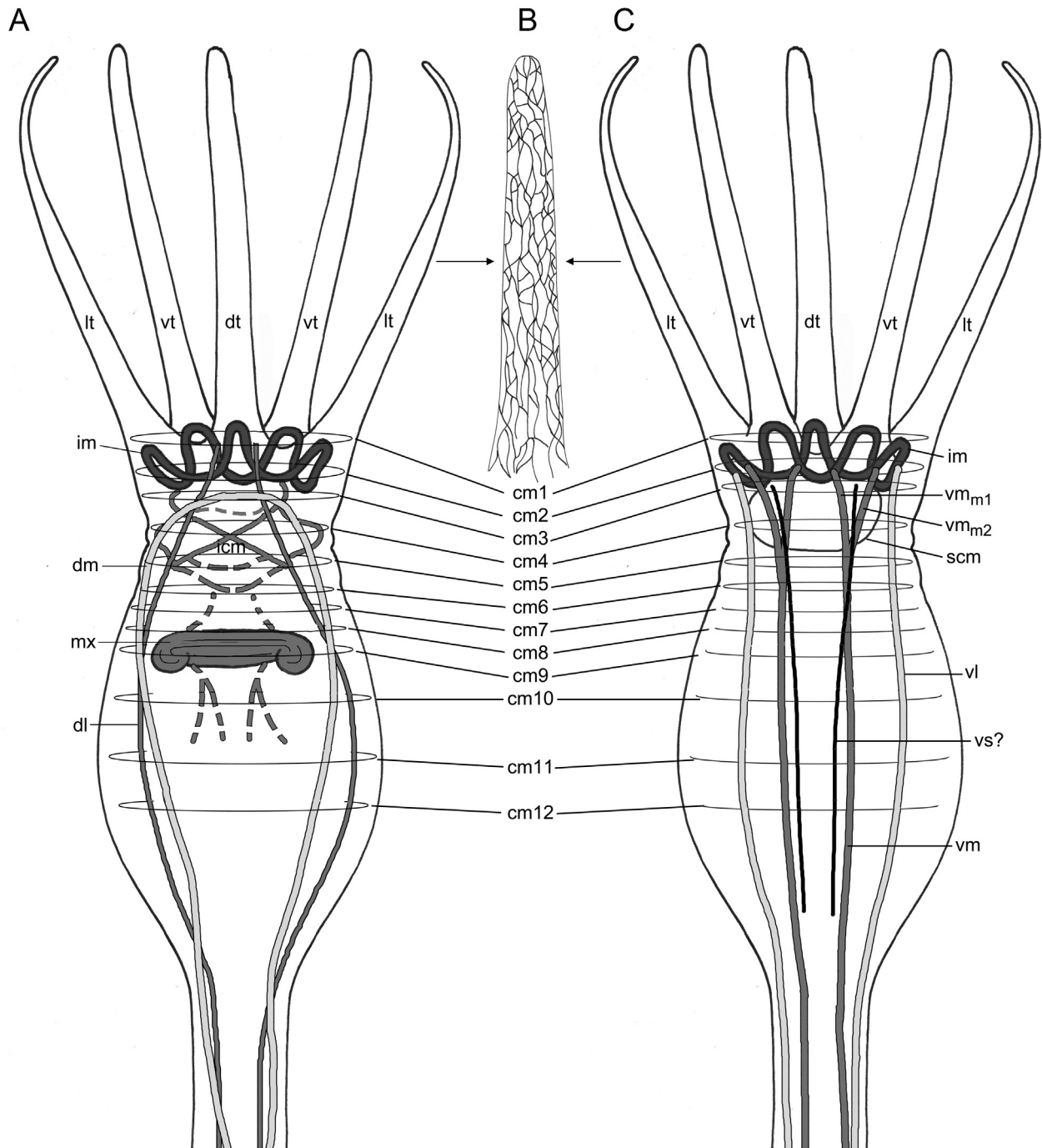


Fig. 6. Schematic of the muscle patterns in the adult female *Stephanoceros fimbriatus*. Splanchnic muscles that are not directly affiliated with the infundibulum are represented by dashed lines. Potentially homologous muscles of the adult and larva (Fig. 4) have identical grey scale coding. (A) Dorsal muscles. (B) Example of the pattern of muscles in the infundibular tentacles. (C) Ventral muscles. Abbreviations: cm1–12, somatic circular muscles from anterior to posterior; dl, dorsolateral longitudinal muscle; dm, dorsal longitudinal muscle; dt, dorsal tentacle; icm, dorsal cross-over muscles of the infundibulum; im, infundibular muscle; lt, lateral tentacle; mm, mastax musculature; scm, semicircular muscles of the infundibulum; vl, ventrolateral longitudinal muscle; vm, ventral longitudinal muscle; vm_m, first medial branch of the vm; vm_{m#}, subsequent branches of the vm; vs, ventral segmental muscle; vt, ventral tentacle.

