Invertebrate Biology

Invertebrate Biology x(x): 1–8.

DOI: 10.1111/ivb.12085

# Ultrastructure of the rotifer integument: peculiarities of *Sinantherina socialis* (Monogononta: Gnesiotrocha)

Rick Hochberg,[1,a] Adele Hochberg,[1] and Courtney Chan[2]

[1] Department of Biological Sciences, University of Massachusetts Lowell, Lowell, Massachusetts 01854, USA
[2] Andover High School, Andover, Massachusetts 01810, USA

**Abstract.** The rotifer integument is a well-described syncytium that contains an apical intracytoplasmic lamina (ICL) that functions for both skeletal support and muscle insertion. To date, there is limited information on the structure of the integument in species of Gnesiotrocha, a diverse subclade of Monogononta that consists of solitary, colonial, sessile, and planktonic species. In this study, we examined the ultrastructure of the integument in the colonial rotifer *Sinantherina socialis* to determine how it corresponds to that of other monogononts. The integument of *S. socialis* was broadly similar to that of other rotifers, consisting of a thickened glycocalyx, multilaminate ICL, and syncytial epidermis. However, it was different in several regards. The ICL consisted of three distinct layers from apical to basal: layer 1 consisted of at least two electron-dense laminae; layer 2 was a thickened matrix of amorphous, electron-dense material or was fibrous; and layer 3 was an electron-dense lamina of varying thickness that covered the underlying syncytium. Significantly, layers 1 and 2 formed a ridge-and-groove like system of finger-like projections across the trunk surface that has not been observed in other rotifers. A voluminous syncytial cytoplasm (up to 3 μm thick) was present beneath the ICL and was mostly electron lucent and with few organelles. Bundles of potential microtubules were scattered throughout the syncytium. We hypothesize that the voluminous cytoplasm with microtubules serves as skeletal support for the rotifer's sessile lifestyle, while the external ridges may function as a texture-based deterrent to predators, or serves to trap secretions from the species' defensive glands. Basally, the epidermis was highly folded and bordered by a thin basal lamina that separated the plasmalemma from the blastocoel. Membrane-bound vesicles were present throughout the integument's cytoplasm and are hypothesized to function in the secretion of extracellular matrix and in the maintenance of the ICL.

*Additional key words:* cuticle, defense, epidermis, Rotifera, ultrastructure

Rotifera consists of mostly microscopic, free-living invertebrates that inhabit the plankton and benthos of freshwater lakes, where they form an important part of the microbial loop (Wallace et al. 2015). Two defining rotiferan features, a complex ciliated head (corona) and a muscular pharynx with intricate jaws (mastax), have been examined in detail across the three major clades, Bdelloidea, Monogononta and Seisonacea, and have been important in defining their systematics and interpreting their evolutionary history. A third feature, and one that links the Rotifera *sensu strictu* with parasitic Acanthocephala (Rotifera *sensu lato*), is the structure of the integument (Lorenzen 1985), which is generally described as a syncytium with an internal "cuticle" of fibrous proteins that create an intracytoplasmic lamina (ICL). Significant variation is present in the ultrastructural organization of the ICL across all four clades including the Bdelloidea (Koehler 1965, 1966; Storch & Welsch 1969; Clément & Wurdak 1991), Monogononta (Koehler 1966; Clément 1969, 1977, 1980; Storch & Welsch 1969; Brodie 1970; Clément & Wurdak 1991), Seisonacea (Ahlrichs 1997), and Acanthocephala (reviewed in Dunagan & Miller 1990). While the function(s) of this variation is largely speculative, the integumental organization is still considered homologous throughout Rotifera (Clément & Wurdak 1991; Funch et al. 2005).

---

[a] Author for correspondence.
E-mail: rick_hochberg@uml.edu

Clément & Wurdak (1991) have provided the most comprehensive review of integumental ultrastructure throughout the Rotifera (excluding Acanthocephala), and described the epidermis as a form of thickened peripheral skeleton covered by a thin extracellular cuticle (glycocalyx) that itself serves no skeletal function. The epidermis is entirely syncytial and generally contains a pair of distinct laminae. In bdelloids, the laminae include a thin outer electron-lucent layer and a thicker inner electron-dense region, the latter of which functions in skeletal support. In monogononts, the two laminae are reversed in organization and are more diverse in structure, with a variable but thickened electron-dense outer region and a thinner and more electron-lucent inner region. In sesionoids, the more basal of the two laminae is thickest (as in bdelloids) and separated from the apical lamina by a thin region of cytoplasm. Membrane-bound vesicles are present throughout the integumental cytoplasm of most species and expel secretion products to the outside of the body, although the nature of the secretions and their functions are unknown.

