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ROTIFERA XV

When heads are not homologous: the coronae of larval and adult collothecid rotifers (Rotifera: Monogononta: Collothecaceae)

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Abstract Rotifers are diverse and abundant aquatic micrometazoans that rely on their ciliated apical end (corona) for locomotion and feeding. In order Collothecaceae, which includes mostly sessile species, larval rotifers go through a complex metamorphosis after settlement wherein they replace their corona with an unusual cup-shaped head that functions exclusively in food capture. This new head, called the infundibulum, consists of morphological elaborations such as lobes or tentacles that develop precociously in the larval stage; in adults they function in ambush and sit-and-wait predation. Here, we provide evidence from brightfield and transmission electron microscopy that the infundibulum of collothecid rotifers is derived from the larval foregut and not the larval corona, suggesting that the adult head of collothecids is a morphological novelty and therefore not homologous with the rotifer corona as classically defined. The wide variety of morphologies of the infundibulum suggests that selection to maximize foraging may have driven the evolution of head form in these sessile species, and that future studies of collothecid rotifers should consider the infundibulum as a unique form of food-collection device that is evolutionarily distinct from the rotifer corona.

Keywords Foraging · Development · Gnesiotrocha · Morphology · Sessile

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Introduction

Most rotifers are aquatic micrometazoans that use their anterior crown of cilia, called the corona, for food collection and swimming. The corona often bears two circumapical rings of cilia—the inner trochus and outer cingulum—whose form varies widely among taxa and is thought to reflect differences in trophic mode and lifestyle, e.g., benthic, epizoic, periphytic, planktonic, semi-terrestrial, and sessile (de Beauchamp, 1907; Remane, 1933; de Beauchamp, 1965; Melone & Ricci, 1995; Melone, 1998, 2001; Wallace

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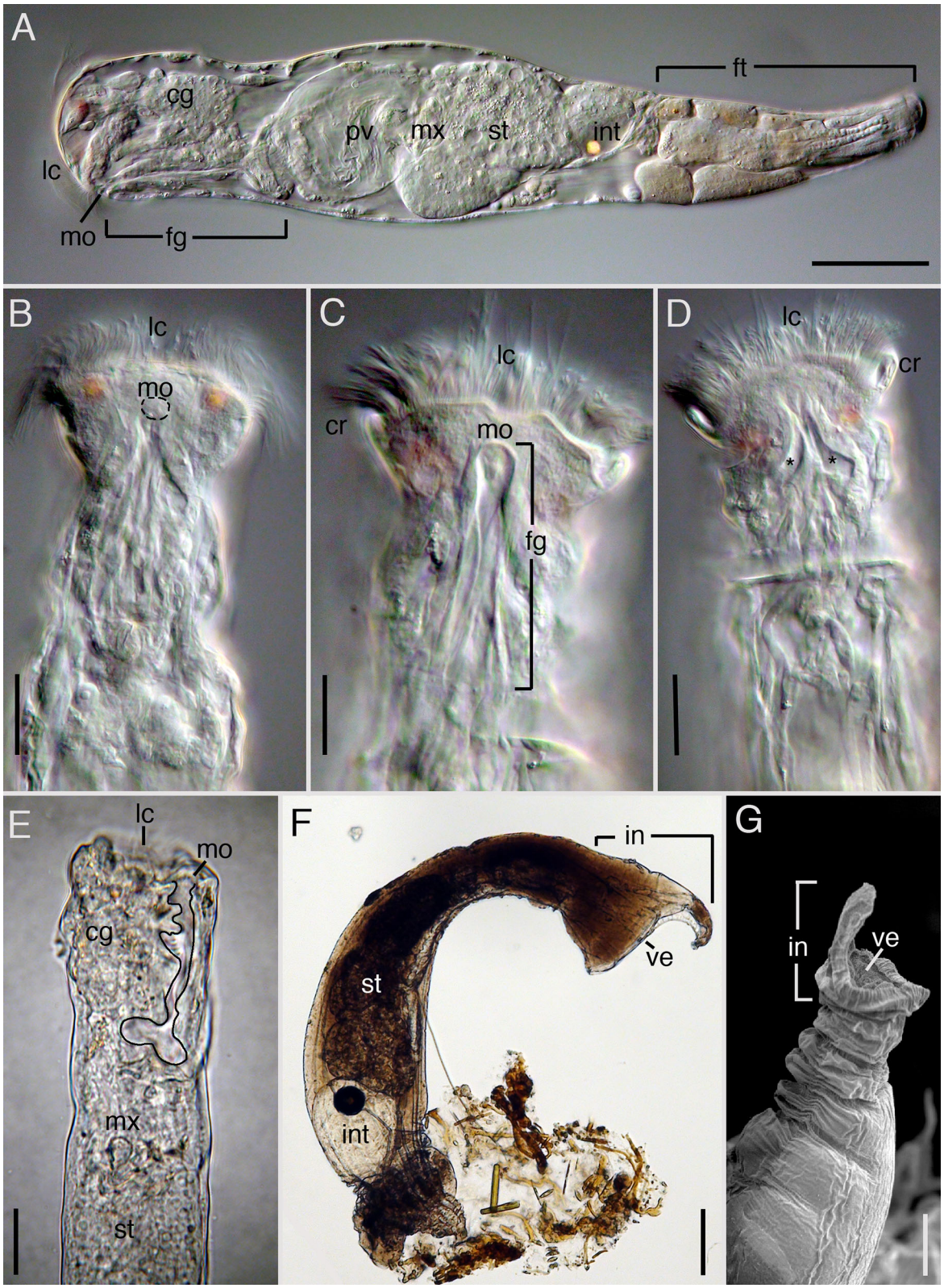
& Smith, 2013; Fontaneto & De Smet, 2015; Wallace et al., 2015). While many species of the superorder Gnesiotrocha are planktonic and highly mobile, some are strictly sessile as adults, with the only mobile stages being the larval females and short-lived males. In the order Flosculariaceae, the sessile females live on submerged plants and use their ciliated corona to collect microscopic prey such as bacteria and microeukaryotes (e.g., algae, protists) from the water. Another order, the Collothecaceae, also contains sessile females, and though taxonomic diversity is lower in this group (Segers, 2007), the morphology of their head is highly divergent and in all cases, so is their mode of foraging. Some species function as ambush predators that swallow whole animals (Vasishist & Dawar, 1969; Bevington et al., 1995), while others function as sit-and-wait predators that rely on incidental contact by smaller eukaryotes to stimulate cilia on their elaborate head, which then traps them like a Venus fly trap (Wright, 1958). This elaborate head is widely regarded as a homologue of the traditional rotifer corona, though details on its development are poorly known (Hochberg et al., 2010; Wallace et al., 2015). Importantly, phylogenetic relationships between collothecids and other rotifers have never been explored in detail (see e.g., Sørensen, 2002; Sørensen & Giribet, 2006), meaning that the evolution of the corona and its relationship to the divergent head of collothecid rotifers are also unknown.

Sessile species of both Flosculariaceae and Collothecaceae engage in indirect development, wherein eggs develop into free-swimming, non-feeding, female larvae that function in dispersal (Young et al., this volume). These larvae possess a ciliated cap that has been equated to the corona of most other rotifers and has been regarded as homologous for more than a century (Mantell, 1846; Cubitt, 1870; Wallace et al., 2015). However, only in Collothecaceae do the larvae undergo a complex metamorphic process that appears to replace the larval corona with an unusual head called the infundibulum (Kutikova, 1995; Hochberg & Hochberg, 2015, 2017). The infundibulum is morphologically elaborate and may form large lobes or elongate tentacles adorned with stiff setae (cilia) that surround an expansive mouth cavity (e.g., Wright, 1955). The functional significance of the infundibulum and its evolution remain poorly known, in part because of the limited information on its structure and development (Montgomery, 1903; Vasishist & Dawar, 1969; Kutikova, 1995; Hochberg & Hochberg, 2015, 2017). Nevertheless, the infundibulum has been hypothesized as a homolog of the standard rotifer corona despite its unique morphology and function in food collection.

