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The ultrastructure of the integument and proventriculus in the raptorial rotifer *Cupelopagis vorax* (Monogononta: Collothecaceae: Atrochidae)

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Abstract. Members of the sessile rotifer species *Cupelopagis vorax* are unusual ambush predators that live permanently attached to submerged freshwater plants. Previous light microscopical research has revealed several uncommon features in this species including a stellate-patterned integument and an expansive foregut region called the proventriculus. In this study, we apply transmission electron microscopy to explore the ultrastructure of both the integument and foregut to determine how they differ from other rotifers. Our results reveal that the integument is covered by a thick glycocalyx and is patterned with tubercles that originate from the intracytoplasmic lamina (ICL) within the syncytial epidermis. The ICL forms an apical layer within the syncytium, is electron dense and mostly amorphous, and forms tubercles up to 2.3 μm; these tubercles probably account for the patterned appearance of the integument and are similar to what has been found in other gnesiotrochan rotifers. The basal cytoplasm is highly granular and contains two types of membrane-bound vesicles: large ovoid vesicles (320–411 nm) with amorphous, opaque contents, and secretory bulbs (110–264 nm) with electron-lucent cores and occasionally electron-dense contents. Only the secretory bulbs were observed to form connections to the apical plasmalemma, and so are probably exocytotic. Internally, the proventriculus is a large distensible sack that connects the anterior pharyngeal tube to the posterior mastax. The proventricular epithelium is a thin syncytium mostly covered with a dense glycocalyx and a strong brush border of microvilli underlain by a thin terminal web. The cytoplasm contains few organelles and there is no evidence that it is either secretory or has features (e.g., ICL) that might aid in maceration. We hypothesize that the thick glycocalyx might serve a protective function against the movements of live prey and/or against enzymes released from the rotifer's gastric glands that become regurgitated during feeding.

Additional key words: digestion, Gnesiotrocha, predatory, sessile, zooplankton

Rotifera comprises more than 2000 species of aquatic microinvertebrates (Segers 2007), most of which use their ciliated head (corona) to simultaneously create feeding currents and swim through the plankton or glide along the benthos and submerged vegetation. Within the Rotifera, Superorder Gnesiotrocha contains several species in which a ciliated corona is present only during the larval stage; adults of these species have a large, cup-shaped head called an infundibulum that develops internally during metamorphosis (Kutikova 1995). Many gnesiotrochans of Order Collothecaceae possess an infundibulum that is elaborated into lobes or tentacles often adorned with long setae that function to capture planktonic prey. Other species use their large unadorned infundibulum to capture small epiphytic animals and protists (Wallace et al. 2015). A curious member of the latter group is *Cupelopagis vorax* (Leidy 1857), individuals of which are ambush predators that have been examined in some detail because of their unusual body form and ability to detect vibrations produced by prey (Vashist & Dawar 1969; Koste 1973; Bevington et al. 1995).

The stout, ovoid body in individuals of *C. vorax* separates the species from other members of the Collothecaceae, as well as from the two other vermiform members of its family, Atrochidae (Cori 1925;

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Vashist & Dawar 1969). In their descriptions of the anatomy of *C. vorax*, Vashist & Dawar (1969) note that the rotifer possesses some other unique features including the structure of its body wall and how it processes its prey. For example, the rotifer's integument appears to possess a thin and colorless cuticle that may be highly variable in terms of thickness, at least along the dorsal surface, as well as possessing tubercles that produce a stellate appearance. As the rotifer integument is known to contain a cuticle *within* its syncytium (aka intracytoplasmic lamina; Clément & Wurdak [1991]), and not as an extracellular secretion, this description of *C. vorax* appears to be unusual. Moreover, while many rotifers are known to possess some uncommon body wall elaborations (Hochberg et al. 2015), stellate tubercles are unknown from other species. In terms of predation behavior, members of *C. vorax* are also unique in how they survey their surroundings for vibrations from prey prior to a rapid ambush attack on organisms such as protists, crustaceans, gastrotrichs, nematodes, and other rotifers (Bevington et al. 1995). Once captured by the infundibulum, prey is forced into an expansive region of the foregut called the proventriculus. The proventriculus functions as a temporary storage organ before prey is grasped by the trophi (jaws) within a muscular mastax and deposited into the stomach, where extracellular digestion takes place (Vashist & Dawar 1969).

To date, *C. vorax* has not been studied at the ultrastructural level to determine whether its body wall or digestive tract departs from what has been described in other rotifers. Therefore, we undertook this investigation to determine how the ultrastructure of *C. vorax* compares to other rotifers, and whether the anatomical observations at the light microscopical level can be verified with transmission electron microscopy (TEM). Hochberg et al. (2015) have shown that light microscopical observations of rotifers provide an incomplete view of the body wall; EM research should also provide additional details that might be relevant for understanding both the functional biology of a species and its evolutionary history (Hochberg et al. 2015). Thus, we posit that our studies of the proventriculus will shed light on its evolutionary origins, since such a region is unknown from rotifers outside Order Collothecaceae, which otherwise have a standard foregut.