To date, most studies of the rotifer integument have focused on the solitary free-living forms, and there are no formal descriptions of the integument in sessile or colonial species that make up the bulk of the diversity within the subclade Gnesiotrocha (Monogononta). While the monophyly of Gnesiotrocha remains speculative, the lineage contains a diversity of species with peculiar lifestyles and body plans (reviewed in Wallace et al. 2015), the latter of which may present unusual features such as the absence of a corona (adults of Collothecaceae), the presence of locomotory appendages (e.g., some species of Flosculariaceae), the presence of a hard or soft extracorporeal tube (e.g., species of Flosculariacea and Collothecacae), or the presence of defensive epidermal spines or warts (e.g., species of *Sinantherina*). It is this latter peculiarity—unusual surface features—that is the focus of the current investigation. Hochberg & Lilley (2010) provided a description of the neuromuscular system of *Sinantherina socialis* (LINNAEUS 1858), a sessile rotifer that forms large colonies on submerged vegetation. In their study, the authors revealed that the integument of *S. socialis* had an unusual surface microtexture not documented for any other rotifer. In this study, we provide a more detailed description of this unusual integument using electron microscopy, and speculate on its potential functions.

## Methods

*Sinantherina socialis* was collected on submerged plants in Mascuppic Pond in Tyngsboro, MA in the summer months of 2013 and 2014. Living specimens were photographed with a Sony Handycam mounted on either a Zeiss Stemi stereomicroscope or Zeiss A1 compound microscope. Individual specimens were extracted from colonies with "000" insect pins and then anaesthetized with 1.5% $MgCl_2$ in pond water for 10–15 min in a 5 mL embryo dish.

For scanning electron microscopy (SEM), ten colonies still attached to their substratum (grass) were anesthetized, fixed in 2.5% glutaraldehyde in 0.1 mol $L^{-1}$ phosphate buffer (PB, pH 7.3) for 2 h, rinsed in 0.1 mol $L^{-1}$ PB for 1 h, postfixed in 1% $OsO_4$ in 0.1 mol $L^{-1}$ PB for 1 h, and rinsed in 0.1 mol $L^{-1}$ PB for 1 h. Colonies were dehydrated in an ethanol series (70%, 90%, 100% ×2) for 10 min each step, and subsequently critical point dried in a Tousimis SamDri PVT-3D, coated with gold, and viewed on a JEOL JSM 6390 SEM.

Approximately fifty individual specimens for transmission electron microscopy (TEM) were fixed in 2.5% glutaraldeyde in 0.1M sodium cacodylate buffer (pH 7.3) for 2 h, postfixed in 1% $OsO_4$ in 0.1 M sodium cacodylate for 1 h, and subsequently rinsed in the same buffer for 1 h. Following an ethanol dehydration (70%, 90%, 100% ×2), specimens were placed in propylene oxide (PO) for 20 min (×2), and slowly infiltrated in a PO:epoxy resin mixture (Araldite, EMbed 812; Electron Microscopy Sciences) in ratios of 3:1 for 1 h, 1:1 for 1 h, and 1:3 for 1 h. Specimens were placed in pure epoxy resin overnight (12 h) on a rotator and then embedded in pure epoxy resin the next day. The resin was cured in an oven at 60°C for 24 h, and five specimens were sectioned at 70–90 nm on a Reichert ultracut ultramicrotome. Sections were stained in uranyl acetate and lead citrate and examined on a Phillips EM 400T electron microscope equipped with an Advantage HRL-B bottom mounted 1.3 Megapixel CCD camera and a Pentium computer. All image capture was digital, and no alterations to the photographs were made except for cropping and changes to brightness and contrast.

## Results

### External morphology

Colony members had a smooth body wall when examined with brightfield microscopy (Fig. 1A,B).