Fig. 1 Light micrographs of larval and adult *Acyclus inquietus*. ▶ **A** Lateral view of a larva, dorsal is top. **B–D** Successive focal planes of the larval head during some minor contractions. **E** Lateral view of a specimen fixed for TEM showing the position of the mouth and the foregut cavity (artificially outlined) **F** Lateral view of an adult removed from a colony of *Sinantherina socialis*. **G** Lateral view of an adult view with SEM showing the hood-like infundibulum and large mouth cavity (vestibulum). *cg* cerebral ganglion, *cr* rim of larval corona, *fg* foregut region, *ft* foot, *in* infundibulum, *int* intestine, *lc* larval corona, *mo* mouth, *mx* mastax, *pv* proventriculus, *st* stomach, *ve* vestibulum. Scale bars: **A** 40 μm; **B–D** 21 μm; 20 μm; **F–G** 45 μm

Here, we provide new data on development of the infundibulum in four sessile collothecid rotifers: *Acyclus inquietus* Leidy, 1882, *Collotheca coronetta* (Cubitt, 1869), *Collotheca campanulata* (Dobie, 1849), and *Stephanoceros millsii* (Kellicott, 1885). Species of *Collotheca* and *Stephanoceros* feed passively by collecting small planktonic organisms (e.g., algae and protists) using an infundibulum that possesses lobes (*C. campanulata*), short tentacula (*C. coronetta*), or tentacles (*S. millsii*). *Acyclus inquietus* is a sessile predator that is often found inhabiting colonies of another sessile rotifer, *Sinantherina socialis* (Linnæus, 1758). It possesses a relatively large infundibulum with a wide mouth, which functions to capture and ingest eggs and larvae of *S. socialis*. Here, we provide detailed light microscopical observations of the development of the infundibulum in both species of *Collotheca*, and supplement light microscopical observations with electron microscopy studies to describe the development of the infundibulum in larvae of *S. millsii* and *A. inquietus*. Our intention here is not to document the complete development of the infundibulum in these species because the process appears to be extremely complex. Instead, we test the hypothesis that the collothecid infundibulum is a morphological novelty and therefore not homologous with the rotifer corona as defined in most references. Additionally, we provide ideas for future consideration of the study of collothecid rotifers that aim to increase our understanding of the functional significance of the infundibulum and its importance in trophic ecology.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Methods

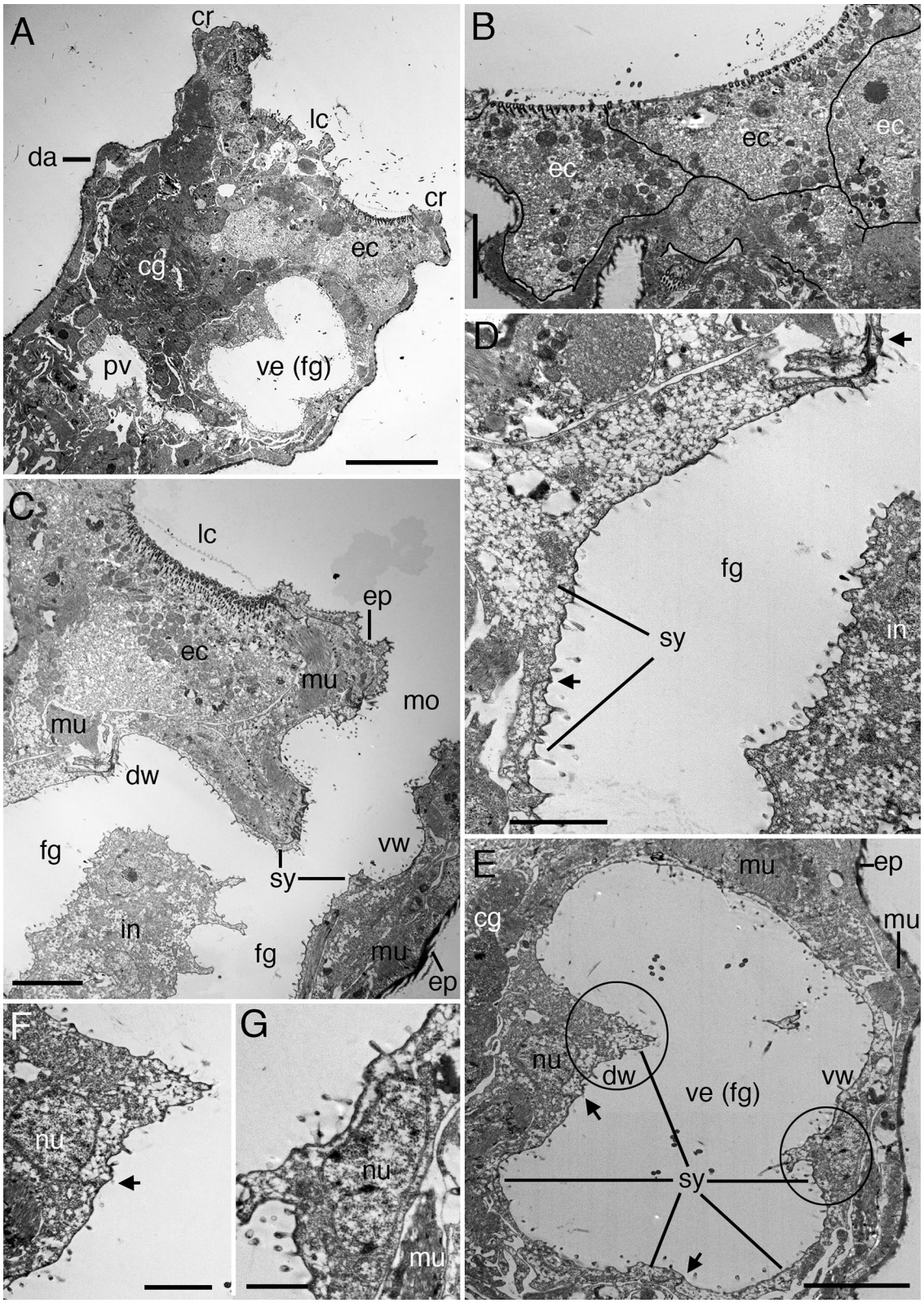
Species of *Acyclus inquietus*, *Collotheca campanulata*, *C. coronetta*, and *Stephanoceros millsii* were collected from Flint Pond, Tyngsboro, Massachusetts USA (42°40′29.00″N, 71°25′32.21″W) from June–August in 2014–2017. Specimens of *A. inquietus* were gently removed from colonies of *S. socialis* that were attached to species of *Utricularia*; colonies are also known from species of *Myriophyllum* (Surface, 1906; Felix et al., 1995), *Ceratophyllum* (Felix et al. 1995), Elodea (RLW, pers. obs.), and artificial substrata (Champ, 1978). The general procedures for removing sessile rotifers from their substratum have been described by Wallace & Edmonson (1986) and Wallace et al. (2006). Animals were cultured in native pond water for 1–3 weeks. Brightfield photographs were taken on a Zeiss A1 compound microscope equipped with differential interference contrast (DIC) and a Sony Handycam digital camera. Larval specimens of *A. inquietus* and *S. millsii* were anaesthetized with 0.5% bupivacaine for 10–30 min and processed for transmission electron microscopy (TEM). Specimens were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) for 2–4 h, rinsed in buffer (4 × 15 min), postfixed in 1% OsO_4 in 0.1 M cacodylate buffer for 1 h, rinsed in buffer (4 × 15 min), and dehydrated in an ethanol series (50%, 70%, 90%, 100%, 100%) for 10 min each. Larvae of *A. inquietus* were processed in propylene oxide (2 × 15 min) and then transitioned through a propylene oxide:epon resin mixture (Araldite Embed 812, Electron Microscopy Sciences) in the following ratios: 2:1 for 1 h, 1:1 for 1 h, and 1:2 for 2 h. This was followed by a 12-h infusion in pure resin and then embedment in pure resin in a BEEM capsule overnight at 60°C. A single free-swimming larva and an attached metamorphosing juvenile of *S. millsii* were processed in a similar fashion but embedded in Spurr's low viscosity resin. Resin blocks were trimmed, sectioned on a Reichert ultramicrotome at 70 nm, and sections collected on copper grids. Grids were stained with uranyl acetate (3 min) and lead citrate (3 min).

