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Stoma and intestine ultrastructure of the marine free-living nematode *Odontophora deconincki* (Nematoda: Araeolaimida: Axonolaimidae)

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Summary – The fine morphology of the buccal capsule and intestine (midgut) of the marine free-living nematode *Odontophora deconincki* was investigated. The cheilostome is armed with six equal claw-like odontia that can evert radially by opening the mouth. Light-refracting accessory buccal structures within the cheilostomatal cuticle alternate with odontia and consist of two elements: anterior armilloids and posterior granular armilliths. The buccal cavity (pharyngostome) is surrounded by a complex of longitudinal and oblique muscles partially attached to the cheilostome cuticle at the sites of the accessory buccal structures and enabling a wide opening of the mouth. With the described stoma condition, the nematode probably scrapes food particles from the substrate surface. In cross-section, the midgut consists of 5-7 cells that appear uniform throughout its length. An extracellular matrix (glycocalyx) over the microvillar brush varies in thickness and stratification depending on presence or absence of food content in the lumen. Abundant spherocrystals (globular inclusions with concentric striations) were present in all gut cells. No indication of endocytosis or digestive vacuoles was observed in the gut cells and extracellular digestion predominates. Most specimens had a gut content formed from a long cylinder of compressed flocculent material with some barely identifiable components and few spherocrystals expelled from the enterocytes. We assume that the nematode diet comprises a wide range of objects, mainly eukariotic epigrowth organisms, which are shorn off and scraped from the surface of sand grains and then ingested.

Keywords – alimentary tract, buccal capsule, feeding type, morphology, TEM study.

Free-living nematodes are the most abundant metazoan organisms in the marine sediments and play an important role in benthic ecosystems (Moens *et al*., 2014). The taxonomic diversity of marine nematodes has been calculated to be at least 6900 species (Appeltans *et al*., 2012) and has since been increased. Actual species diversity in the oceans of the world is predicted to be over 1 000 000 species (Lambshead & Boucher, 2003). Despite a uniform general body plan and an apparently featureless impression when looking at the nematode under the low magnification of a stereomicroscope, high-resolution optical microscopy reveals a vast variety of morphological details in cuticular sculpture, sensory organs, mouth armament, pharynx construction, copulatory apparatus, supplementary organs, *etc*. Even higher morphological diversity can be discerned at the ultrastructural level when similar looking simple structures turn out to be different. However, the fine structure of the free-living, and especially the marine nematodes has been studied in far less detail compared to the model species *Caenorhabditis elegans* or parasitic species of medical and economic importance. We believe that ultrastructural studies may promote a deeper insight, not only in fine morphology, but also in the taxonomy, physiology and ecology of marine free-living nematodes.

The goal of the present work was to investigate the alimentary tract of *Odontophora deconincki* Galtsova, 1976, a common species in the intertidal and upper sublittoral zones of northern Europe and the White Sea. Our study was focused on two regions: *i*) the food-procuring apparatus, *i.e.*, structure of the mouth and buccal cavity; and *ii*) the food-digesting intestine or midgut. The feeding and diet of the majority of marine nematode species remains under-reported or at best hypothesised from general considerations. Direct observation of feeding in the majority

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of marine species is not possible because of their cryptic lifestyle in sediments. Other approaches, including investigation of gut content, stable isotope analysis, and fatty acid composition analysis, have their own limitations and often may not provide a full and precise knowledge of the range of food items in the diet. We expect that the data obtained on the fine morphology of the buccal structures and intestine will also provide some information on the feeding technique and prey items of marine free-living nematodes.

Materials and methods

Material was collected from intertidal sediments at the White Sea Biological Station located on the Karelian coast of Kandalaksha Bay (66°33′N, 33°06′E) of the White Sea, Northern Russia, in August 2015-2019.