Methods

Specimens of *Cupelopagis vorax* were collected from Moon Lake (Marquette Co., WI, USA; 43°48′09″N, 89°22′11″W). Rotifers were photographed alive using a Zeiss A1 compound microscope equipped with DIC (Zeiss, Thornwood, NY, USA) and a Sony Handicam digital camera (Sony Corp., New York, NY, USA). Three specimens were relaxed in 0.5% bupivacaine or 1% $MgCL_2$ and fixed in 2.5% glutaraladehyde in 0.1 mol L^{-1} sodium cacodylate buffer (pH 7.3) for 2 h at room temperature. Specimens were next rinsed four times in 0.1 mol L^{-1} sodium cacodylate buffer (15 min each), postfixed in 1% OsO_4 in 0.1 mol L^{-1} cacodylate buffer for 1 h, and rinsed again in the same buffer (4×15 min). Fixed animals were dehydrated in an ethanol series (70%, 90%, 100%, 100% ×10 min) followed by two rinses in propylene oxide (15 min each) and then transitioned through a propylene oxide: epoxy resin mixture (Araldite, EMbed 812; Electron Microscopy Sciences, Hatfield, PA, USA) series of 2:1 (1 h), 1:1 (1 h), and 1:2 (1 h), followed by an overnight infusion in pure epoxy resin in a microcentrifuge tube on a rotator at room temperature. Specimens were then placed in pure resin in BEEM capsules and transferred to a 60°C oven for 24 h to cure. Epoxy resin blocks were trimmed and sectioned on a Reichert ultramicrotome and sections collected on copper grids. Sections were stained with uranyl acetate and lead citrate and examined on a Philips CM10 equipped with Gatan Orius 813 digital camera (Gatan Inc., Pleasanton, CA, USA) at the Core Electron Microscope Facility at the University of Massachusetts Medical School in Worcester, MA. Specimens were examined at 80 kV. Digital images were not manipulated except for basic cropping and some changes in brightness and contrast.

Results

Our descriptions of the integument follow the terminology of Clément & Wurdak (1991), which follows standards based on earlier studies (e.g., Koehler 1965, 1966; Dickson & Mercer 1967; Clément 1969; Schramm 1978). Descriptions of the digestive tract in individuals of *Cupelopagis vorax* vary among authorities and differ from what is observed in most other rotifers. Using Edmonson (1959: p. 431–432) and traditional animal anatomy as a guide, we described the following alimentary structures in individuals of *C. vorax*: infundibulum, pharynx (pharyngeal tube, proventriculus, and mastax with trophi), esophagus, stomach, intestine, cloaca, and anus. This series differs from Vashist & Dawar (1969) and Koste (1973), who refer to the pharyngeal tube as an esophagus, which differs from

its more traditional use as a postpharyngeal structure (Clément & Wurdak 1991; Wallace & Smith 2013).

Integument

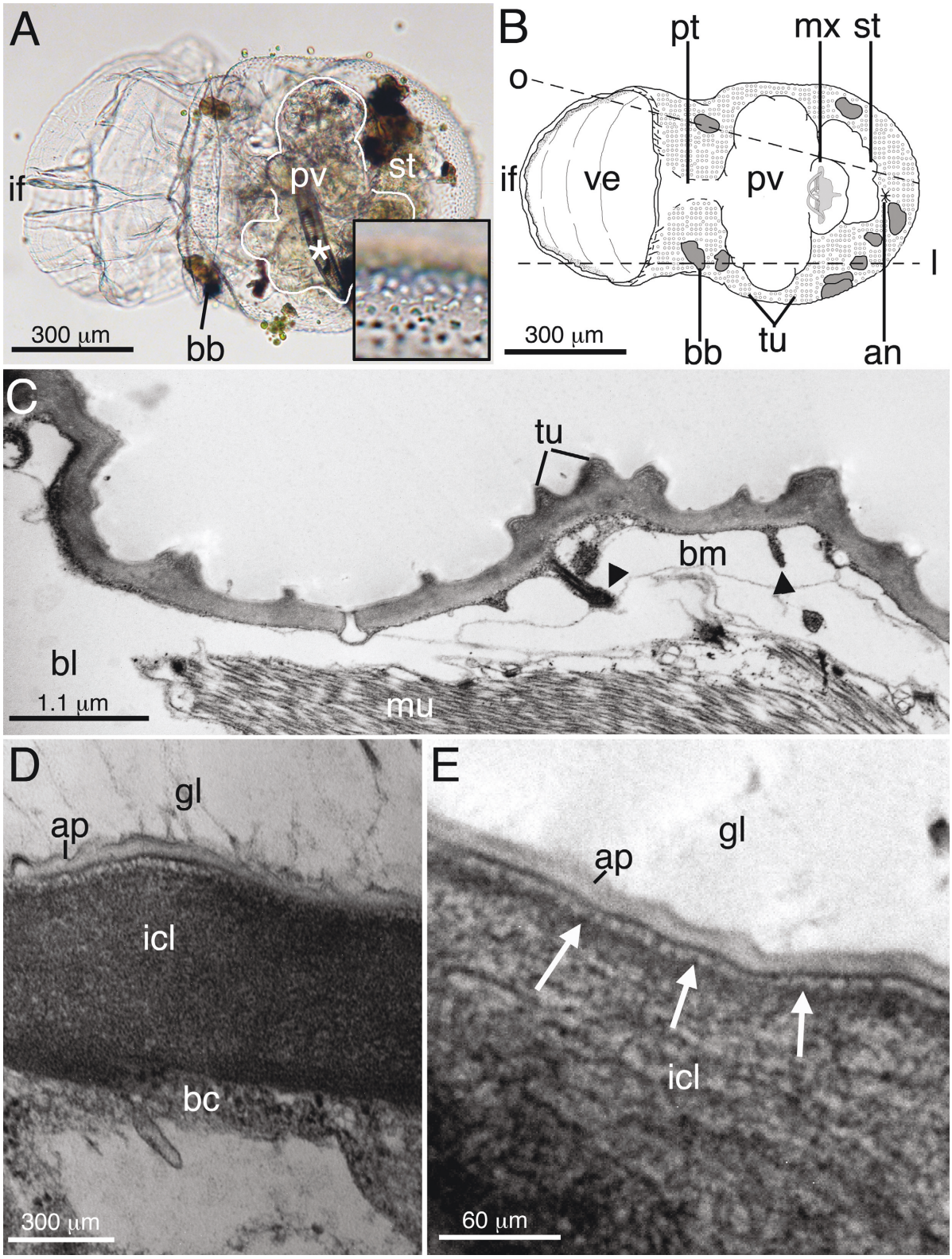
Members of *Cupelopagis vorax* have translucent body walls with very small tubercles (bumps) that were difficult to see with brightfield microscopy, but easily observed with DIC microscopy (Fig. 1A). The tubercles were most noticeable along the dorsal and ventral body wall of the trunk when animals were contracted (Fig. 1A); they were not evident on the integument of the infundibulum. At the ultrastructural level, these tubercles were abundant in all sections of the trunk (Fig. 1C), but not present on the dorsal side of the infundibulum. The integument consisted of three general layers: outer glycocalyx, syncytial epidermis with apical intracytoplasmic lamina (ICL) atop a more basal granular cytoplasm, and a basement membrane (Fig. 1C–E). Together, all three layers were 500–2300 nm thick across most of the body; variations in thickness were either due to differences in the thickness of the glycocalyx or the ICL around regions of tubercles. The glycocalyx was present over the entire animal. Anteriorly, the glycocalyx was extremely thin, ~40–56 nm, while posteriorly across much of the trunk, it could be extremely thick, from 300–900 nm, and was often thickest in between tubercles (Figs. 1D,E, 2). The glycocalyx often appeared as wispy vertical lines arising from the apical plasmalemma (Fig. 1D). Immediately beneath the glycocalyx was the apical plasmalemma, which often had a distinctly trilaminate (dark-light-dark) appearance and was generally 22–27 nm thick, although in sections of some tubercles it was up to 43 nm thick (Fig. 1D,E). Artifacts were noted in some sections; for example, a distinction between the apical membrane and the glycocalyx could often not be discerned (Fig. 1D).