TEM sections were examined on a Philips CM10 TEM and photographed with a Gatan Orius 813 digital camera (Gatan Inc., Pleasonton, CA, USA) at the University of Massachusetts Medical School in Worcester, Massachusetts. Digital images were cropped for size and enhanced minimally for brightness and contrast.

A single adult female of *A. inquietus* was processed for scanning electron microscopy (SEM). This specimen was relaxed, fixed, and dehydrated as above, but then further dehydrated in a critical point dryer, mounted on a carbon-coated SEM stub, and sputter coated with gold. It was examined on a JEOL JSM 6390 SEM at the Materials Characterization Laboratory at the University of Massachusetts Lowell.

A single adult female of *C. coronetta* and *S. millsii* were processed for confocal laser scanning microscopy (CLSM). Both specimens were relaxed in 0.5% bupivacaine for 30 min and then fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3) for 2 h, rinsed in buffer (1 h), and then stained in Alexa Fluor 488 phalloidin (Thermofisher Scientific) for 2 h at 4°C on a rotator. Specimens were removed from the stain, rinsed briefly in buffer, and then mounted on glass microscope slides in ProLong Diamond Antifade Mountant (Invitrogen). Specimens were examined on an Olympus FV 300 CLSM equipped with a multi-argon laser. Confocal stacks were collected and processed with Olympus software.

Fig. 2 Sagittal TEM sections through the head of a larval *Acyclus inquietus*. **A** Section through coronal region showing a portion of the larval foregut (to become the adult mouth and vestibulum). **B** Close up of the hypodermal/epithelial cushion cells of the corona, artificially outlined. **C** Section through the region of the larval mouth and foregut, just ventral of the corona. Dorsal is to the left. **D** Close up of the walls of the foregut revealing their syncytial structure. **E** Close up of the foregut cavity (vestibulum) from **A**. Arrows point to junctions between separate syncytial cells within the vestibulum. Circled areas correspond to **F** and **G**. **F** Close up of a region of the dorsal wall showing a nucleus and intercellular junction (arrow) that is reminiscent in shape to the adult head. **G** Close up of a region of the ventral wall showing a nucleus and syncytium with apical microvilli. *cr* rim of larval corona, *da* dorsal antenna, *dw* dorsal wall of the foregut, *ec* epithelial cell that corresponds to the epithelial/hypodermal cushions of Clément & Wurdak (1991), *ep* syncytial epidermis with electron-dense intracytoplasmic lamina (cuticle), *fg* foregut, *in* infundibulum, *lc* larval corona with cilia, *mu* muscle, *nu* nucleus, *sy* syncytium, *vw* ventral wall of the foregut. Scale bars: **A** 10 μm; **B** 3.5 μm; **C** 4 μm; **D** 3 μm; **E** 6 μm; **F**–**G** 3 μm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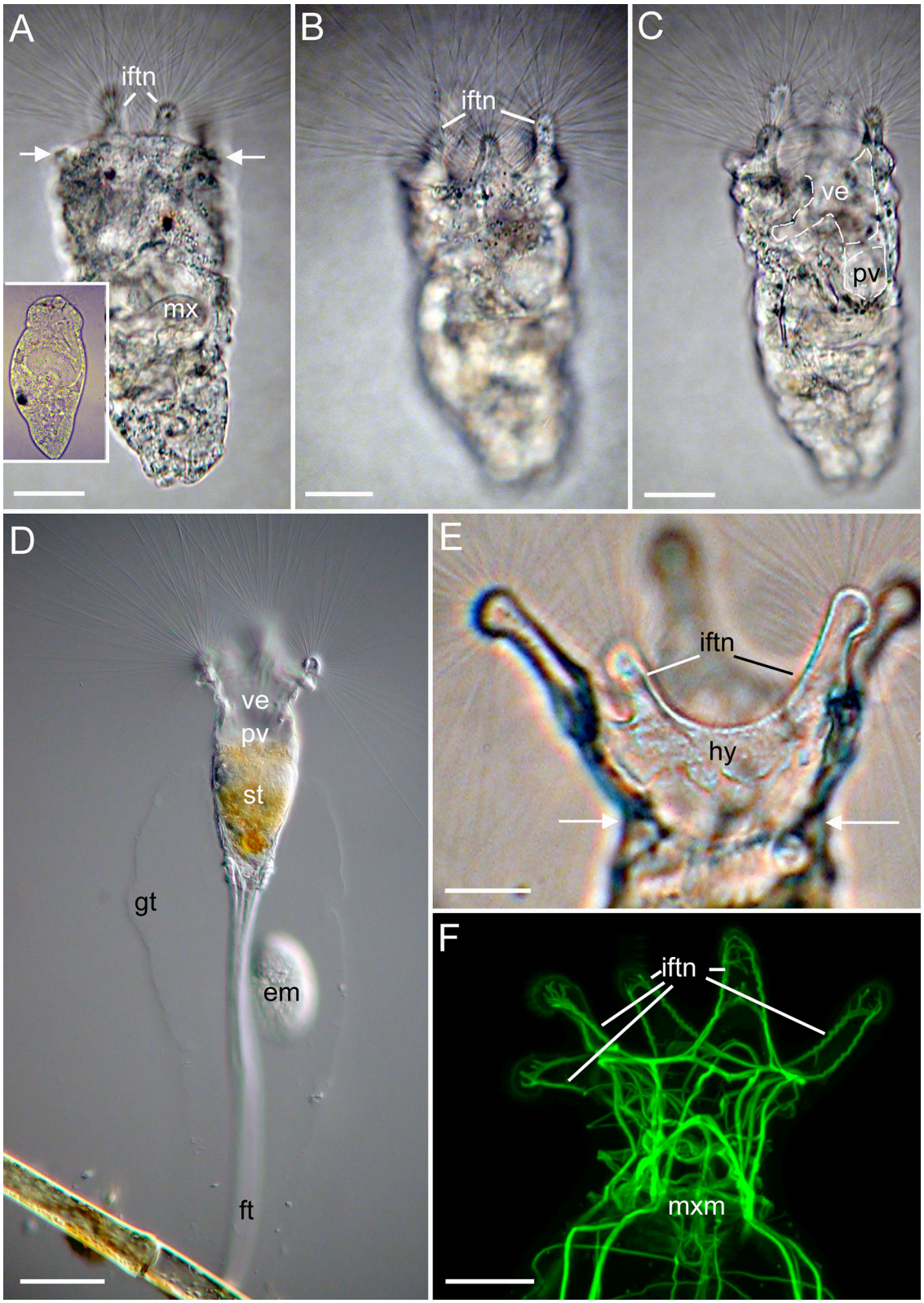
Results

Acyclus inquietus (Figs. 1, 2)

Three larvae were documented with brightfield microscopy and two of the specimens were prepared for TEM; one adult was prepared for SEM. The larval corona could be observed in late-stage embryos, but the infundibulum could not be seen. Immediately after hatching, larvae appeared to possess an elongate foregut cavity that connected the mouth to the developing proventriculum and mastax (Fig. 1A–D). In live specimens viewed from the ventral side, a circular mouth was located directly ventral of the corona, which had an indentation in its ventral margin (Fig. 1B, C). The foregut cavity appeared relatively straight along the ventrum (Fig. 1C, E), but a bilateral pair of bow-shaped cavities appeared to branch from the foregut cavity as the plane of focus was moved medially (Fig. 1D). In fixed specimens viewed laterally, the foregut had an uneven (lobed) dorsal wall and a relatively straight ventral wall (Fig. 1E). The foregut cavity was relatively straight for approximately 25% of the body length and then expanded into a multilobed cavity positioned anterior and ventral to the mastax (Fig. 1E). We could not identify the structure of the infundibulum in the foregut cavity, nor relate the shape of the cavity to the formed infundibulum that would eventually emerge after metamorphosis (Fig. 1G, H). Larvae would not settle and metamorphose in the absence of the host rotifer, *Sinantherina socialis*. This made observations of metamorphosis very difficult because colony members of *S. socialis* continued to move and obscured our line of sight. Based on limited observations, larvae appeared to go through multiple contractions prior to the emergence of the cup-shaped infundibulum. We could not determine the fate of the coronal cilia and did not directly witness emergence of the infundibulum.