Light microscopy

Live nematodes were prefixed with 4% formaldehyde made up in filtered sea water for several days, then transferred into a solution of distilled water (70%), 95° ethanol (29%) and glycerin (1%), and eventually processed at 40°C by the slow evaporation method to pure glycerin (Seinhorst, 1959). The nematodes were then mounted on glycerin slides sealed with beeswax-paraffin with glass bead supports and observed with a Leica DM 5000 microscope equipped with differential interference contrast, Leica Application Suite Version 3.8.0 software and a Leica DFC 425 C digital camera.

Scanning electron microscopy (SEM)

Specimens were prefixed in 4% formalin in filtered sea water and then dehydrated in a graded series of ethanol-acetone solutions. Specimens were critical point-dried with carbon dioxide. Dried specimens were mounted on a stub, coated with gold-palladium mixture, and examined with a CAMScan S-2.

Transmission electron microscopy (TEM)

Specimens were prefixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (PBS) for 12 h and then post-fixed for 6-8 h in 1% OsO_4. After fixation, the specimens were gradually dehydrated in an ethanol-acetone series and embedded in Epon 812 epoxy resin (mostly according to the method of Jennings & Colam, 1970). Longitudinal and transverse sections 50-70 nm thick were made using a Leica ultramicrotome with a diamond (Diatome) or glass knife and mounted on formvar-coated single slot copper grids. Sections were stained with uranyl acetate (40 min) and lead citrate (15 min). TEM observations were performed using a JEM-1011.

Confocal microscopy

Specimens were prefixed in 4% PFA in 0.1 M phosphate buffer, washed in PBS, exposed to acetone (−20°C, 30-60 s), washed once more in PBS, stained with Phalloidin for 6-8 h and DAPI for 30 min, washed in PBS after staining and transferred to glycerin (30-60-90%) (generally according to De Ley *et al.*, 1995). Finally, the specimens were mounted on glycerin slides and observed with a Nikon A1 microscope JEM-1011. 3D reconstruction was done using Amira and ImageJ software.

Results

Head and buccal capsule

Under the light microscope, the stoma appeared to have two compartments (Fig. 1). Both parts appear in optical section as two cones or pyramids connected through their bases. This structure is typical for most genera of the Axonolaimidae. The anterior compartment of the stoma

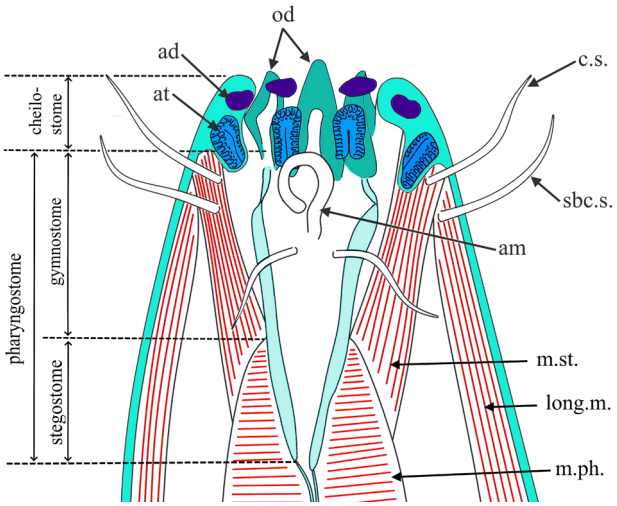


Fig. 1. Schematic drawing of head of *Odontophora deconincki*. Abbreviations: ad = armilloids; am = amphidial fovea; at = armilliths; c.s. = cephalic setae; od = odontia; sbc.s. = subcephalic setae; long.m. = longitudinal muscles; m.ph. = pharyngeal muscles; m.st. = muscles of stoma.