The ICL lay below the apical plasmalemma and was electron dense throughout the integument, including on the trunk and infundibulum (Figs. 1D, E, 2, 3). The ICL was often amorphous in appearance, especially at low magnifications (Fig. 1C), although in several areas it contained an abundance of longitudinally oriented fibers (Fig. 1E) and electron-dense dots similar to the granular cytoplasm (Fig. 3B). Fibers were most visible in regions of the integument that were pinched or bent, and in the tubercles (described below). Apically, the ICL contained vertical striations just beneath the apical plasmalemma (Fig. 1E). The striations were often quite small (e.g., 26–35 nm long) but easily observed due to the light–dark banding pattern (Fig. 1E). The striations appeared to arise from a thin (~20–25 nm) longitudinal band of electron-dense ICL (Fig. 1E), often giving the entire apical region of the integument a bilayered appearance (see Figs. 2A–C, 3C). The remaining portion of the ICL was 170–400 nm thick through most of the body where tubercles were absent (i.e., between tubercles). Most of the ICL was consistently homogeneous in appearance (Fig. 1C), but other regions displayed a dark basal ICL just above the granular cytoplasm (Fig. 1D), and still others had dark bands in various regions (Fig. 2A,D).

Tubercles appeared as small apical protrusions of the ICL and were present across most of the body, including the dorsal and ventral sides of the trunk and also inside the mouth cavity (vestibule) of the infundibulum (but not on the dorsal side of the infundibulum) (Fig. 1). The tubercles were thickenings of the ICL only in that there was no evidence that they contained the granular cytoplasm that

Fig. 1. *Cupelopagis vorax*. **A.** DIC image of a live relaxed specimen in dorsal view; anterior is to the left and shows the cup-shaped infundibulum. An asterisk denotes a diatom contained within the proventriculus (artificially outlined). Brown pigment spots of unknown function are present in the animal. Inset: close-up of tubercles on dorsal integument. **B.** Illustration of the same specimen in ventral view to show the shape of the mouth cavity beneath the infundibulum. The mouth leads to a short pharyngeal tube that expands to become the proventriculus. The mastax and stomach are illustrated to show their position only. Dashed lines indicate planes of section for TEM: longitudinal plane (l) and oblique plane (o). **C.** Ultrastructure of the body wall, longitudinal section. Apical tubercles of the intracytoplasmic lamina project outward. Basal projections (arrowhead) of the cytoplasm are surrounded by basement membrane and project into the blastocoel and toward a skeletal muscle. **D.** Longitudinal section of the integument showing glycocalyx, apical plasmalemma, intracytoplasmic lamina, and thin layer of basal cytoplasm. **E.** Detail of integument, longitudinal section; arrows point to a dark line of intracytoplasmic lamina that is consistently present beneath the apical plasmalemma (and separated from it by an electron-lucent layer often filled with electron-dense dots). The intracytoplasmic lamina contains longitudinally oriented fibers. an, anus; ap, apical plasmalemma; bb, brown pigment spots; bc, basal cytoplasm; bl, blastocoel; bm, basement membrane; gl, glycocalyx; icl, intracytoplasmic lamina; if, infundibulum; mu, skeletal muscle; mx, mastax; pt, pharyngeal tube; pv, proventriculus; st, stomach; tu, tubercle; ve, vestibule.

formed a 'layer' below the ICL. (Note: the ICL is in fact present *within* the cytoplasm of the syncytium, but it is distinctly different in appearance from the more basal portion of the cytoplasmic syncytium and appears as a separate layer.) The tubercles generally took the shape of small rounded



humps or in some cases rectangles with flat tops (Figs. 1C, 2). Most tubercles were less than 1 μm high, but some measured up to 2.3 μm. The thickest glycocalyx existed between tubercles, and was often as thick as the tubercles were high. The ICL at the base of a tubercle was generally indistinguishable from the ICL of the surrounding integument (Fig. 2A,B), but within the body of the tubercle itself, the ICL matrix often appeared more fibrous, mesh-like, and less electron dense (Fig. 2). The top of many tubercles was often speckled with electron-dense dots close to the apical plasma membrane (not shown).