Two larvae prepared for TEM were sectioned in the sagittal plane. The larval corona was apical and consisted of several multiciliated hypodermal cells (epithelial/hypodermal cushions sensu Clément & Wurdak, 1991). A thin glycocalyx covered the integument. Individual cilia of the hypodermal cells each possessed a pair of striated rootlets. The hypodermal cells were very large (Fig. 2A–C) and consisted of numerous mitochondria, membrane-bound vesicles, endoplasmic reticulum, and at least one nucleus. The apical field between the multiciliated cells was syncytial. The integument of the body, not including the ciliated coronal region, had an electron-dense intracytoplasmic lamina (cuticle) that appeared as a wavy outline across the syncytium (ep, Fig. 2B, E). Directly below the corona on the dorsal side was the cerebral ganglion (cg, Fig. 2A, E), which consisted of numerous electron-dense cells. The dorsal antenna was close to the cerebral ganglion (da, Fig. 2A). Ventrally, the mouth opened into the foregut, which was lined by a syncytial integument. The syncytium was broken into multiple "strips" as indicated by the presence of plasma membranes that demarcated one strip of syncytium from another (arrowheads, Fig. 2D, E, F). The syncytia had numerous apical microvilli along both the dorsal (roof) and ventral walls (floor) of the foregut. A portion of the dorsal foregut wall was highly vacuolated and contained numerous membrane-bound vesicles (Fig. 2D). The vesicles were present but less numerous in other regions of the foregut. Muscles and other unidentified tissues were observed beneath the syncytia of the foregut. In one specimen, a portion of the developing infundibulum was present in the foregut cavity as a large mass of tissue (Fig. 2C). This mass had numerous microvilli, was multinucleate, and contained abundant mitochondria and electron-lucent, membrane-bound vesicles. We could not determine which portion of the adult infundibulum that this mass of tissue was to become, nor which wall of the foregut it was derived from. In another specimen, the dorsal wall of the foregut formed a triangular shape (Fig. 2E) reminiscent of the eventual shape of the adult infundibulum (Fig. 1F, G), but we could not confirm its identity. We did not

Fig. 3 Larvae and adults of *Collotheca coronetta*. **A** Metamorphosing larva showing the slow emergence of the infundibular tentacula. Arrows point to the rim of the larval corona; larval cilia have already been shed. Inset: squash specimen of larva prior to metamorphosis. **B** Same specimen as **A** in a different focal plane. **C** Same specimen as **A** with a focus through the body wall showing the vestibulum and proventriculus (artificially outlined). **D** Adult specimen with embryo. **E** Close up of an adult's infundibulum. Arrows point to rim of larval corona that remains after metamorphosis. **F** Confocal image of a specimen stained with phalloidin to visualize the musculature of the infundibulum. *em* embryo, *ft* foot, *gt* gel tube, *hy* hypodermal cells of the infundibulum, *iftn* infundibular tentacula, *mxm* mastax musculature, *pv* proventriculus, *st* stomach, *ve* vestibulum. Scale bars: **A**–**C** 45 μm; **D** 40 μm; **E**, **F** 35 μm ▶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examine the ultrastructure of the proventriculus (Fig. 2A).

Collotheca coronetta (Fig. 3)

Free-swimming larvae (1–3 h old) had a ciliated corona (Fig. 3A inset); cilia were shed at settlement and prior to emergence of the infundibulum (Fig. 3A–C). After settlement, larvae underwent a series of longitudinal contractions (time not measured) and the larval mouth could no longer be observed. Eventually, long cilia (setae) and five short tentacula of the infundibulum could be observed projecting from an apical depression at the anterior end of the now-juvenile specimen (Fig. 3A). This shallow depression was encircled by the rim of the larval (cilia-less) corona (Fig. 3A, arrows). At the base of the depression was the floor of the infundibulum, which was continuous with the rim of the larval corona. The syncytial floor formed five short tentacula with slightly swollen tips and bearing numerous elongate cilia (Fig. 3B–C) similar to what was present in reproductive adults (Fig. 3D, E). The tentacula were lined by a thin ciliated syncytium (nuclei and cell bodies were not observed) and contained muscles (not shown). In reproductive adults, there was a distinct hypodermal cell body (with nucleus) closely aligned to the base of each tentaculum (Fig. 3E); the cells appeared interconnected and may have formed a syncytium. These cells were not observed in the developing infundibulum of the free-swimming larva. The rim of the larval corona was still present in the juvenile as a constriction at the base of the infundibulum (Fig. 3E, arrows). Muscles were present inside each adult tentaculum (Fig. 3F). Their orientations and homologies with other rotifer muscles were not evaluated.

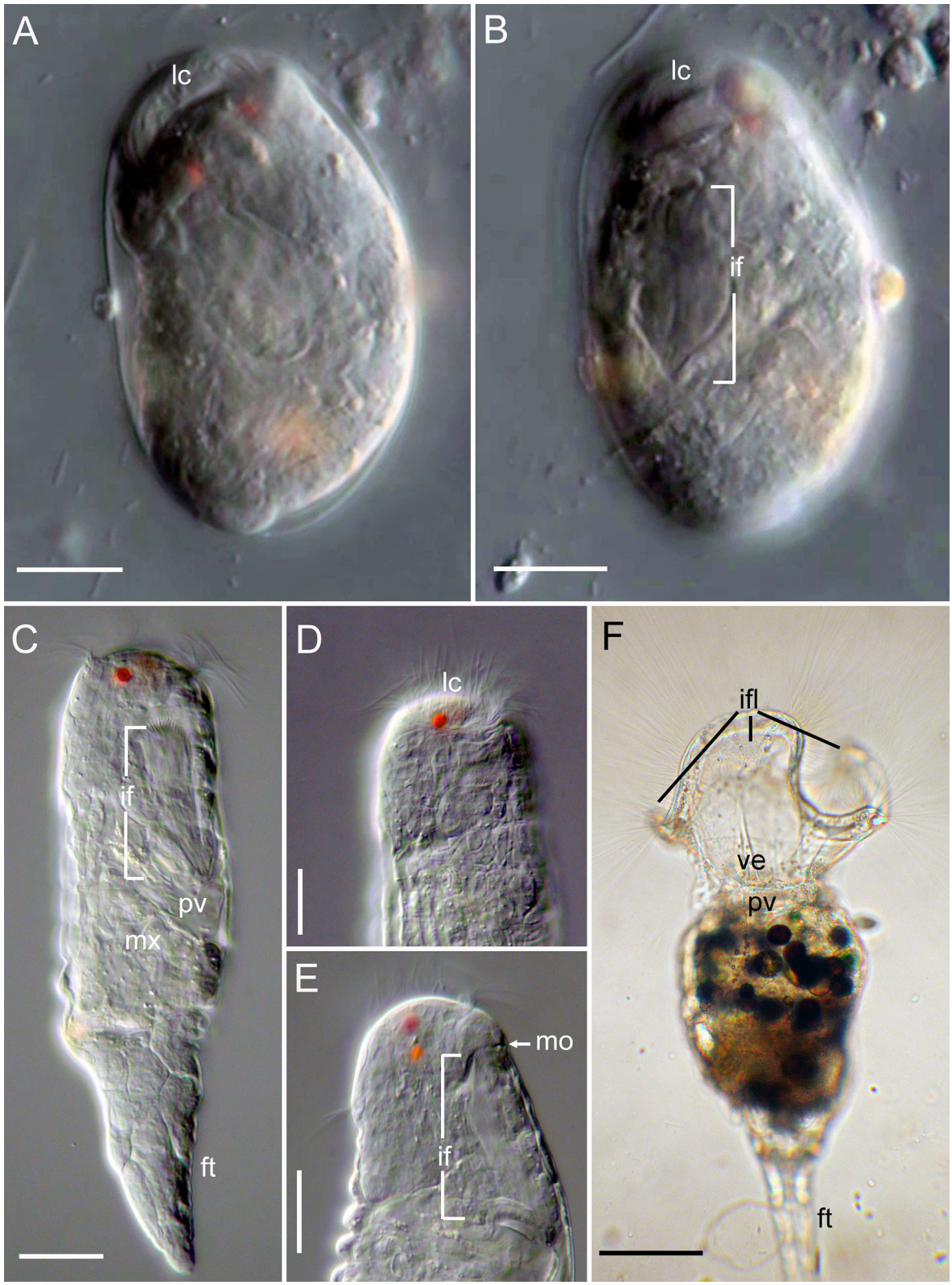
Collotheca campanulata (Fig. 4)