gitudinal muscles that supply the im (Figs. 5 and 6). The tentacles contained a prominent meshwork of muscle fibers that had both longitudinal and perhaps diagonal orientations: the latter orientation was difficult to determine with precision, but the muscle fibers were clearly not circular (Figs. 5 and 6 B). The patterns of muscle fibers in each tentacle were extremely similar (Fig. 6B).

Muscles that were closely associated with the im in the larva – the semicircular muscle (scm) and the strapped shaped muscles that crossed at the midline (icm) – were still present in the adult (Figs. 5 D and 6). Both sets of muscles were present posterior of the im: the scm was positioned at the ventroposterior margin of the im, and the icm was positioned at the dorsoposterior margin (Figs. 5 and 6).

4. Discussion

Sessile rotifers are largely confined to orders Collothecaceae and Flosculariaceae (superorder Gnesiotrocha), although the homology of the sessile condition within the Gnesiotrocha remains to be determined. Seemingly tied to the sessile condition is the presence of an indirect (larviparous) lifecycle, with female adults producing larvae via parthenogenesis. Larvae are mostly non-descript and vermiform, with a ciliated corona and trophi, but otherwise do not closely resemble their adult relatives. Presumably, the larva functions to disperse the species across water bodies since adults are permanently sessile and there is no free-spawning of gametes. Curiously, larval anatomy has not received a great deal of attention beyond some basic light microscopy (see de Beauchamp, 1965), all the more remarkable as the body plan changes, often dramatically, after metamorphosis (see Kutikova, 1995). This is especially true in species of Collothecaceae, where metamorphosis leads to an extreme elongation of the foot and a complete reorganization of the anterior end. By all accounts, the larval head (corona) is not homologous to the adult head (infundibulum), since the adult head does not develop from the larval corona and instead has a separate anatomical origin, most likely in the foregut (pers. observations, see also de Beauchamp, 1965). In fact, the infundibulum can be observed to develop precociously in the larval body, and upon settlement and metamorphosis, everts out the anterior end, thereby replacing the larval corona (see Fig. 1). Our observations indicate that this eversion likely takes place through a larval mouth that is terminally displaced during metamorphosis; however, these observations need to be confirmed, preferably via SEM of larvae or early metamorphosing individuals. We are also uncertain about the fate of the larval corona, as there is no indication of any obvious resorption of the corona or anatomical waste (sloughing off of the corona). The use of apoptosis markers would be beneficial in this determination.

Larval development in collothecid rotifers has been examined in several species (Kutikova, 1995), but, to date, there are few details about the precise anatomical changes that occur during metamorphosis. It is clear that the infundibulum (adult head), develops inside the foregut, and that in *S. fimbriatus*, this infundibulum bears five elongate tentacles adorned with setae (see Figs. 1–3). Previously, we revealed that despite this dramatic development, there is no significant change in the structure of the serotonergic nervous system (Hochberg and Hochberg, 2015). The number and distribution of serotonergic cells and neurites are effectively maintained from larva to adult, which is remarkable considering the radical anatomical changes that occur. We had hypothesized that since serotonin is a known modulator of ciliary activity in invertebrates (Hochberg and Hochberg, 2015), and that collothecid larvae rely on coronal cilia for swimming, that we would see changes in the serotonergic network that supplies the head. Adults do possess cilia (setae) on their tentacles, but these cilia are not obviously innervated, and unlike the coronal cilia, do not have a locomotory function.