Beneath the ICL was a highly granular cytoplasm (Figs. 1D, 2–4) that was distinctly different from the cytoplasm of any other epithelium including the stomach (st, Figs. 3D, 4) and proventriculus (Fig. 4). The cytoplasm ranged 60–400 nm thick. No nuclei were observed, but there were occasional mitochondria, endoplasmic reticulum, and various types of vesicles (below). The cytoplasm was bordered basally with a somewhat indistinct plasma membrane. In several regions of the body, the cytoplasm that bordered the plasmalemma formed thin protuberances that projected into the blastocoel (Fig. 1C). These protuberances were always much more electron dense than the granular cytoplasm and ranged 150–700 nm in length. A thin basement membrane ~12–20 nm thick covered the basal plasmalemma including all basal protuberances. No direct anatomical connections between these protuberances and other organs (e.g., muscles, protonephridia) were observed, although they often contacted the basement membrane of some muscles (Fig. 1C). It was unknown whether these points of contact were incidental or not, but plaques or other evidence of junctional complexes were not evident.

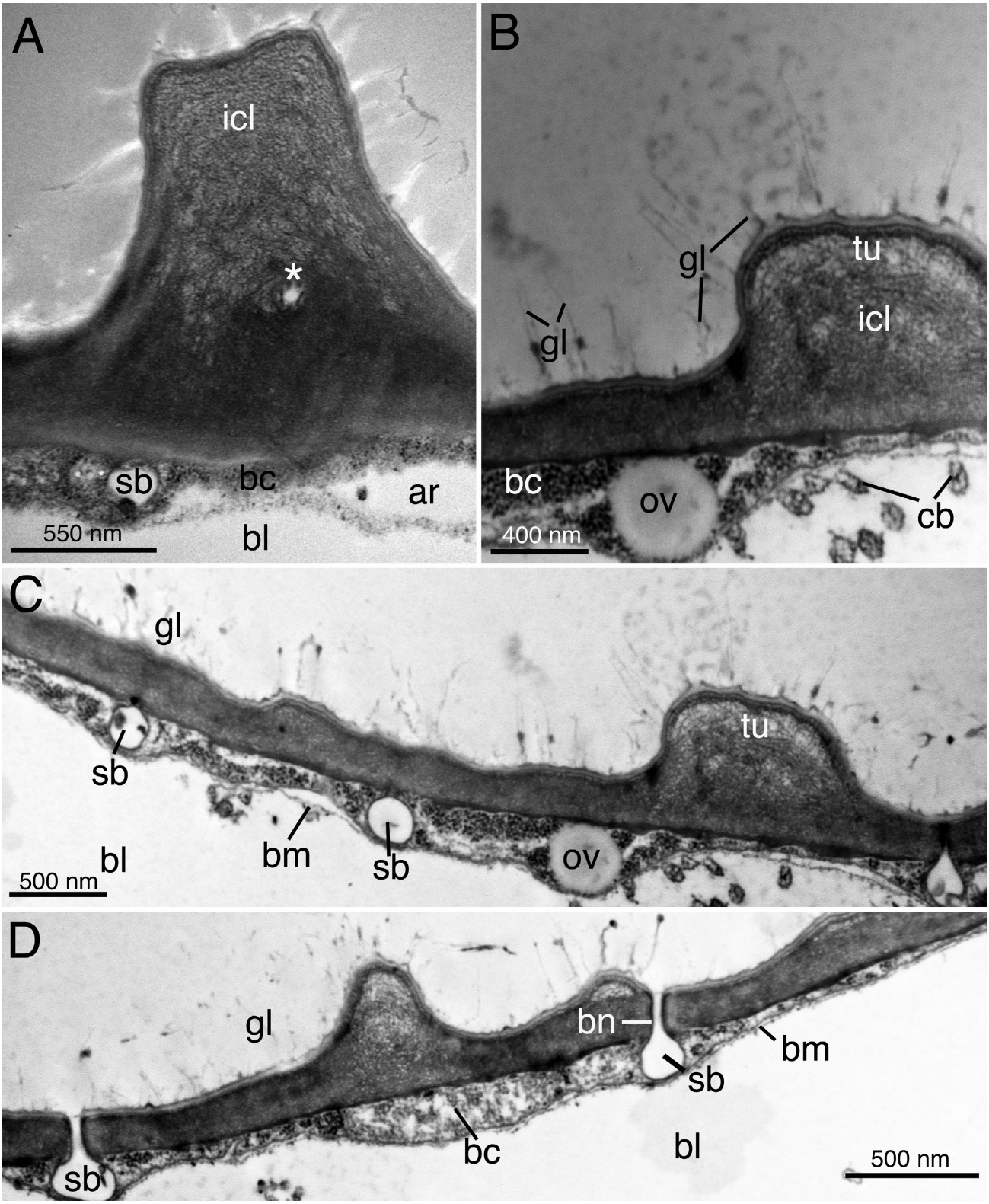
Two types of membrane-bound vesicles were present in the basal cytoplasm only: secretory bulbs (sb, Fig. 3A–C) and ovoid vesicles (ov, Fig. 2B,C). No membrane-bound vesicles were present in the apical ICL, although secretory bulbs did form necks that extended through the ICL. The secretory bulbs varied in size (110–264 nm diameter) and most had an electron-lucent core, but several were also observed to contain undetermined electron-dense contents (Fig. 2C). Secretory bulbs formed elongated necks that fused with the apical plasmalemma (bn, Figs. 2D, 3C). In several sections, bulbs without elongated necks were present (Fig. 2C), but these are hypothesized to be secretory bulbs viewed from a different plane of section that did not show the neck. The necks were up to 120 nm in diameter (including an electron-lucent core up to 76 nm wide). Several regions of the ICL also contained small vacuole-like structures with an outer rim of six, radially arranged, electron-dense spots (asterisk, Fig. 2A). The source of these structures is unknown, but may be either cross-sections through the neck of a secretory bulb or pores in the integument. Other vesicle-like structures were also present in the basal cytoplasm. One type (ar, Fig. 2A) was rare, always hollow, and lacked a delimiting membrane, and therefore interpreted as an artifact of fixation. Another type (ov, Fig. 2B,C) had a thin but distinctive membrane and always contained opaque, amorphous contents. This vesicle was 320–411 nm in diameter, only present in the basal cytoplasm, and never observed to fuse with the apical or basal plasmalemma.