Late-stage embryos had a larval corona at the apical end and an elongate foregut posterior to it on the ventral side (Fig. 4A, B). The elongate foregut contained active cilia and appeared to be the early stage of infundibulum development. Protonephridial cilia were also active within the embryo. Free-swimming larvae had active coronae and the cilia in the foregut cavity were also active, though the latter could only be observed when mounted for high magnification (Fig. 4C–E). Larvae settled on the glass surface of a bowl or even upside down on the surface tension of the water. Larvae went through multiple longitudinal contractions > 30 min before the long infundibular cilia could be seen protruding from the larval mouth. One specimen (1 h post-hatch) was mounted for high magnification and observed > 30 min. Larval cilia appeared to be shed during this time, and the highly elongate cilia of the infundibulum projected from the apical end and were active. The large infundibular lobes of the adult (Fig. 4F) were not observed in the infundibular cavity of the larva.

Fig. 4 Ontogenetic stages of *Collotheca campanulata*. **A** Late-stage embryo in its eggshell with a focus on the larval corona. **B** Same as **A** but with a focus on the developing infundibulum. **C** Free-swimming larva (not the same as in **A**), lateral view, dorsal is to the left. **D** Focus on larval corona. **E** Focus on developing infundibulum. **F** Adult specimen, approximately 5 days old. *ft* foot, *if* infundibulum, *ifl* infundibular lobes, *lc* larval corona, *mo* mouth, *mx* mastax, *pv* proventriculus, *ve* vestibulum. Scale bars: **A**–**B** 18 μm; **C** 15 μm; **D** 10 μm; **E** 13 μm; **F** 35 μm

Stephanoceros millsii (Figs. 5, 6, and 7)

Late-stage embryos had noticeable eyespots, larval corona, and developing infundibulum with highly mobile cilia (Fig. 5A). Newly hatched larvae also showed evidence of the proventriculus (Fig. 5B), and the developing infundibulum occupied up to 40% of the body length in relaxed specimens (Fig. 5C). The mouth was on the ventral side and led directly to the foregut cavity (to become the adult vestibulum) and infundibulum (Fig. 5D). The larvae swam around for several hours prior to settlement and metamorphosis on the bottom of a glass dish. Two individuals were mounted on glass slides and observed with a compound microscope. In one specimen (Fig. 5E), the infundibulum could be seen emerging from the anterior end of the larva. Coronal cilia were not observed, but several cells had fragmented from the animal, which were likely a result of coverslip pressure and not a natural process. A second specimen appeared similar to the first, but with small tentacles emerging from the anterior end (not shown). The infundibulum consisted of five tentacles, all with highly elongate and active cilia. The cavity in the center of the infundibulum (the vestibulum) was apparent. The distinction between vestibulum and proventriculus, which was easy to visualize in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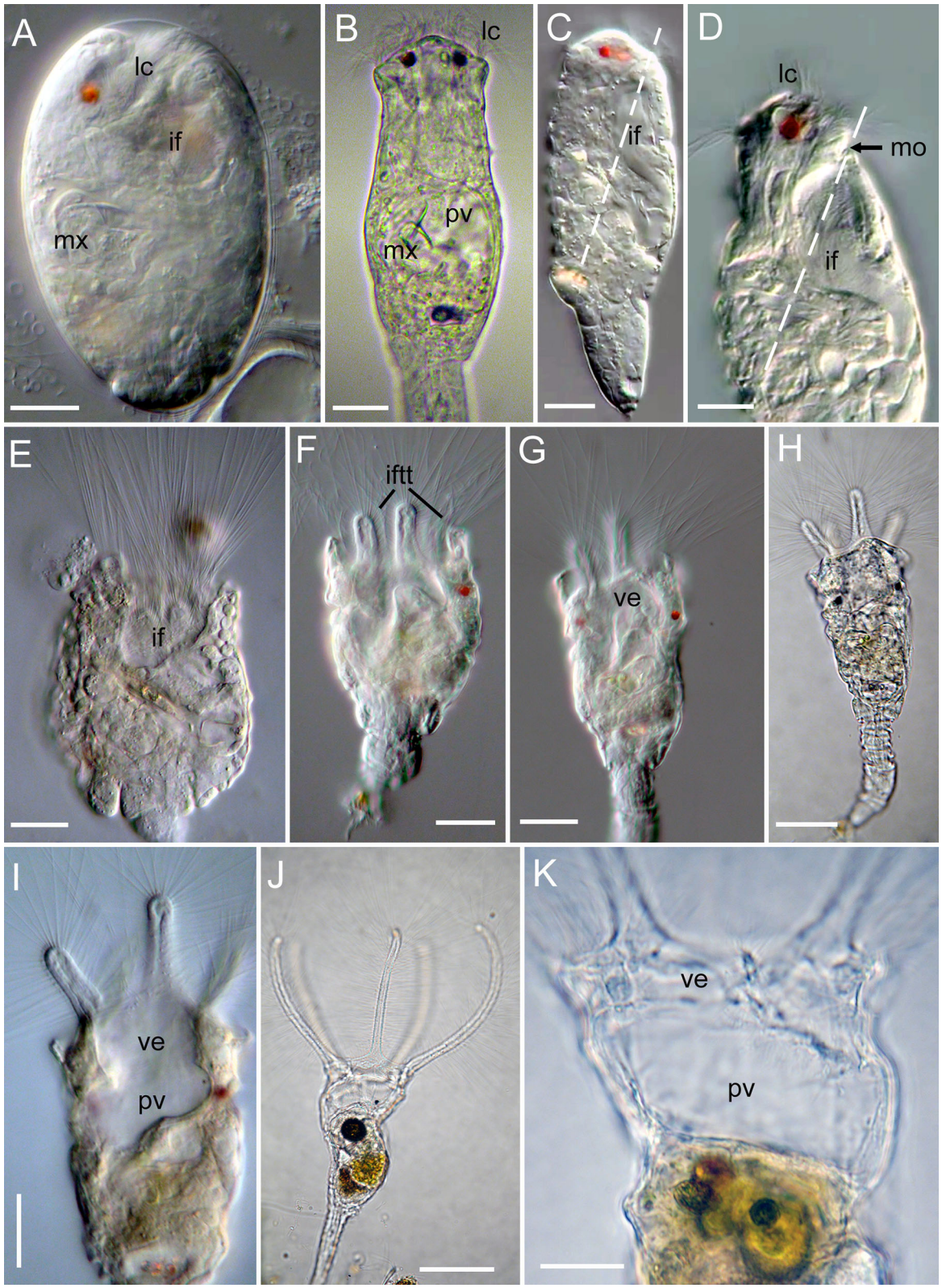
juveniles and adults (Fig. 5J, K), was not apparent in metamorphosing larvae until the tentacles had nearly completely emerged (Fig. 5I). The process of tentacle and foot growth occurred over the next 48 h.