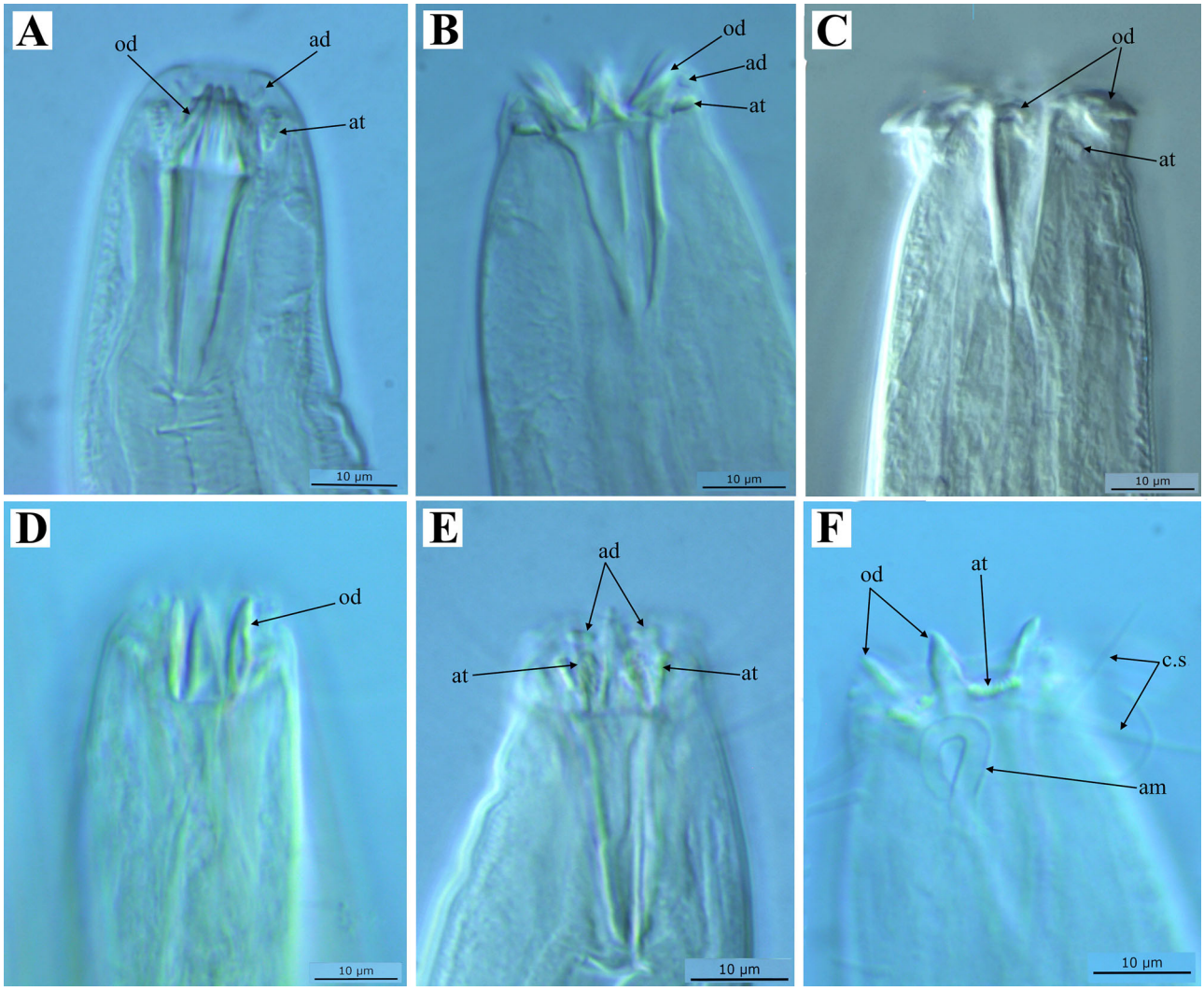


Fig. 2. *Odontophora deconincki* head at different stage of mouth opening, light microscope. A: Mouth closed; B: Mouth partly opened; C: Mouth opened; D: Odontia position in partly opened mouth; E: Armilliths; mouth closed; F: Armilliths; mouth opened. Abbreviations: ad = armilloids; am = amphid; at = armilliths; c.s. = cephalic setae; od = odontia.

is shorter than the posterior one and is in the form of a low blunted cone (Figs 1, 2A). Six equal claw-like odontia have their bases inserted into the stoma walls. However, in many specimens fixed by glutaraldehyde that had the mouth opened widely, the anterior stoma compartment becomes cylindroid or even expanding anteriorly (Fig. 2B-F). When the mouth is open, the odontia turn outwards perpendicular to the longitudinal axis, thus resembling a hexactinal flower shape (Figs 2C, F; 3A, C, D). The posterior stoma compartment is shaped like an elongate triangular pyramid turned upside down with the summit directed posteriad and continuing further into the narrow pharynx lumen (Figs 1, 2A-C).