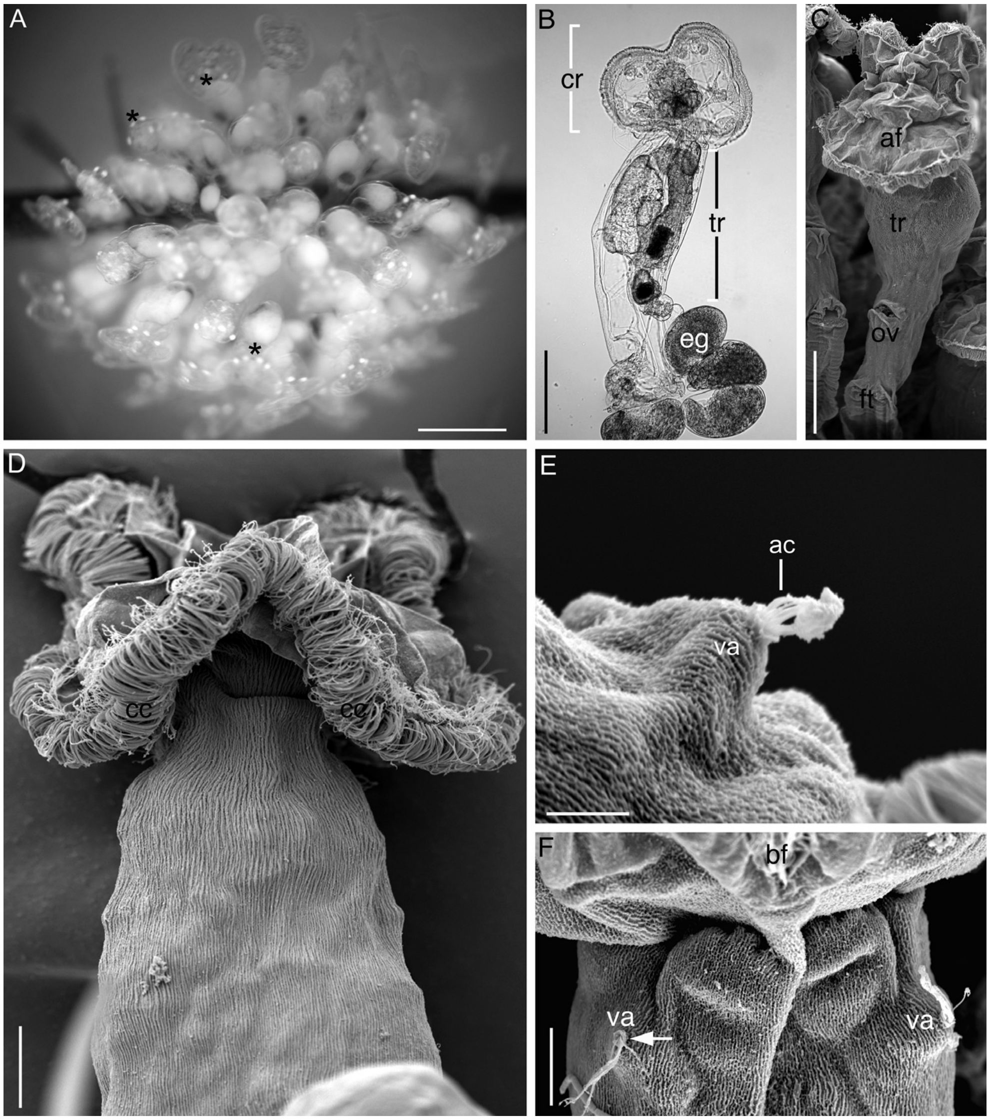

**Fig. 1.** Adults of *Sinantherina socialis*. **A.** Brightfield image of colony on submerged vegetation. Defensive warts (*) are present on all specimens. Scale bar=500 µm. **B.** Brightfield image of a single adult with eggs (e.g) extracted from the colony and showing a smooth body wall. The corona (cr) and trunk (tr) are visible, while the foot is contracted and partly hidden by the eggs. Scale bar=100 µm. **C.** Scanning electron micrograph (SEM) of a single adult within a colony. The texture of the integument appears less smooth with increasing magnification. The ridged microtexture is only apparent on the trunk (tr) and around the oviferon (ov), but is absent from the foot (ft) and apical field (af) of the corona. Scale bar=500 µm. **D.** SEM of the anterior trunk between the oviferon and coronal cilia (cc), dorsal view. Scale bar=20 µm. **E.** SEM of a ventral antennae (va) with cilia (ac) extending out the apical pore. Scale bar=4 µm. **F.** SEM of the ventral integument posterior of the corona (not shown) and buccal field (bf). The only regions of the integument that lack microtexture are the openings to the ventral antennae (arrow). Scale bar=10 µm.

Traces of the ridge-like microtexture were barely visible with low magnification SEM (Fig. 1C) but could be readily observed at higher magnifications on the dorsal side (Fig. 1D) and ventral side (Fig. 1E,F). Posteriorly, the integument of the foot (between the site of substratum attachment and the oviferon; see Fig. 1C) was wrinkled and without noticeable texture (data not shown). The integument of the trunk had a very dense ridge-and-groove like microtexture that formed longitudinal lines along the surface but with numerous interdigitations between lines that gave them a zipper-like appearance at high magnification (Fig. 2A). The only trunk regions that lacked the ridge-and-groove microtexture were the openings to the ventral antennae, which were formed of a ring of smooth integument around the sensory cilia (arrow, Fig. 1F).