Two late-stage larvae (with developing infundibula) were examined with transmission electron microscopy (see dashed lines of Fig. 5C, D for approximate region of sectioning, but performed in the frontal plane). Both specimens were contracted, making identification of many organ systems and their orientations difficult. One larva had a contracted corona, which created a depressed region of coronal cilia (Fig. 6A). The apical field was entirely syncytial and had an undulating appearance that was probably a result of the contraction (Fig. 6A, C). Coronal cilia arose directly from the syncytium and appeared to have ciliary rootlets that extended parallel to the syncytium (not shown). Several cell bodies were proximal to the apical field and coronal cilia, but the state of contraction prevented an easy determination of their anatomical relationship to the larval corona. Only a small section of the infundibulum was present, identified by the highly elongate cilia (in, Fig. 6A). The second specimen was cut in the region of the infundibulum, and though contracted, included the larval mouth (Fig. 6B, D, E). The mouth led to a large vestibulum containing the infundibular cilia (Fig. 6B), and depending on the region of the section, lateral tentacles (Fig. 6D). Sections with other tentacles always overlapped the grid crossbars and so photographs could not be evaluated. The floor of the infundibulum had a slightly undulating appearance, was syncytial, and ciliated (Fig. 6B, D). The lateral tentacles were also highly ciliated and syncytial. Their cytoplasm contained mitochondria and membrane-bound vesicles with electron-lucent cores. Two pairs of large cells—herein called tentacular cells (te, Fig. 6D)—were present below the mouth rim. Each of the two cell bodies had a highly granular and electron-dense appearance and were filled with mitochondria and secretion vesicles with electron-dense cores. Both cell necks extended toward the lateral tentacles based on their adjacent plasma membranes (Fig. 6D, E). The distinction between plasma membranes became unclear at the base of the tentacles (Fig. 6D, E), and so appeared to form a syncytium that produced the tentacle body.

To confirm the syncytial nature of tentacles in adults, and determine whether muscles supplied them as they do in *S. fimbriatus* (Hochberg & Hochberg, 2017), we stained (Fig. 7A) and sectioned two specimens (Fig. 7B–D). The tentacles were supplied with several muscles that appear to be extensions of the somatic muscles of the body (Fig. 7A). Each tentacle contained several muscle fibers, though the precise number was not determined. Minimally, two longitudinal muscles supply two sides of each tentacle, though within the tentacle body there appeared be a complex network of muscle fibers (Fig. 7A). At the ultrastructural level, these tentacles were syncytial and hollow, with a blastocoel that was continuous with the body. The syncytial integument contained abundant organelles including mitochondria, membrane-bound vesicles, and cilia; nuclei were not observed. Each cilium had a horizontal and vertical striated rootlet (Fig. 7B, D). Muscles inserted at various points along the length of the tentacles; the epitheliomuscular junctions often appeared as electron-dense spots (Fig. 7D).

The proventriculus was located below the infundibulum and vestibulum. This large cavity was demarcated from the vestibulum by a multilayered tissue that appeared to comprise the syncytial floor of the infundibulum, basal lamina, muscle, and the syncytial roof of the proventriculus (Fig. 6A, D). Cilia projected from the roof of the proventriculus, but the floor of the proventriculus was not ciliated (Fig. 6B). Our photomicrographs were not of sufficient quality to accurately determine the structure of tissues that separated the floor of the infundibulum from the roof of the proventriculus. Also, we did not

Fig. 5 Ontogenetic stages of *Stephanoceros millsii*. **A** Late-stage embryo in its eggshell. **B** Newly hatched (minutes) larva, dorsal view. **C** Larva seen in lateral view, dorsal to the left. Dashed line shows approximate plane of section for TEM. **D** Close up of larval head (different specimen than **C**) showing the mouth. Dashed line shows approximate plane of section for TEM. **E**–**H** Larvae in the early stages of metamorphosis displaying the slow emergence of the elongate cilia (setae) of the infundibulum (**E**) and eventually the tentacles (**F**–**H**). **I** Focus on the large alimentary cavities of the larva. J. Juvenile specimen, approximately 24–36 h after metamorphosis. **K** Close up of the infundibulum of a juvenile showing the large cavities of the alimentary canal. *if* infundibulum, *ifft* infundibular tentacles, *lc* larval corona, *mo* mouth, *mx* mastax, *pv* proventriculus, *ve* vestibulum. Scale bars: **A** 13 μm; **B** 25 μm; **C** 25 μm; **D** 20 μm; **E** 38 μm; **F** 40 μm; **G** 41 um; **H** 43 μm; **I** 35 μm; **J** 65 μm; **K** 30 μm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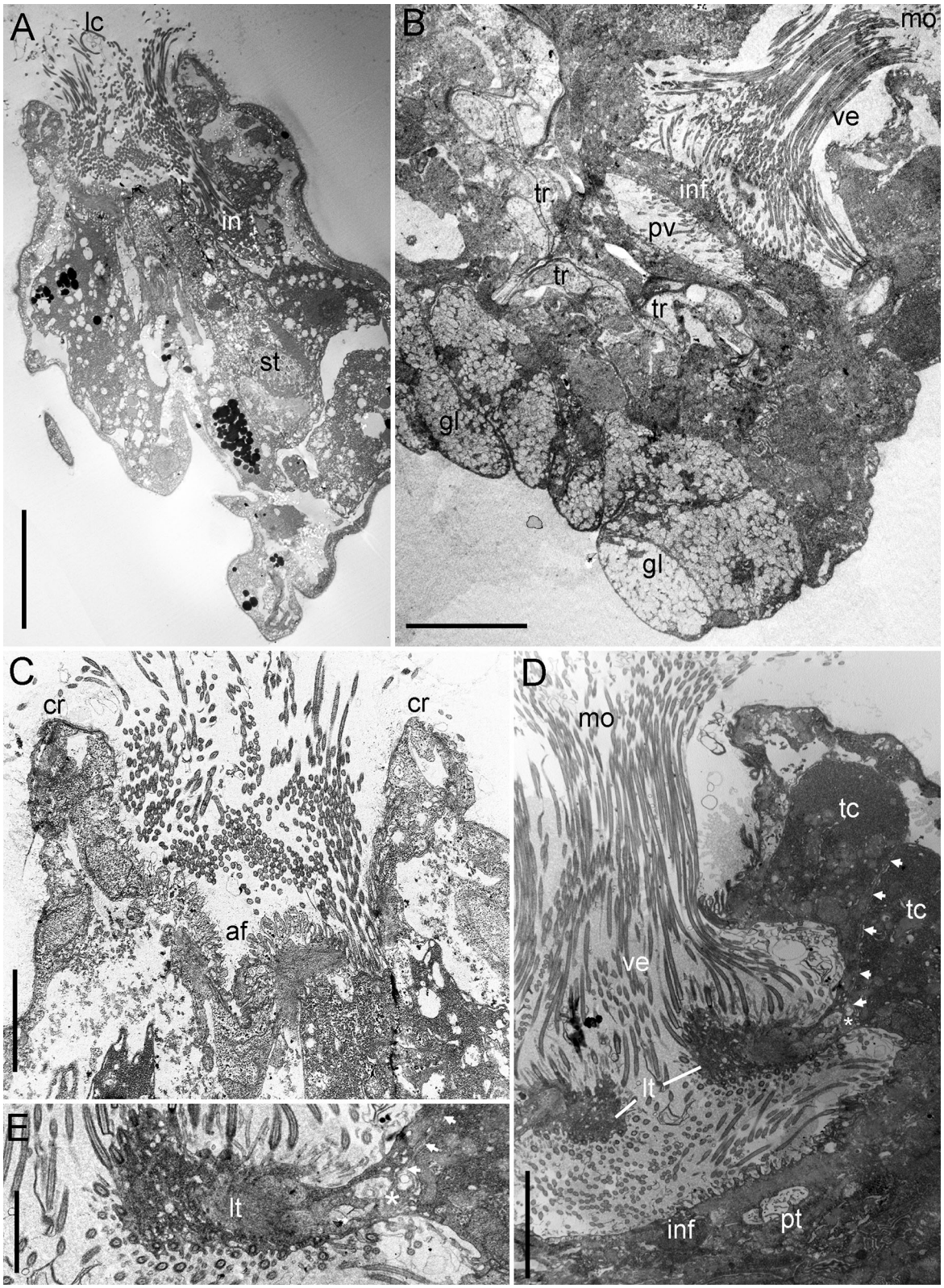
locate the opening of the vestibulum to the proventriculus in our sections.