The cuticle of the anterior part of the body is covered by a dense layer of fine filaments oriented radially (Fig. 4A, l.f.f.). The somatic cuticle is 0.25 μm thick and annulated, the annuli being 0.8 μm apart (Fig. 4A). The somatic cuticle is defined in this work as the body wall cuticle and its derivatives such as the cuticle of the cheilostome. The cuticle consists of four distinct zones: epicuticle, cortical zone (exocuticle) 0.1 μm wide, median zone (mesocuticle) 0.1 μm wide, and basal zone (endocuticle)

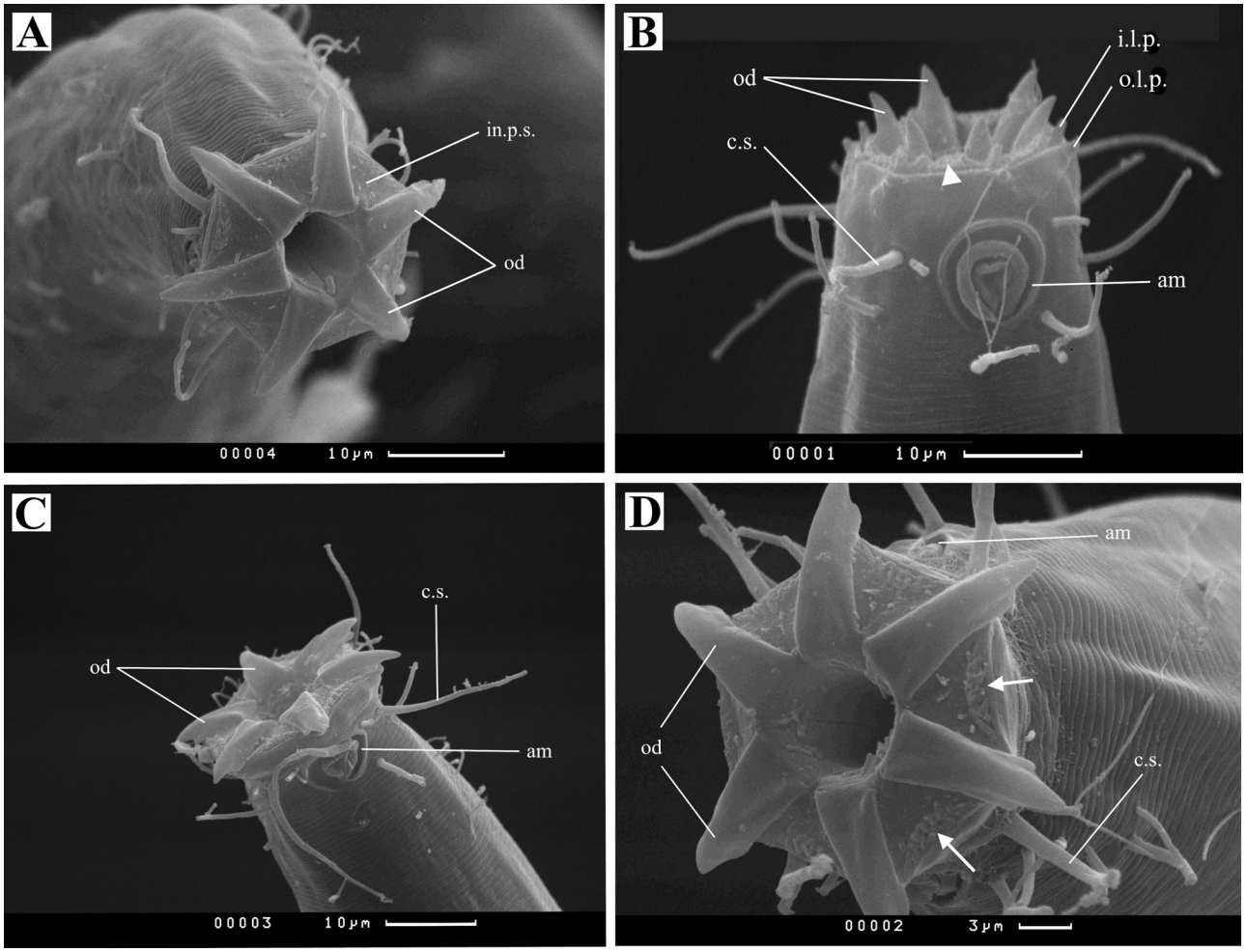
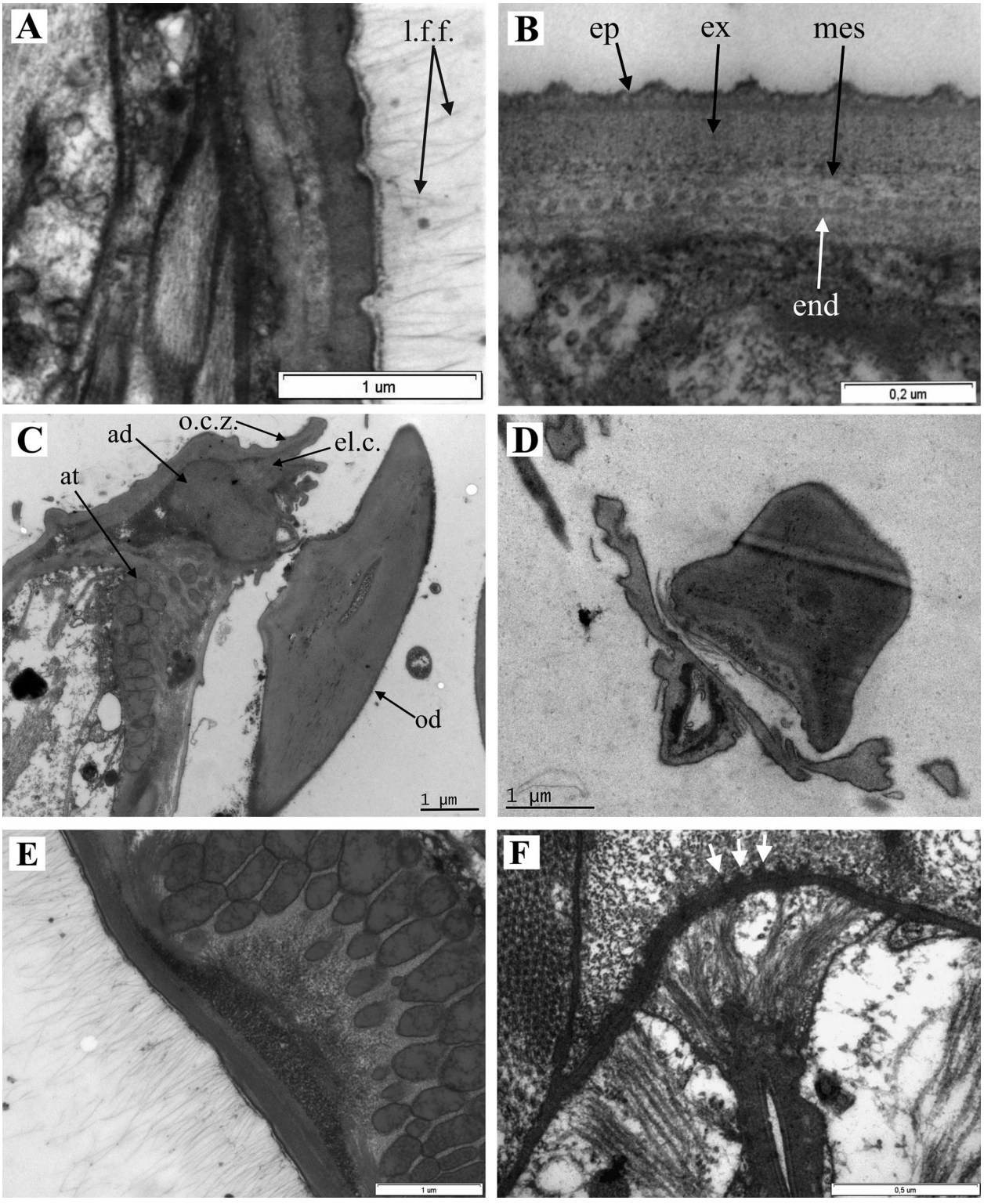