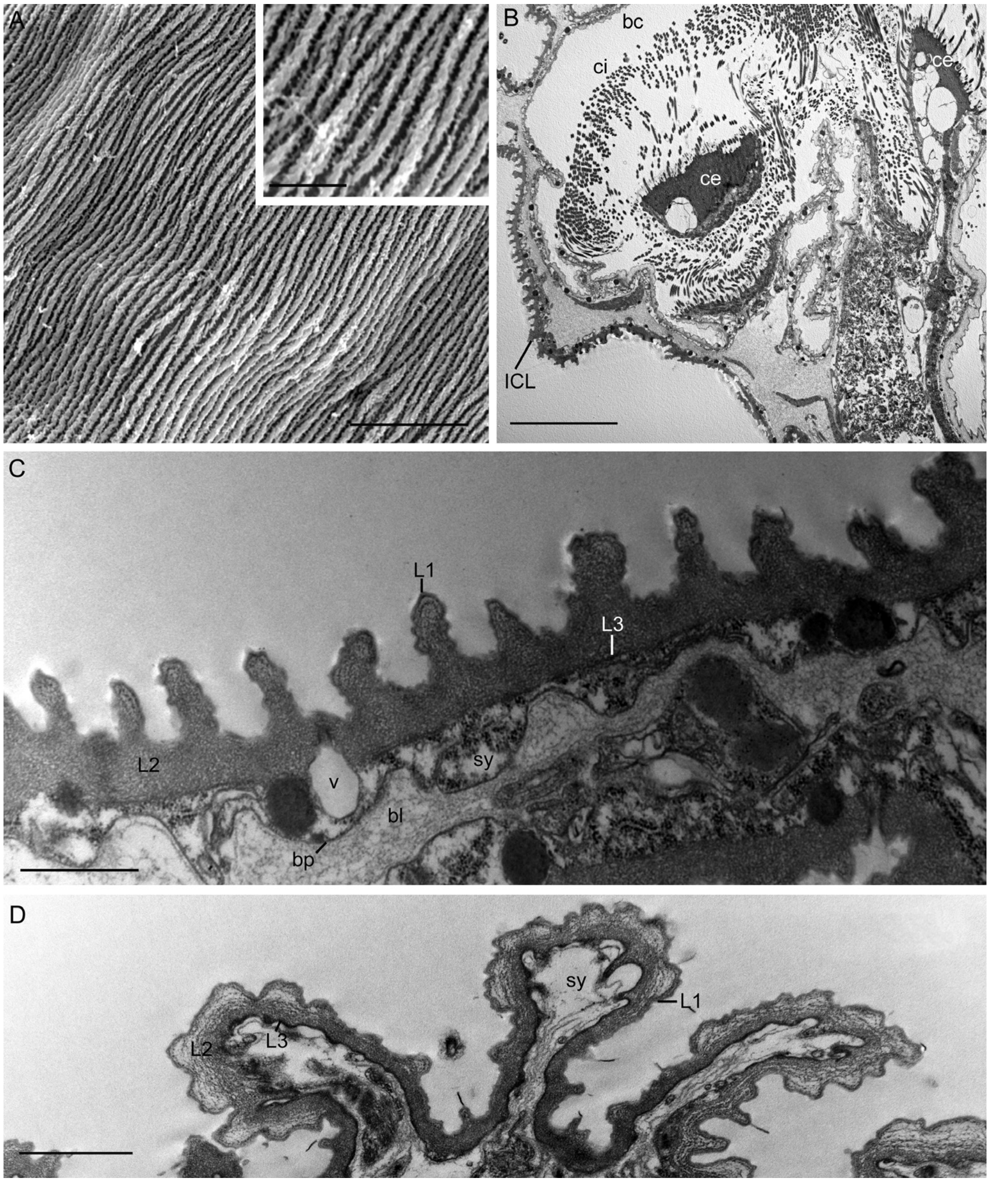

**Fig. 2.** Ultrastructure of the integument of an adult specimen of *Sinantherina socialis*. **A.** Scanning electron micrograph of the trunk epidermis showing the ridge-and-groove microtexture. Scale bar=7 μm. Inset: closeup of the integument showing the zipper-like appearance. Inset scale bar=3 μm. **B.** Transmission electron micrograph (TEM) of an oblique section through the anterior trunk region with the corona retracted into the body. The cilia (ci) and epidermal ciliary cushions (ce) can be seen inside the blastocoel (bc). The ridge-and-groove microtexture of the intracytoplasmic lamina (ICL) is present around this portion of the trunk. Scale bar=8 μm. **C.** TEM of the ICL showing the three layers: layer 1(L1) is the apical cell border; layer 2 (L2) is the mostly granular matrix; and layer 3 (L3) is the electron-dense layer that borders the underlying cytoplasmic syncytium (sy). A vesicular bulb (v) is proximal to L2, but its function (exocytotic or endocytotic) is unknown. A thickened basal lamina (bl) is present below the basal plasmalemma (bp). Scale bar=700 nm. **D.** TEM of the folded body wall of the anterior trunk region. The folds show the stretched regions of the ICL, which highlight the fibrous matrix of L2 in this region. L3 is present as a very electron-dense membrane. Scale bar=1 μm.

Anteriorly, the surface below the coronal cilia and on either side of the buccal field also had a ridged integument (Fig. 1F). The apical field of the corona was smooth and lacked microtexture (Fig. 1C).