Proventriculus

The proventriculus was a highly distensible dorsal sack that extended laterally on both sides of the body above the stomach (Fig. 1A,B). A short pharyngeal tube (esophagus of Vashist & Dawar 1969; Koste 1973) is proposed to connect the mouth to the proventriculus, but this was not observed in our sections. After capture of prey such as gastrotrichs (*Lepidodermella squamata* [Dujardin 1841]) and protists, the proventriculus could be seen to expand and contract, although this movement did not appear to be a result of

Fig. 2. Longitudinal sections of the integument of *C. vorax* with a focus on the tubercles, intracytoplasmic lamina, and the different types of vesicles. **A.** Close-up of a tubercle, revealing the fibrous apical portion of the intracytoplasmic lamina. Note the presence of a small pore or vacuole with radially arranged electron-dense dots (*). A secretory bulb is present in the basal cytoplasm, below which is the blastocoel. Large vacuoles were present in some sections and are interpreted as artifacts because no delimiting membrane was present. **B.** Close-up, the integument reveals wisps of glycocalyx in between and on top of a tubercle. A large ovoid vesicle is present in the basal cytoplasm. Cytoplasmic blebs were rare features in sections of the integument. **C.** Portion of the dorsal integument revealing the two types of vesicles. **D.** Ventral integument showing secretory bulbs with necks that traverse the intracytoplasmic lamina. The basement membrane was always present. ar, vacuole; bc, basal cytoplasm; bl, blastocoel; bm, basement membrane; bn, neck of secretory bulb; cb, cytoplasmic bleb; gl, glycocalyx; icl, intracytoplasmic lamina; ov, ovoid vesicle; sb, secretory bulb; tu, tubercle.

the proventriculus itself, but rather movements of either the contained prey (gastrotrichs) or contractions of the somatic musculature and/or pumping of the more posterior mastax. Both longitudinal and oblique sections of the proventriculus were observed (Figs. 1A,B, 4), but its highly folded



nature (e.g., Fig. 4A) prevented a complete characterization of the entire structure, so we are uncertain whether the epithelium is homogeneous throughout. Sections revealed numerous eukaryotic organisms (e.g., diatoms, flagellates; Figs. 1, 4A,B) in the proventricular cavity, as well as other materials from larger organisms, but these were unidentifiable because of the plane of section and/or partial degradation of the organism. The proventricular epithelium was often in close contact with skeletal muscles and the stomach, but not obviously joined to either through cell junctions (Fig. 4). The epithelium was syncytial and without any evidence of ICL. The lumenal side of the syncytium was mostly covered by a brush border of microvilli embedded in a thick glycocalyx (Fig. 4B–D). The glycocalyx had a fibrous or granular appearance (Fig. 4D) and was up to 420 nm thick and covered all microvilli. The microvilli were at least 65 nm wide and up to 410 nm long, but most were only 250–300 nm in length, which might be the result of the sections not containing the complete length of each microvillus. A noticeable, electron-lucent halo surrounded all microvilli where they projected into the glycocalyx (Fig. 4D). In several sections, the epithelium was pinched and folded (Fig. 4B).

Just below the plasma membrane was a terminal web, evidenced by longitudinal striations that were not present anywhere else in the cytoplasm (not shown). The rest of the cytoplasm was mostly electron lucent with some mitochondria, ribosomes, and limited smooth endoplasmic reticulum (Fig. 4D). There was no evidence of secretory activity along the length of the syncytium: that is, there were no invaginations of the apical or basal plasmalemma (indicating endo- or exocytosis) and no secretory bulbs like those of the integument. Below the basal plasmalemma was a thin basement membrane. The basement membrane of the proventricular epithelium remained close to the epithelium despite the folding of the epithelium and its contact with muscles and the stomach (Fig. 4B).