Fig. 3. *Odontophora deconincki* head, SEM. A: Apical view of widely opened mouth; B: Lateral view of partially closed mouth (arrowhead pointing to cuticular folds of cheilostome); C: Lateral view of opened mouth; D: Apical view of widely opened mouth, arrows pointing to projecting armilliths. Abbreviations: am = amphid; c.s = cephalic setae; i.l.p. = inner labial papillae; in.p.s. = internal part of stoma; od = odontia; o.l.p. = outer labial papillae.

0.05-1.0 μm wide. A thin epicuticle (0.02 μm thick) covers the transverse annules and the furrows between them. Transverse sections reveal tiny projections (Fig. 4B) that contain an electron-dense core in their apical part. These are likely to be cross- sections of the fine longitudinal cuticular ribs, although there was no evidence of longitudinal ribs in SEM.

The mouth opening is encircled by a cuticular fold (Figs 3B (arrowhead); 4C). The fold includes both cortical and median zones of the cuticle. Longitudinal sections show that the outer cuticular zone consists of electron-dense material whereas the core is electron-light (Fig. 4C, o.c.z, el.c.). The perioral somatic (cephalic) cuticle anterior to

Fig. 4. *Odontophora deconincki*, TEM. A: Longitudinal section of somatic cuticle in anterior part of pharynx; B: Transverse section of somatic cuticle at midgut level; C: Longitudinal section of anterior part of stoma (cheilostome); D: Transverse section of anterior part of odontia; E: Transverse section of armillith; F: Part of transverse section of posterior part of stoma; white arrows point to conjunctions between outer muscles and pharyngeal basal membrane. Abbreviations: ad = armilloids; at = armilliths; el.c. = electron light cuticle layer; end = endocuticle; ep = epicuticle; ex = exocuticle; l.f.f. = layer of the dense finest filaments; mes = mesocuticle; o.c.z. = outer cuticle zone; od = odontia.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the amphids is widened and modified. Both cortical and basal zones diverge due to the expansion of the median zone. The structures of all three zones are modified by comparison with the subcephalic somatic cuticle. The walls of the anterior stoma compartment are formed by the expanded perioral somatic cuticle.

The anterior stoma compartment is armed with two lateral, two latero-dorsal and two latero-ventral odontia of equal shape and size (8-10 μm long). All six odontia are inserted into the stoma walls (Figs 5, 6A). The pointed anterior ends of the odontia are drawn closely together when the mouth is closed (Fig. 2A). When the mouth is open, the odontia evert at right angles to the longitudinal axis of the body, the open mouth then appearing as a hexaradiate star (Fig. 3A, C, D). In transverse section, the odontia have a rectangular outline 6 μm wide (Figs 4D; 6A) and seem to be hollow (Figs 2D; 5). The cuticle of the odontia is homogenous and is clearly solid (Figs 4C, D; 5). The core of this structure contains a pulp composed of arcade cell processes.