## Ultrastructure of the integument

**Intracytoplasmic lamina.** The ridge-and-groove-like microtexture of the integument (Fig. 2A) was a prod-

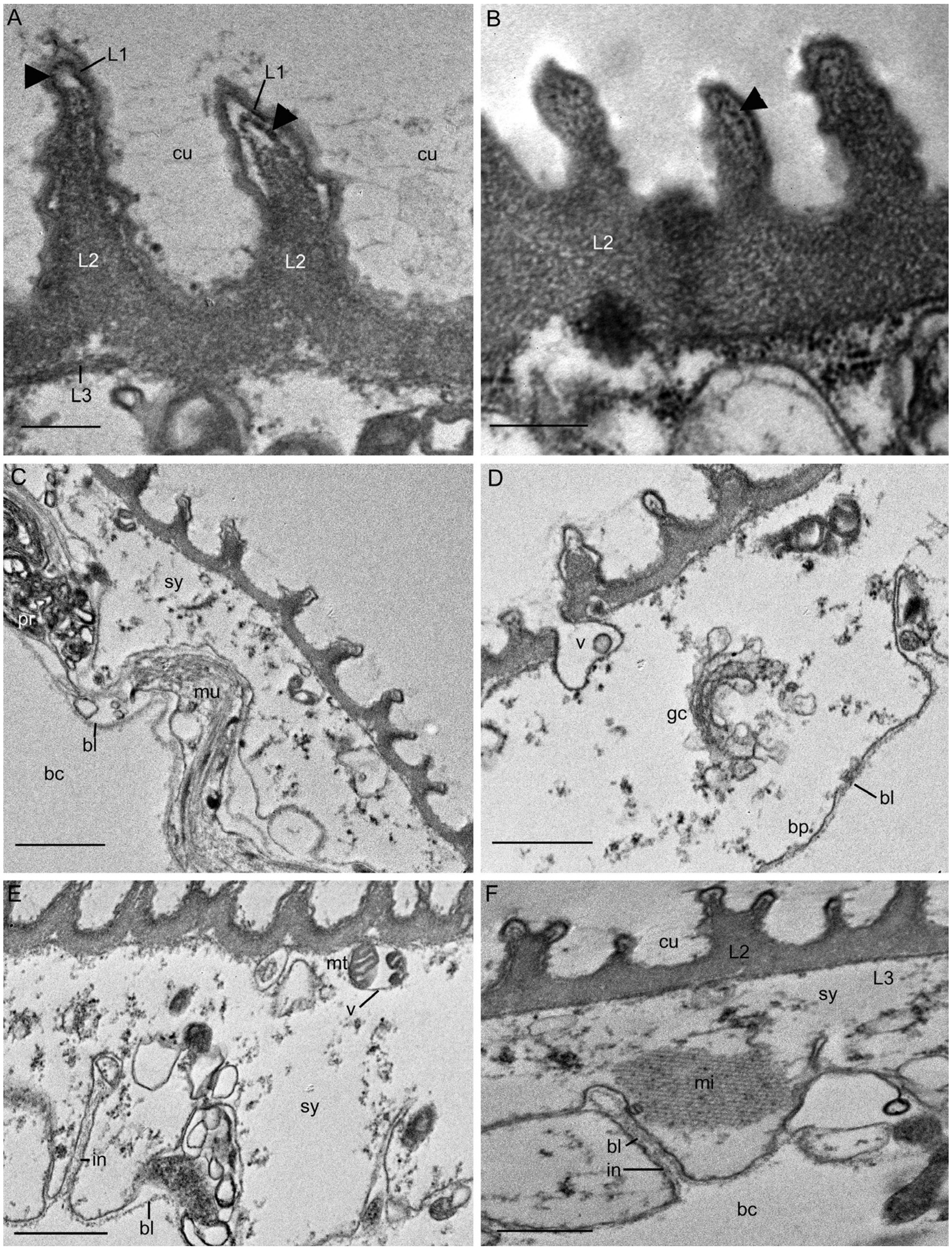

**Fig. 3.** Transmission electron micrographs (TEMs) of sections through the integument of *Sinantherina socialis*. **A.** Close-up of the ridge-and-groove system showing the three layers of the ICL (L1, L2, L3). Electron-dense dots (arrowheads) are present close to the base of L1 or within the matrix of L2. The glycocalyx is present as a thickened extracellular cuticle (cu). Scale bar=150 nm. **B.** TEM of a section through the ICL showing the electron-dense dots within the matrix of layer 2 (L2). Scale bar=150 nm. **C.** TEM of a section through the epidermis of the trunk revealing the voluminous cytoplasm of the syncytium (sy). A muscle (mu) can be seen in the blastocoel (bc) as can a portion of a protonephridium (pr). Both appear enclosed by the basal lamina (bl). Scale bar=400 nm. **D.** TEM of a section revealing the merging of a membrane-bound vesicle (v) with the overlying ICL. The cytoplasm is largely devoid of organelles except for Golgi complex (gc) and apparent ribosomes (dark spots). Scale ba r=500 nm. **E.** TEM of a section through the syncytium (sy) showing invaginations (in) of the plasmalemma at the base of the epidermis. A mitochondrion (mt) is apparent within a membrane-bound vesicle close to the ICL. Scale bar=300 nm. **F.** TEM of a section revealing a bundle of presumed microtubules (mi) within the cytoplasm of the syncytium. Scale bar=500 nm.