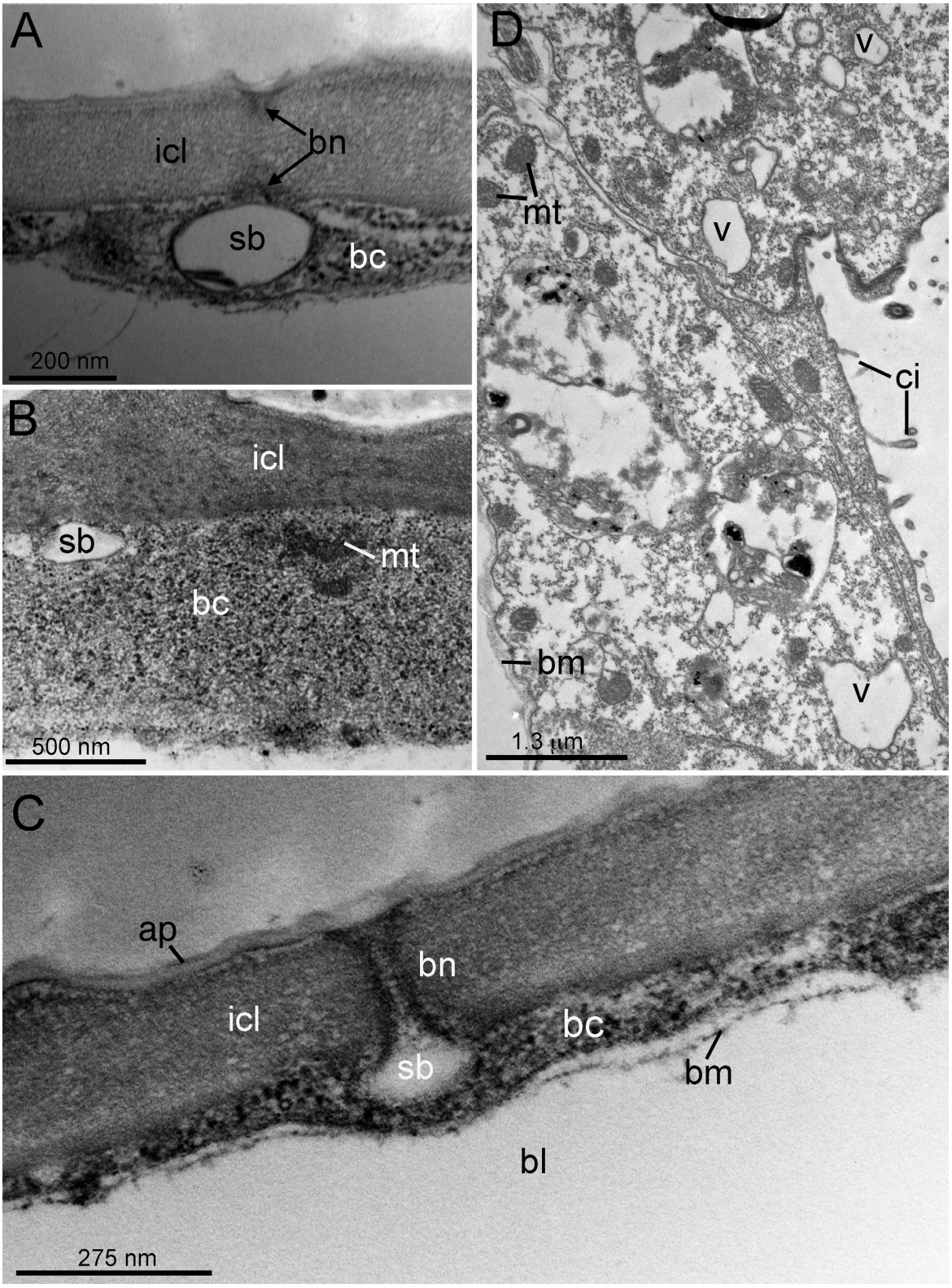
Discussion

Individuals of *Cupelopagis vorax* are sessile and raptorial rotifers of the Collothecaceae, a taxon of ambush predators in the clade Gnesiotrocha (Wallace et al. 2015). Collothecids are defined in part by possessing an infundibulum, which is an expansive region of the head that can fold over and capture live prey. Most of these predators rely on passive contact by small plankton (e.g., protists), but members of *C. vorax* are active predators, which pivot on their permanently attached pedal stalk toward swimming and crawling prey before engulfing them (Bevington et al. 1995). To date, no raptorial gnesiotrochans have been examined at the ultrastructural level, although rotifers from all major clades (Bdelloidea, Ploima, Gnesiotrocha) have received ample attention regarding the structure of the integument and to a lesser degree, their digestive tract (reviewed in Clément & Wurdak 1991; Fontaneto & De Smet 2015). Here, we examined portions of both organ systems to determine whether and how they differ from other rotifers.

Our data reveal that the integument of individuals of *C. vorax* is covered by glycocalyx that differs in thickness across the body, ranging up to 900 nm thick in between the series of protuberances that project from the surface of the integument. The thick glycocalyx might be the cuticle that is referenced by Vashist & Dawar (1969) in their description of the species' anatomy. The protuberances are present across the entire trunk of the animal and probably correspond to the tubercles also described by Vashist & Dawar (1969). The authors noted a stellate pattern of tubercles on their examined specimens, and while we did not observe this species of rotifer with scanning electron microscopy, we also noted the presence of tubercles with light microscopy (DIC). These tubercles are products of the intracytoplasmic lamina (ICL), like similar protuberances in another gnesiotrochan examined with TEM, *Sinantherina socialis* (Linnaeus 1858) (Hochberg

Fig. 3. Ultrastructural details of the integument and stomach. **A.** Ultrastructure of the integument (longitudinal section) showing secretory bulb with partial section through a neck that traverses the intracytoplasmic lamina; basal cytoplasm is relatively thin. **B.** Oblique section of integument with thicker cytoplasm (compare to A). Note that the intracytoplasmic lamina has numerous electron-dense dots similar to the basal cytoplasm. Mitochondria are present among the granules of the basal cytoplasm, as are secretory bulbs. **C.** Detail of a secretory bulb with bulb neck that traverses the intracytoplasmic lamina, oblique section. **D.** Oblique section of the stomach epithelium for comparison to the integument. Note the highly voluminous cytoplasm, mitochondria, vesicles, basement membrane, and the numerous cilia on the lumenal border. ap, apical plasmalemma; bc, basal cytoplasm; bl, blastocoel; bm, basement membrane; bn, secretory bulb neck; ci, cilium; icl, intracytoplasmic lamina; mt, mitochondrion; sb, secretory bulb; v, vesicles.

et al. 2015). In the latter species, a ridge-and-groove like system characterizes the rotifer's integument and is a result of unique projections of the ICL, as opposed to folds in the integument (Hochberg et al. 2015). Both gnesiotrochans, therefore, can be said to possess an ornamented integument that stands in



contrast to the largely non-textured integument of other (non-gnesiotrochan) rotifers examined with TEM (Koehler 1965, 1966; Clément 1969, 1980; Storch & Welsch 1969; Brodie 1970; Schramm 1978; Hendelberg et al. 1979; Clément & Wurdak 1991; Ahlrichs 1997).