Dense, transversally oval, structures were observed between the odontia in the median zone of the perioral widened cuticle. These structures were described and named as "armilloids" by Smolyanko (1994) and Smolyanko & Belogurov (1995). Under the light microscope, they look like optically dense, homogenous bean-shaped bodies (Fig. 2A, B, E). Under TEM, these bodies also appear homogeneous and solid (Figs 4C; 5). The armilloids are *ca* 2 μm wide (from inner to outer part of head), 1 μm in height (from anterior to posterior side) and about 3 μm long in the transverse axis. Just posterior to the armilloids are light-refracting grainy structures defined by Smolyanko (1994) and Smolyanko & Belogurov (1995) as "armilliths" and visible in light microscopy (Fig. 2A-C, E, F). In TEM images, the armilliths are represented as an aggregation of slightly elongated oval grains arranged together in a caliciform structure with the concave side turned outwards. The grains are inserted in the basal zone of the perioral cuticle and positioned perpendicular to the body axis; they are distinctly separated from their surroundings by a loose matrix of the perioral cuticle (Figs 4E; 6A, B). These armilliths are 5 μm long and 2 μm wide in transverse section. The grains have different sizes in transverse sections of the stoma, varying from 0.4 $\times$ 0.8 to 0.2 $\times$ 0.4 μm. The armilliths change their shape during the mouth opening process, being folded almost double when the mouth is closed (Fig. 2A, E) and straightened out when open (Fig. 2F). In the wide-open mouth, the armilliths are slightly everted and become visible above the surface as a coarse graininess (Fig 3D, white arrows). The stoma myocytes are connected with the armilloid-armillith region (Fig. 5, white arrow).

Arcade tissue surrounds the anterior stoma compartment and also partially surrounds the anterior part of the posterior stoma compartment. The part of the buccal cavity surrounded by arcade tissue is designated as the gymnostome (De Ley *et al*., 1995). This tissue extends posteriorly to the basal lamina of the pharyngeal tissue (Fig. 5). The cuticle of the buccal capsule is narrowed between the anterior and posterior compartments (Fig. 5, black arrow), thus functioning as a circular articulation permitting the opening the mouth and the divergence of the odontia. The cuticle (cuticular walls) of the walls of the buccal cavity is similar in width and structure in both anterior and posterior compartments. As is evident in the cross sections, the posterior compartment of the stoma consists of three walls of a tetrahedral pyramid (one dorsal and two lateroventral walls) with the summit turned posteriad ('down'); the walls are 0.6-1.0 μm thick (Fig. 6C) and are joined longitudinally by a folded thinner perradial cuticle 0.10-0.11 μm thick (Fig. 6C, white arrow). Both the cuticle and enveloping tissues of the posterior stoma compartment are precisely triradial in transverse section. At the middle of the posterior stoma compartment (beginning of stegostome) (Fig. 6C), the pharyngeal tissue envelope consists of six cell units: three perradial units in the two latero-median and medioventral angles, and three adradial, dorsal and two ventro-sublateral units. The perradial units are wider and more convex and enclose angular cuticle folds (Fig. 6C, white arrow). Perradial units include subradial myofilaments, which, by their contraction, expand the stoma and thereby contribute to food swallowing. The adradial cell units are very thin (0.2 μm) and are densely penetrated by many tonofilaments that join the basal lamina with the cuticle. The pharyngeal tissue becomes progressively wider in the posterior direction (Fig. 6D). The perradial units are bifurcated because of the marginal cells that appear at the angles of the internal cuticular lining. Muscular perradial cell areas become wider and expand to the adradial area, their myofilaments joining to the basal membrane of the pharyngeal muscles (Fig. 4F, white arrows point to the myofilament conjunctions).

Buccal cavity muscles

The buccal cavity is surrounded by three sets of paired myocytes and one unpaired dorsal myocyte (Fig. 7A, B). Altogether there are 13 longitudinal myocytes in the posterior stoma compartment. All the muscle