uct of the highly folded intracytoplasmic lamina (ICL) (Figs. 2B–D, 3A–F), which was the electron-dense region at the apical surface of the syncytium. The ICL, in total, consisted of three layers atop the syncytial cytoplasm. Layer 1 was the most external layer (cell membrane) and was thin and mostly electron dense (Figs. 2C, 3A–F). In some sections, this layer had "peeled" away from layer 2, which permitted a better view of its multilaminate substructure (Fig. 3A,D): the apical lamina of layer 1 was granular, moderately electron dense, and up to 30 nm thick. The underlying lamina of layer 1 was more electron dense and 14–16 nm thick. Layer 2 was a thickened, mostly homogeneous layer of electron-dense matrix that varied between being amorphous and somewhat granular (most of the trunk; Figs. 2C, 3) to highly fibrous (just below the corona; Fig. 2D). The amorphous matrix of layer 2 at the tips of the ridges and just below layer 1 often contained electron-dense dots (arrowheads; Fig. 3A, B). In some sections, it was difficult to determine if these electron-dense dots were part of layer 1 (arrowhead, Fig. 3A) or layer 2 (arrowhead, Fig. 3B). The fibrous regions of layer 2 were only noted in highly folded areas of the neck, and so might have been the result of layer 2 being stretched at these folds. Layer 2 had a consistent thickness of ca. 150–200 nm in the grooves across the trunk, but thickened at the ridges. The grooves were mostly smooth in appearance, although several contained small swellings (probably of layer 1) that were also prominent on the sides of the ridges (data not shown) and may correspond to the zipper-like appearance of the integument when viewed with SEM (see Fig. 2A).

The external ridges prominent in SEM (and appearing as finger-like projections in TEM) were folds of layers 1 and 2 that formed extensions to 700 nm above the surface of the integument. In some sections, the ridges were bifurcated (Fig. 3F) while others were swollen at their tips (Fig. 3D). These might be artifacts of sectioning or fixation.

Layer 3 was an electron-dense membrane subjacent to layer 2 and directly on top of the syncytium. This layer was moderately electron dense and appeared finely granular or somewhat fibrous in the trunk region (see Fig. 3A,B). Anteriorly, this layer was much more electron dense (Fig. 2C,D). Thickness of this layer was highly variable, from 30 to 60 nm in most regions of the trunk, but occasionally up to 120 nm in some sections. Electron-lucent spaces were occasionally present in between layers 2 and 3, but the nature of this space (artifact or not) could not be determined.

**Syncytium.** The syncytium was voluminous throughout the trunk (1000–3000 nm thick) but thinned out considerably in the neck and coronal regions (compare Figs. 2C and 3C–E). The cytoplasm was mostly electron lucent and contained relatively few organelles other than ribosomes, Golgi, and membrane-bound vesicles (Fig. 3D). The vesicles appeared to be exocytotic because many had apparently fused with the overlying ICL where they had released their contents (Fig. 3D). Several vesicles were noted to contain electron-dense materials and even mitochondria (Fig. 3D,E), while others were electron lucent and without any observable contents (Fig. 2C). Bundles of filaments were present in scattered positions throughout the syncytium in the trunk. The bundles contained more than 50 hollow filaments, each of which was ca. 23–29 nm in diameter; the bundles were never consistently associated with other organelles, although they were always positionally close to the basal plasmalemma (10–12 nm thick), which was highly infolded (Fig. 3F). A basal lamina 30–40 nm thick was present below the plasmalemma and within the invaginations of the basal surface (Fig. 3F). The basal lamina was considerably thicker in the more anterior portions of the trunk where the cytoplasm was less voluminous (compare Figs. 2C and 3D).

**Cuticle.** The cuticle is defined as the thickened extracellular matrix (glycocalyx) that serves no skeletal function (see Clément & Wurdak 1991). In *S. socialis*, the cuticle appeared as a lightly flocculent material up to 800 nm thick (Fig. 3A,F). It was present in all specimens although it was not consistently present in all body regions. It was generally present in the trunk and often absent towards the anterior end immediately below the corona.

## Discussion

Koehler's (1965, 1966) seminal research on the rotifer integument was important in establishing the unique structure of the intracytoplasmic lamina (ICL) and in showing that it is not, in fact, an extracellular cuticle, despite its appearance. He noted that exocytotic vesicles (hypodermal bulbs of Koehler 1965, 1966) are often abundant in the cytoplasm and merge with overlying ICL to release their contents, which suggests that the ICL is not extracellular or, in fact, solid, but instead permits movement of vesicles through its matrix. Subsequent studies have confirmed his findings in a variety of species and revealed additional structural variation in the ICL (Clément 1969, 1980; Storch & Welsch 1969; Brodie 1970; Schramm 1978; Hendelberg et al. 1979;

Clément & Wurdak 1991; Ahlrichs 1997). According to Clément & Wurdak (1991), and integrating studies of Ahlrichs (1997), there are three general types of ICL in rotifers: (1) the bdelloid type, which consists a thin external lamina (hypodermal membrane of Koehler 1966) over a thickened internal lamina (dense layer of Koehler 1966); (2) the monogonont type (containing 3 subtypes), which consists of a thickened external lamina on top of a thinner basal lamina; and (3) the seisonoid type, which consists of a thin external lamina (sublayer 1 of Ahlrichs 1997) separated from the thicker basal lamina (sublayer II of Ahlrichs 1997) by a region of cytoplasm.