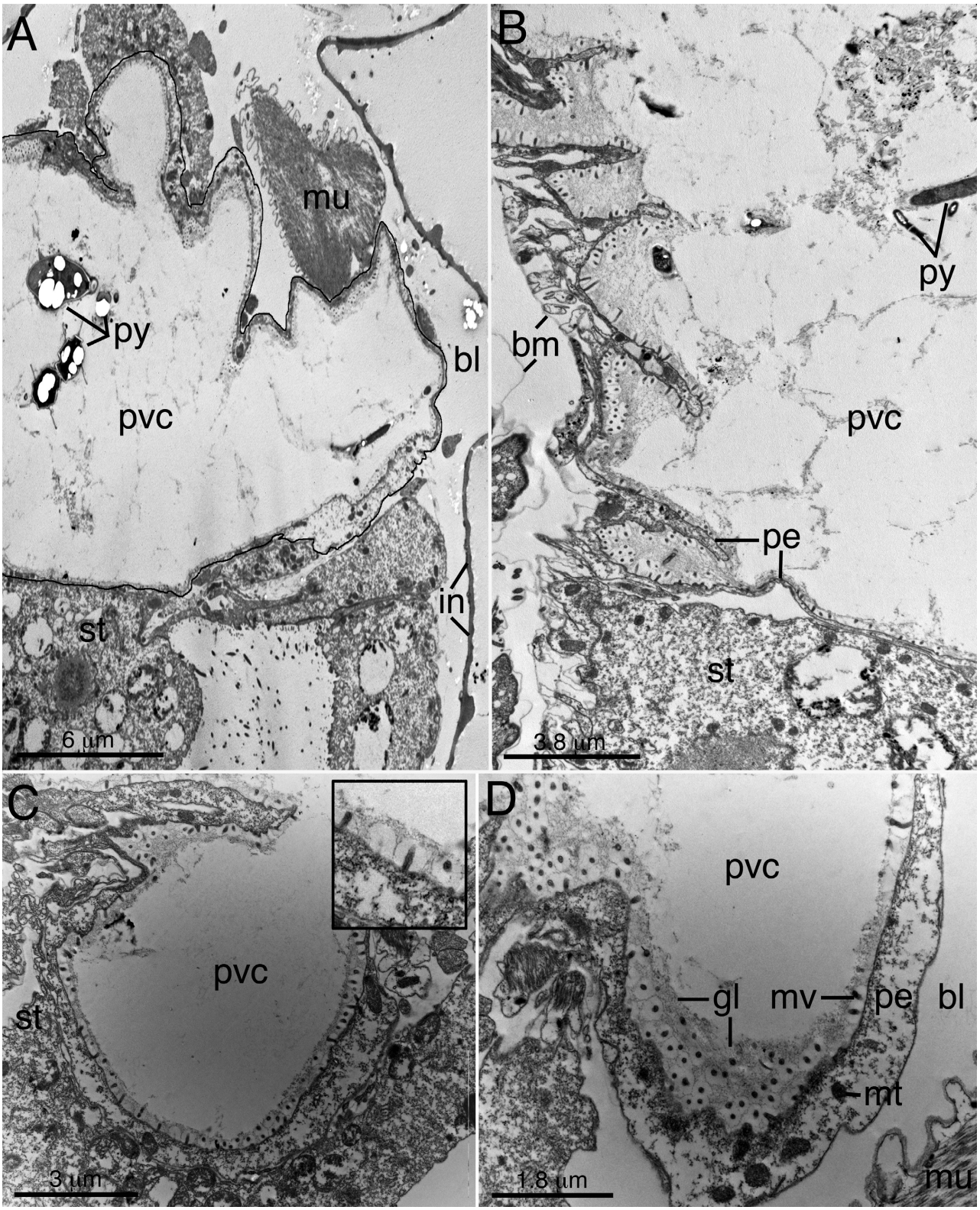
The syncytial epidermis of *C. vorax* fits broadly within the ultrastructural 'type' revealed for other monogonont rotifers (reviewed in Clément & Wurdak 1991; Fontaneto & De Smet 2015): a thickened apical region that contains keratin-like proteins (Bender & Kleinow 1988; Kleinow 1993) atop a thinner basal region that consists mostly of electron-lucent cytoplasm and various organelles (Clément & Wurdak 1991). The ICL itself may have two distinct layers as revealed in *S. socialis* (Hochberg et al. 2015), but in *C. vorax*, the ICL consists of a single relatively homogeneous layer. This layer is always electron dense, but the density may vary slightly as evidenced in some sections (see Fig. 2). In general, the ICL is mostly amorphous, but it has a fibrous appearance in the region of the apical tubercles; this fibrous region is similar in appearance to the fibrillar region of the ICL in *S. socialis* (Hochberg et al. 2015).

Beneath the ICL is an extremely thin and highly granular cytoplasm that forms a distinct (but not separate) layer; the granularity is probably the result of abundant ribosomes, although the endoplasmic reticulum was never abundant in any of the sectioned material. This region differs significantly from the cytoplasmic region in *S. socialis*, which is highly voluminous and electron lucent. Hochberg et al. (2015) hypothesized that the voluminous cytoplasm in *S. socialis* might function as a secondary hydrostatic skeleton (in addition to the blastocoel) to stiffen the elongated body that always assumes an upright posture in this sessile species. Alternatively, most other rotifers, including *C. vorax*, have a very thin and unremarkable cytoplasm, save for the highly granular nature. Also, like many other rotifers including bdelloids, monogononts, and seisonids (Koehler 1965, 1966; Clément 1969, 1980; Storch & Welsch 1969; Brodie 1970; Schramm 1978; Hendelberg et al. 1979; Clément & Wurdak 1991; Ahlrichs 1997), specimens of *C. vorax* did have an abundance of vesicles within their cytoplasm. The two most common types of membrane-bound vesicles—secretory bulbs and ovoid vesicles—were both restricted to the basal cytoplasm. Neither vesicle was observed to be connected to either endoplasmic reticulum or Golgi, so their origins remain to be determined. The major differences between the two were observed in their sizes (ovoid vesicles were always larger; see Fig. 2C), the opacity of their contents (ovoid vesicles were always lightly stained, while secretory bulbs were clear or contained small electron-dense inclusions; Fig. 2C), and the presence of a membrane-bound neck that connected to the apical plasmalemma (only observed in secretory bulbs; Figs. 2D, 3C). Presently, there is no classification system for the types of vesicles present in rotifers, although several types are shown or described in the literature (Koehler 1965, 1966; Clément 1969, 1980; Storch & Welsch 1969; Brodie 1970; Schramm 1978; Hendelberg et al. 1979; Clément & Wurdak 1991; Ahlrichs 1997). While it is possible that the two types of vesicles observed in *C. vorax* are the same but have a different appearance depending on the amount of contained secretory material (e.g., see Koehler [1965] and Schramm [1978]), it is also possible that they have separate functions (transport different contents). In fact, as noted by Koehler (1965) for *Asplanchna sieboldii* (Leydig 1854), the secretory bulbs appear to only transport ICL material. Future research on the rotifer integument would be advised to apply histochemical tests to differentiate the contents of the vesicles and determine their functions, such as DAB incubation for catalase or $K_3Fe(CN)_6$ for glycogen (Schramm 1978).