Discussion

The infundibulum of collothecid rotifers represents a highly unusual head morphology that appears to be adapted for a different mode of foraging compared to most other rotifers. Observations suggest that collothecids are either ambush predators that feed on whole organisms such as algae, gastrotrichs, other rotifers, and protists (Vasishist & Dawar, 1969; Bevington et al., 1995) or function as sit-and-wait predators that can trap smaller eukaryotic prey in the confines of the infundibular cilia (Wright, 1958). In either case, the infundibulum is only present in species of Collothecaceae and may therefore be considered a synapomorphy i.e., a unique evolutionary character of the order. Yet, historical diagnoses of the Collothecaceae have often described the infundibulum as a modified corona, e.g., as "… an aberrant form of the trochal disc …" by Cubitt (1870, p. 242), as "coronal arms" by Gosse (1862), and as a "coronal cup" by Montgomery (1903). Even contemporary taxonomic keys list a modified corona as diagnostic to the order (Wallace et al., 2006), and while such keys rely solely on observable characters and make no proposals about homology, consistent use of the term corona (or even modified corona) in current descriptions (e.g., Meksuwan et al., 2013) reinforces the idea that the infundibulum is a derived corona. Confounding this issue is a lack of information on phylogenetic relationships within Collothecaceae or between the order and other taxa (Sørensen, 2002; Sørensen & Giribet, 2006; Meksuwan et al., 2015), meaning that we cannot reconstruct the ancestral condition of the rotifer head and determine the relationships between the classical rotifer corona and the infundibulum. Nevertheless, our results make a strong case for the non-homology of the infundibulum with the coronae of other rotifers, and therefore that the adult head of collothecid rotifers is a morphological novelty.

The rotifer corona is often defined as an anterior field of cilia that functions for locomotion and feeding (Wallace et al., 2015). In many species, the corona consists of two circumapical bands of cilia that surround a naked apical field. Beyond this generic description, it is important to note that the pattern of ciliation and the structure of the apical field can be highly variable in size, shape, and structure as seen throughout the Rotifera (de Beauchamp, 1965), to the exclusion of Acanthocephala (Herlyn et al., 2003). Moreover, there is limited information on development of the corona in any species (e.g., Fontaneto et al., 2003), and similarly, relatively few ultrastructural details that can be compared to the results in our study (Wurdak et al., 1983; Clément & Wurdak, 1991). Perhaps the most comparable description of development and metamorphosis in a rotifer comes from the study by Fontaneto et al. (2003) of *Floscularia ringens* (Linnæus, 1758), a sessile species that engages in indirect development and undergoes metamorphosis after settlement. Here, the larval form possesses a ciliated corona and other morphological characters typical of the adult; the major differences between the two life stages are that the adult possesses an elongate foot and larger, more elaborate corona. Based on that study, the larval corona goes through allometric growth after settlement, and this gradual metamorphosis eventually leads to a larger and more complex corona that contains two rings of cilia (trochus, cingulum), a labium, and a specialized pellet-forming organ (modulus). The modulus functions in building a specialized extracorporeal tube (Wright, 1950). Importantly, the larval corona develops into the adult corona. Similar examples exist from less-detailed studies of Ploima and Bdelloidea that have direct development, wherein a neonate (juvenile) has a corona that is similar to, and develops into, the

Fig. 6 Frontal sections through two larvae of *Stephanoceros millsii*. **A** Larva with a contracted corona. **B** Ventral region of another specimen showing the developing infundibulum. Elongate cilia project from the lateral walls and floor of the infundibulum. The mouth is at the top. **C** Close up of the larval corona with a focus on the apical field with its highly wrinkled appearance (same specimen as **A**). **D** Close up of the developing infundibulum showing the lateral tentacles (same specimen as **B**). Arrows point to the border between two large cells with elongate necks that form the lateral tentacles. **E** Close up a developing lateral tentacle. Arrows point to the plasma membranes of two adjacent cells that create the syncytium of the tentacle. The region where the plasma membranes can no longer be observed is indicated by an asterisk. *af* apical field, *cr* rim of the larval corona, *gl* glandular integument of the foot region (highly contracted); *in* infundibulum, *inf* floor of the infundibulum, *lt* lateral tentacle, *mo* mouth, *pv* proventriculus, *st* stomach, *tr* trophus sclerites, *tc* tentacular cell with long neck that supplies the tentacles, *ve* vestibulum. Scale bars: **A** 15 μm; **B** 9 μm; **C** 6 μm; **D** 7 μm; **E** 2.2 μm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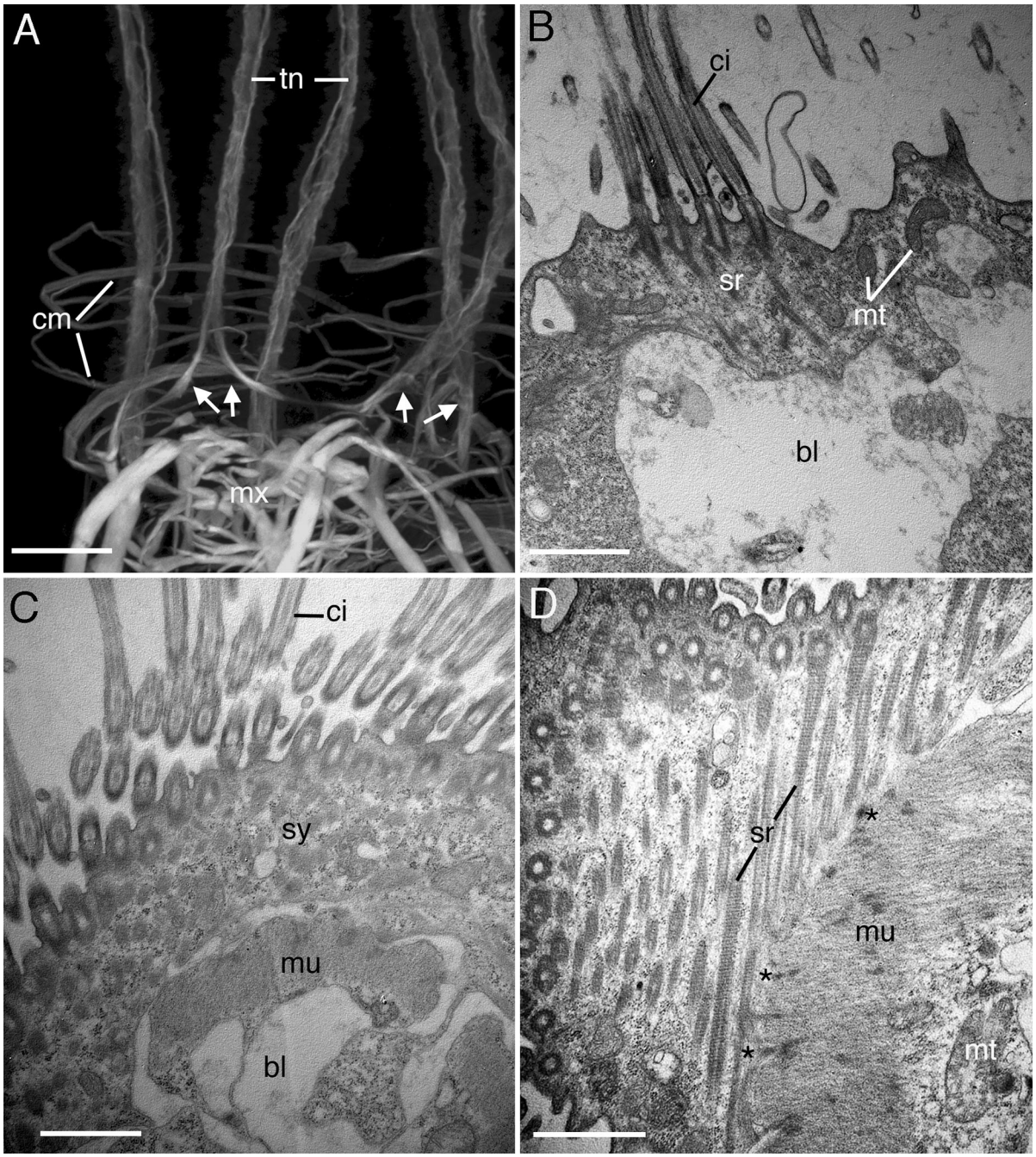