The microtexture of the ICL in *Sinantherina socialis* is unique as described below, but its ultrastructure is broadly similar to the ICL of other monogononts, consisting of a thin outer membrane (hypodermal membrane of Koehler 1966), thickened matrix layer, and a relatively thin basal layer. In both cases, the thin apical layer (layer 1 of *S. socialis*) corresponds to the outer cell membrane, although the substructure of this membrane (or membranes, see Fig. 3A) differs among the different groups of rotifers including *S. socialis*. In *S. socialis*, this membrane possessed a clear substructure, where there was a finely granular lamina (30 nm thick) atop a thinner electron-dense lamina (14–16 nm). Curiously, layer 1 was clearly different in substructure and thickness from the basal plasmalemma (10–13 nm), which makes its categorization as a standard cell membrane difficult to interpret. In two other rotifers, *Asplanchna sieboldi* Leydig 1854 (Monogononta) and *Habrotrocha rosa* Donner 1950 (Bdelloidea), the hypodermal membrane also shows an unusual substructure: *A. sieboldi* has five layers reaching 20 nm in thickness (Koehler 1965), while *H. rosa* has a trilaminar membrane 17 nm in thickness (Schramm 1978). Both researchers note that the cellular membranes of their respective species are thicker than typical unit membranes. Unfortunately, there is little qualitative or quantitative data on the substructure of cell membrane in other rotifers, so a more detailed comparison is not permissible.

The more internal and thickened layer of the ICL of *S. socialis* was either amorphous or finely granular, but always became more fibrous toward the anterior end. This amorphous or finely granular substructure is similar to what is known for bdelloids (Koehler 1966) and some species of Monogononta (subtype 3 of *Keratella* and *Trichocerca*: Clément & Wurdak 1991). Interestingly, in *S. socialis*, the matrix of layer 2 appeared to change toward the anterior end of the animal, becoming distinctly more fibrous (compare Fig. 2C,D). It is unknown if this structural change is due to stretching of the ICL around folds in the body wall or if it is always present. It is worth noting that all sections through the anterior end of five separate specimens were highly folded. Layer 3 of the ICL in *S. socialis* was distinct from layer 2 and appeared finely granular or occasionally fibrous (Fig. 3A,F). Anteriorly, this layer was much more electron dense than in the trunk (compare Figs. 2D, 3). Regardless, this layer appeared grossly similar to the internal layer of other rotifers, but was generally much thinner being only 30–60 nm thick compared to 200 nm in bdelloids (Koehler 1966); quantitative data for monogononts is absent.

Layers 2 and 3 made up the unique ridge-and-groove microtexture of the integument of *S. socialis*. To date, no other rotifers have been shown to possess such a unique microtexture, and while several monogononts have spines or thickened ICLs that form a lorica and are derived from the external lamina (described in Clément & Wurdak 1991; equivalent to layer 2 of *S. socialis*), none create the unusual surface patterns seen in *S. socialis*. It is tempting to hypothesize that this unique pattern may impart a form of texture-based deterrent to predators, since the species is regularly rejected in studies of predation by fish (Felix et al. 1995) and invertebrates (Walsh et al. 2006). In these studies, it is hypothesized that the four wart-like glands posterior of the corona (asterisks, Fig. 1A) secrete defensive compounds that make the rotifers unpalatable, but to date, the nature of the secretions has not been determined. It is also possible that the ridge-and-groove microtexture of the integument functions to capture any secreted fluids from the glands, which may theoretically accumulate within the grooves especially if gland secretion is constitutive and the secretions are not water-soluble.

In addition to the unique ICL of *S. socialis*, we note that the syncytium was much more voluminous in this species (up to 3000 nm thick in portions of the trunk) than in any other described rotifer. In most species, the cytoplasm is extremely thin and less than 100 nm thick (Koehler 1965, 1966; Schramm 1978), although it reaches 1000 nm in preoral and caudal regions of *H. rosa* (Bdelloidea) (Schramm 1978). We hypothesize that the voluminous cytoplasm in the trunk of *S. socialis* acts synergistically with the ICL to provide skeletal support to animals that are regularly “upright” in posture as they feed and interact with other colony members. To this end, the bundles of filaments that are sparsely distributed throughout the cytoplasm might also make a skeletal contribution, though we have few details on the exact position, abundance, or lengths of these filaments. Longitudinal sections through these bundles were never observed, but the diameter of individual filaments (23–29 nm) roughly corresponds to the diameter of microtubules present in other invertebrates (de-Thé 1964; Chalfie & Thomson 1979), hence, our interpretation of the filaments as microtubules. We do note that the general appearance and regular arrangement of the filaments is similar to other types of cell inclusions such as protein crystalloids (Threadgold 1965; Reger 1969), but their hollow substructure is much more similar to microtubules. To date, the only other rotifer known to possess bundles of microtubules in its epidermis is the marine ectosymbiont *Seison nebaliae* Grube 1861 (Ahlrichs 1997). In this species, the microtubules are also present in the trunk epidermis (*S. nebaliae* has a cellular epidermis), but their substructure was not examined, thus their precise identity remains to be determined.