In addition to our investigation of the integument, we also examined the ultrastructure of the proventriculus, which is a unique sack-like

Fig. 4. Proventriculus of *C. vorax*. **A.** Portion of the proventricular cavity on the left side of the body, artificially outlined (dark line), oblique section. Numerous prey are present in the cavity. The cavity is surrounded by skeletal muscles present in the blastocoel. The proventriculus does not contact the integument in any sections, but is often in contact with the stomach. **B.** Portion of the proventriculus where its epithelium creates large lumenal folds, longitudinal section. The basement membrane of the proventriculus occurs on the blastocoel side. **C.** 'Pocket' of proventriculur epithelium where it contacts the stomach, longitudinal section. Inset: close-up of the microvilli showing the electron-lucent halo around each microvillus. **D.** Detail of the proventricular epithelium showing electron-lucent cytoplasm and mitochondria, longitudinal section. The numerous microvilli and thick glycocalyx occur on the lumenal side of the proventriculus. bl, blastocoel; bm, basement membrane; gl, glycocalyx; in, integument; mt, mitochondrion; mu, skeletal muscle; mv, microvilli; pe, proventricular epithelium; pvc, proventricular cavity; py, prey; st, stomach.

expansion of the foregut that functions to hold prey after capture by the large infundibulum. As noted by Vashist & Dawar (1969), the proventriculus might also function in maceration and thereby prepare prey for easier processing by the mastax (jaws). In point of fact, we have observed pieces of eukaryotic



organisms in the proventriculus, which might be evidence of digestive processes, but this is difficult to verify with ultrathin-sectioned material of organisms we cannot identify. However, we have also observed whole smaller organisms (algal cells, flagellates; Fig. 4A,B) in the proventriculus as well. Based on the presence of both partial and whole organisms, it is difficult to determine whether the cavity does in fact play a role in digestion. One might suspect that if the proventriculus had a hardened or grinding type of epithelium as evidenced by a thick ICL (which it did not), then perhaps only larger prey would be degraded, as our observations may have revealed; if enzymatic activity was also characteristic of the proventriculus, then small organisms would be as equally degraded as larger organisms, which did not appear to be the case. However, our results reveal that the proventricular epithelium is neither stiffened by ICL nor obviously secretory (no evidence of vesicles of any sort), which would be evidence of enzymatic activity. Instead, the syncytium is extremely thin and virtually featureless save for the apical brush border of microvilli embedded in a thick glycocalyx. And while we do note that the proventricular epithelium is not entirely homogeneous—there are some regions that appear to have few microvilli and no glycocalyx—we hypothesize that the proventriculus may play a role in food processing.

We have observed that organisms captured by *C. vorax* are only temporarily (minutes) stored in the proventriculus (also noted by Bevington et al. 1995 for protozoans), and that pumping of the mastax often brings prey within reach of the jaws. The jaws (trophi) of *C. vorax* are of the uncinate type, which function mainly in grasping prey (Wallace et al. 2015). We suspect that the jaws might in fact tear larger prey apart prior to movement into the stomach. If so, then the proventriculus would function for food processing by temporarily storing pieces that require further degradation by the mastax. If secretions by gastric glands also make their way into the proventriculus (by way of the mastax), then it is possible that the cavity also functions in part for extracellular digestion. So how are the microvilli and glycocalyx related to this function? We suspect that the microvilli do not play a role in absorption but instead function to hold the thick glycocalyx in place. The function of the glycocalyx then is to prevent damage either by the living prey or perhaps regurgitated enzymatic secretion.

Finally, we emphasize that the proventriculus is an unusual structure as far as rotifer digestive anatomy is concerned. Most rotifers lack an expansive foregut, and by all accounts, it would appear that one is present only in species of Collothecaceae (Collothecidae and Atrochidae) (see Wright 1958). As stated by Clément & Wurdak (1991), the structure of the foregut is related to the mode of feeding, and the style observed in *C. vorax* certainly differs from that in other raptorial predators that have been examined previously, such as *Asplanchna* (Salt et al. 1978). Most species appear to have a short and cylindrical foregut (pharyngeal or buccal tube) that is often ciliated and surrounded by muscles (referred to as the esophagus by Schramm 1978; the buccal tube by Clément & Wurdak 1991). Unfortunately, we were unable to obtain sections of the pharyngeal tube from specimens of *C. vorax* for comparison to other species or to compare to the proventriculus within specimens, which might provide insights into its origins. Future research on the proventriculus might include studies of other collothecaceans that show similar raptorial feeding styles (e.g., *Acyclus*) or feeding styles that differ in the types of prey consumed (e.g., *Collotheca* and *Stephanoceros* feed on small planktonic prey) to determine the function of the proventricular epithelium.

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