Fig. 7 The tentacles of *Stephanoceros millsii* visualized with CLSM (**A**) and TEM (**B**–**D**). **A** Confocal image of the infundibulum of an adult specimen stained with phalloidin to show the musculature. Each tentacle is richly supplied with longitudinal muscles. Arrows point to at least two longitudinal muscles that supply each tentacle, though internally there appears to be a network of several muscle fibers. **B** Portion of the integument of a tentacle showing its syncytial structure and blastocoel cavity. **C** Close up of a region of the syncytium showing its multiciliated structure. A section through a longitudinal muscle is present in the blastocoel. **D** Close up of a portion of the syncytium where the ciliate rootlets can be observed and are in proximity to a longitudinal muscle. The asterisks point to potential intercellular junctions that appear as electron-dense spots. *bl* blastocoel, *ci* cilia, *cm* circular muscles, *mt* mitochondria, *mx* mastax musculature, *mu* longitudinal muscle, *sr* striated rootlets, *tn* tentacle. Scale bars: **A** 7 μm; **B** 1.2 μm; **C** 1.2 μm; **D** 1 μm

adult form (e.g., Tannreuther, 1920; Pray, 1965; Paez et al., 1988; Boschetti et al., 2005). These direct transformations would seem to fulfill a general criterion of the argument for developmental homology, that two organs have the same embryonic origins (e.g., Gegenbaur, 1859; Wilson, 1896; Wagner, 1989). In the case of the Collothecaceae, we argue that the infundibulum is not a developmental homolog of the rotifer corona because it does not arise from the larval corona through any obvious transformation sequence, but instead develops from tissues of the foregut. Similar observations were also made by Montgomery (1903, p. 389) who noted the following: "It is important to note that these cilia [of the infundibulum] lie within the alimentary canal, i.e., belong to its inner lining and thus cannot represent a cingulum." Montgomery (1903, p. 389) goes on to say, "… the cilia of the infundibulum are at no time a portion of the corona." de Beauchamp (1907, pp. 25–26) made comparable observations on *S. fimbriatus*; he stated that the larvae lose the coronal (trochus) cilia at metamorphosis, and that the five arms (infundibular tentacles) of the adult are not elongations of the corona. Thus, the argument for non-homology of the corona and infundibulum were made more than a century ago.

At the ultrastructural level, we note that larvae of collothecid rotifers possess a ciliated corona made of hypodermal cells, which are similar in appearance to those of ploimid rotifers. Clément & Wurdak (1991) refer to these cells as epithelial cushions or hypodermal cushions, which are defined as bottle-shaped, multiciliated cells with each cilium possessing a single horizontal and vertical striated rootlet. Somatic muscles insert directly on these cells via epitheliomuscular desmosomes and so control orientation of the corona during locomotion. The hypodermal cushions are visible in live specimens of collothecid larvae and we note their presence at the ultrastructural level in *A. inquietus* (Fig. 2A). The somatic longitudinal muscles also supply the epithelial cushions of larval collothecids. This observation reinforces the idea that they are homologues of epithelial cushions in other rotifer taxa: i.e., component parts of a homologous organ as defined by Remane (1956) and elaborated on by Ruppert (1982). Curiously, we also observe the presence of large multiciliate cells in the metamorphosing larvae of *S. millsii*. Here, the cells are close to the rim of the infundibulum, but send long necks to the developing tentacles that emerge in the vestibulum during metamorphosis (Fig. 6). There appears to be at least two cells that contribute to each tentacle, and based on our photomicrographs, their necks appear to coalesce to form a multiciliate syncytium that forms the epithelium of the tentacles. These observations explain the appearance of large hypodermal cells in the same region in adult collothecids (e.g., Figure 3E). Similar observations were made on species of *Collotheca* by Montgomery (1903, pp. 375–376): "The hypodermis of the arms of the corona [lobes of the infundibulum] would therefore appear to be a direct continuation of the cytoplasm of the large hypodermal cells at the base of the arms." While the developmental origins of these cells are unknown, we posit that they are unlikely to be homologous with the epithelial/hypodermal cushions of larval collothecids or other rotifers for two reasons. (1) Their staining properties are much different (compare Fig. 2A–C with Fig. 6D, or to Figs. 1, 4 of Clément & Wurdak (1991)). (2) They appear to be derivatives of the foregut since they are not obviously present in the non-metamorphosing larval stage. Still, muscles do insert on these cells, but instead of inserting on the cell body as in other rotifers (Clément & Wurdak, 1991), they insert on the long syncytial necks that make up the tentacular epithelium.

According to Brigandt (2003, p. 6), developmental homology explains "… how structures emerge in ontogeny, why they are, how they are, and why structures are conserved or transformed in the course of phylogeny." Our primary purposes for making this argument about the non-homology of the collothecid infundibulum with the rotifer corona are to both correct the literature (adult collothecids do not possess a corona) and to stimulate further research into the evolution and trophic ecology of species of Collothecaceae. As noted, there are good historical descriptions of the development and anatomy of many collothecid rotifers, but these descriptions lack the resolution afforded by more contemporary techniques and technologies. Below, we provide a list of research ideas that are worthy of pursuit because they will provide insights on how collothecid development and morphology have changed over evolutionary time and how their morphology is related to their diverse lifestyles and trophic roles.

1. Explore the morphological diversity of the infundibulum across the sessile species, including

those members of the group that have abandoned sessility for a planktonic existence: e.g., *Collotheca libera* (Zacharias, 1894) (Young et al., this volume).
2. Investigate the correlation between infundibulum morphology and prey types and sizes.
3. Determine if different foraging methods among sessile rotifers have different energetic costs, e.g., ciliary collection in species of Flosculariaceae compared to ambush or sit-and-wait predation in species of Collothecaceae.
4. Resolve how the cilia (setae) of species of some collothecid rotifers can move independently or in small groups (Wright, 1958) despite an apparent lack of direct innervation (Hochberg & Hochberg, 2015), i.e., do individual muscles control groups of cilia?

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