We have demonstrated that the fine structure of *S. socialis* is unique among previously examined rotifers, but without further details on other species of Gnesiotrocha, we hesitate to speculate on whether the integument and its peculiarities (e.g., microtexture, cytoplasmic volume, microtubules) are truly exceptional within Rotifera. We expect that with further examination of gnesiotrochans—taking into account their varied morphologies and lifestyles—the characteristics described here will be found in other species and may therefore be useful in defining phylogenetic relationships (e.g., as noted by Clément & Wurdak 1991); alternatively, they may correlate with particular lifestyles (e.g., sessility, coloniality) suggesting convergence.

**Acknowledgments.** This research was supported by the National Science Foundation (DEB 1257110 to RH). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

## References

Ahlrichs WH 1997. Epidermal ultrastructure of *Seison nebaliae* and *Seison annulatus*, and a comparison of epidermal structures within the Gnathifera. Zoomorphology 117: 41–48.

Brodie AE 1970. Development of the cuticle in the rotifer *Asplanchna brightwellii*. Z. Zellforsch. Mik. Ana. 105: 515–525.

Chalfie M & Thomson JN 1979. Organization of neuronal microtubules in the nematode *Caenorhabiditis elegans*. J. Cell Biol. 82: 278–289.

Clément P 1969. Premières observations sur l'ultrastructure comparèedes tèguments de Rotifère. Vie Milieu 20: 461–482.

——— 1977. Ultrastructural research on rotifers. Arch. Hydrobiol. 8: 270–297.

——— 1980. Phylogenetic relationships of rotifers, as derived from photoreceptor morphology and other ultrastructural analyses. Hydrobiologia 73: 93–117.

Clément P & Wurdak E 1991. Rotifera. In: Microscopic Anatomy of Invertebrates, Volume 4, Aschelminthes. Harrisson FW & Ruppert EE, eds., pp. 219–297. Wiley-Liss Inc., New York.

Dunagan TT & Miller DM 1990. Acanthocephala. In: Microscopic Anatomy of Invertebrates, Volume 4, Aschelminthes. Harrisson FW & Ruppert EE, eds., pp. 299–332. Wiley-Liss Inc., New York.

Felix A, Stevens ME, & Wallace RL 1995. Unpalatability of a colonial rotifer, *Sinantherina socialis*, to small zooplanktivorous fish. Invertebr. Biol. 114: 139–144.

Funch P, Sørensen MV, & Obst M 2005. On the phylogenetic position of Rotifera – have we come any further? Hydrobiologia 546: 11–28.

Hendelberg MG, Morling G, & Pejler B 1979. The ultrastructure of the lorica of the rotifer *Keratella serrulata* (Ehrbg.). Zoon 7: 49–54.

Hochberg R & Lilley G 2010. Neuromuscular organization of the freshwater colonial rotifer, *Sinantherina socialis*, and its implications for understanding the evolution of coloniality in Rotifera. Zoomorphology 129: 153–162.

Koehler JK 1965. A fine structure study of the rotifer integument. J. Ultrastruct. Res. 12: 113–134.

——— 1966. Some comparative fine structure relationships of the rotifer integument. J. Exp. Biol. 162: 231–244.

Lorenzen S 1985. Phylogenetic aspects of pseudocoelomate evolution. In: The Origins and Relationships of Lower Invertebrates. Morris SC, George JD, Gibson R & Platt HM, eds., pp. 210–233. Oxford University Press, New York.

Reger JF 1969. Studies on the fine structure of muscle fibres and contained crystalloids in basal sock muscle of the entroproct, *Barentsia gracilis*. J. Cell Sci. 4: 305–325.

Schramm U 1978. Studies of the ultrastructure of the integument of the rotifer *Habrotrocha rosa* Donner (Aschelminthes). Cell Tissue Res. 189: 167–177.

Storch V & Welsch U 1969. Über den Aufbau des Rotatorientegumentes. Z. Zellforsch. Mik. Ana. 95: 405–414.

de-Thé G 1964. Cytoplasmic microtubules in different animal cells. J. Cell Biol. 23: 265–275.

Threadgold LT 1965. The Ultrastructure of the Animal Cell. Pergamon Press, New York. 313 pp.

Wallace RL, Snell T, & Smith HA 2015. Rotifer: ecology and general biology. In: Thorp and Covich's Freshwater Invertebrates. Thorp JH & Rogers DC, eds., pp. 225–271. Elsevier, Waltham, MA.

Walsh EJ, Salazar M, Remirez J, Moldes O, & Wallace RL 2006. Predation by invertebrate predators on the colonial rotifer *Sinantherina socialis*. Invertebr. Biol. 125: 325–335.