The locomotor activity of rotifer larvae is relatively simplistic, and based on personal observations, the larvae do not have an extensive behavioral repertoire that would indicate the need for a complex musculature. Larvae glide through the water and appear to investigate substrata as potential sites of settlement. Beyond some basic twisting and bending of the body when they contact a substrate, they do not engage in any complex movements. A previous investigation of the larvae of *Acylcus inquietus*, another sessile collothecid, revealed a basic orthogonal muscle pattern (outer circular, inner longitudinal) that was mostly maintained after metamorphosis (Hochberg et al., 2010). Longitudinal muscles appear to be the major sources of bending and twisting (in both larvae and adults), and with their insertions on the larval

corona, explains their function in the documented gliding behaviors (muscles insert on the ciliated cells of the corona; Clément and Wurdak, 1991). While this latter study of *A. inquietus* did not investigate the dynamics of metamorphosis, i.e., the infundibulum was not observed inside the larval body, nor was it witnessed during eversion, it did reveal that the major changes after metamorphosis came in the form of novel muscle orientations resulting from the development of the infundibulum. *A. inquietus* lacks tentacles, and does not appear to have an infundibular muscle as in *S. fimbriatus* (current study). However, it is important to point out that the level of detail in the *Acylcus* study does not measure up to the current study of *S. fimbriatus*, and further details may likely arise with more comprehensive observations. Nevertheless, both species do share similar overall muscle patterns: 12–13 somatic circular muscles, fewer than half of which are complete, four pairs of somatic longitudinal muscles, and a complex splanchnic musculature. These observations are similar (but not identical) to some of the earliest observations of the musculature in *S. fimbriatus* using brightfield microscopy (Valentin, 1890; Montgomery, 1903). In total, the arrangement of muscles in these collothecids is broadly similar to what is known for several other rotifers, including benthic bdelloids (Hochberg and Litvaitis, 2000; Leasi and Ricci, 2010), sessile flosculariids (Santo et al., 2005), and planktonic ploimates (Kutikova et al., 2004; Sørensen et al., 2003; Sørensen, 2005a). However, it is worth noting that there are several deviations from this “common” condition, with some species lacking circular muscles (Hochberg and Ablak Gurbuz, 2008), some possessing modified circular muscles (as dorsoventral muscles; Sørensen et al., 2003; Riemann et al., 2009), some possessing mostly segmental longitudinal muscles (Sørensen et al., 2003; Riemann et al., 2009), and others possessing muscles in unique orientations (Santo et al., 2005; Hochberg and Ablak Gurbuz, 2007, 2008).

In this study, we demonstrate that the larva-to-adult transition in a collothecid rotifer is a dramatic process, but with the exception of some changes in the infundibulum, does not involve a radical reorganization of the somatic musculature. In fact, the most notable changes are confined to the development of the five infundibular tentacles, which are supplied with their own intricate musculature. The fine network of muscles in these tentacles appears to originate from within the infundibular muscle, and so may explain why such a muscle is present in *S. fimbriatus* and not present in apparently related species such as *A. inquietus*.

The compound structure of the infundibular muscle is necessary to provide a source of muscle fibers to the tentacles, even though the tentacles themselves do not appear to engage in any extraordinary movements. In fact, the tentacles of *S. fimbriatus* rarely move on their own, and are relatively stationary except for when being retracted toward the trunk (all five are pulled down, with simultaneous contraction of the trunk). The function of the tentacular muscles, therefore, seems to be in skeletal support, i.e., to maintain their constant vertical posture. The infundibular muscle, which also acts as a site of insertion for many somatic longitudinal muscles, might also function in reorientation of the larval head, bringing the larval mouth to a terminal position so the infundibulum can emerge in the correct orientation for prey capture. However, the apparent lack of such a muscle in *A. inquietus* may argue against this, as the adult head is in the proper orientation after metamorphosis (Hochberg et al., 2010).

To date, *S. fimbriatus* is the only rotifer known to possess an infundibular muscle, but we note that a second species, *S. millsii*, has a similar muscle in the same position (pers. obs.). Still, we have not observed this muscle in any other species of Collothecaceae (unpublished), and so its evolutionary origins remain a mystery. One could argue that this muscle is derived from a series of closely-set circular muscles in the ancestor, but this would not explain its contributions to the larval tentacles, which consist of mostly

longitudinal and various other fiber orientations. We expect that future studies on the phylogeny of the order will help to clarify the evolutionary origins of the tentacles and the infundibular muscle, thereby providing information on the adaptive values relative to the non-tentacular condition in other collothecid rotifers. We also expect that embryological observations will be necessary to determine the homology of the infundibulum and rotifer corona, and that ultrastructural analyses will be imperative to determine the anatomical origins (e.g., foregut) of the infundibulum itself.

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