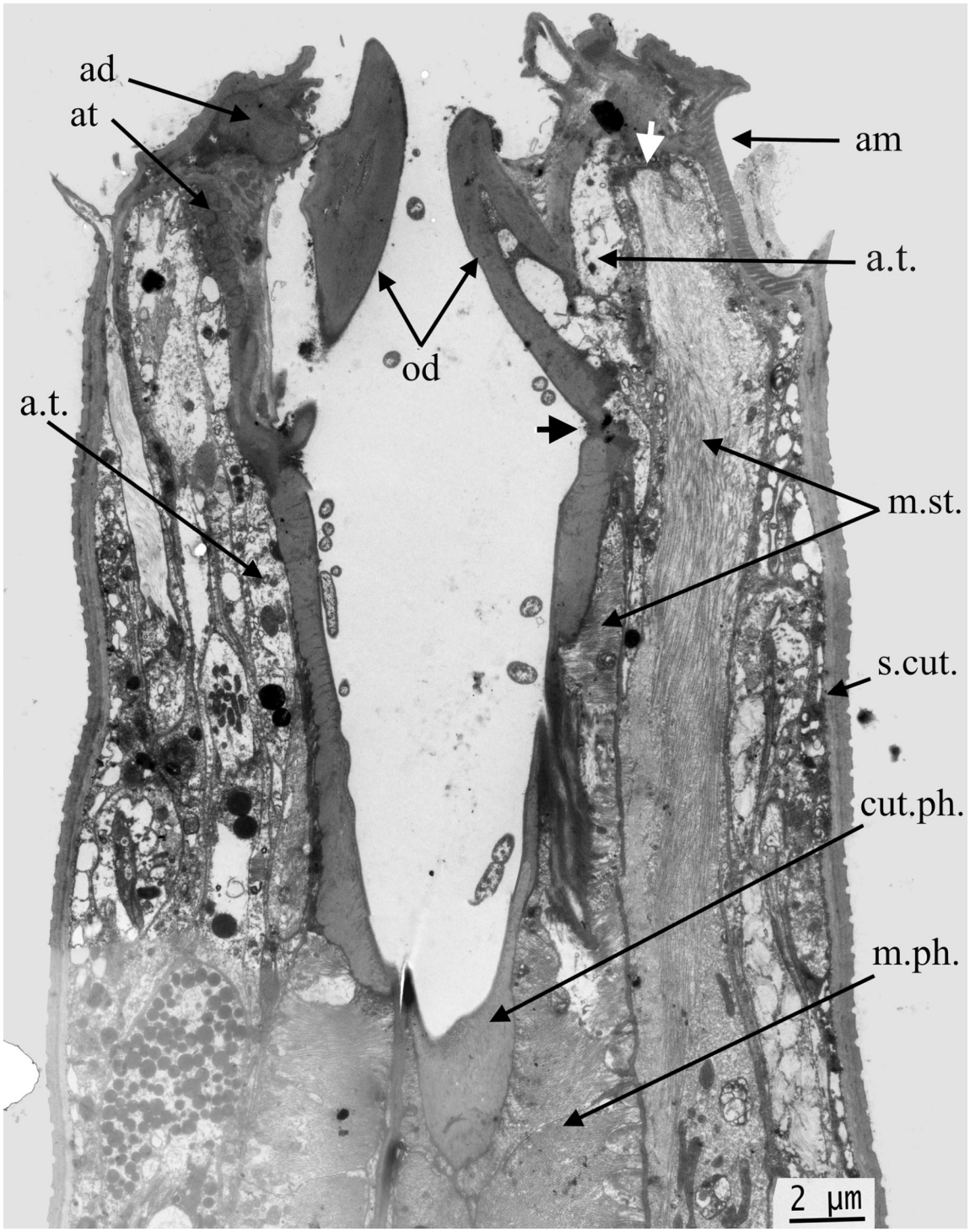

Fig. 5. Longitudinal section of head of *Odontophora deconincki*, TEM. White arrow pointing to connection between muscles and armilloid-armillith part of stoma; black arrow = thin part of cuticle between anterior and posterior part of stoma. Abbreviations: a.t. = arcade tissue; ad = armilloids; am = amphid; at = armilliths; cut.ph. = cuticle of pharynx; m.ph. = muscles of pharynx; m.st. = muscles of stoma; od = odontia; s.cut. = somatic cuticle.

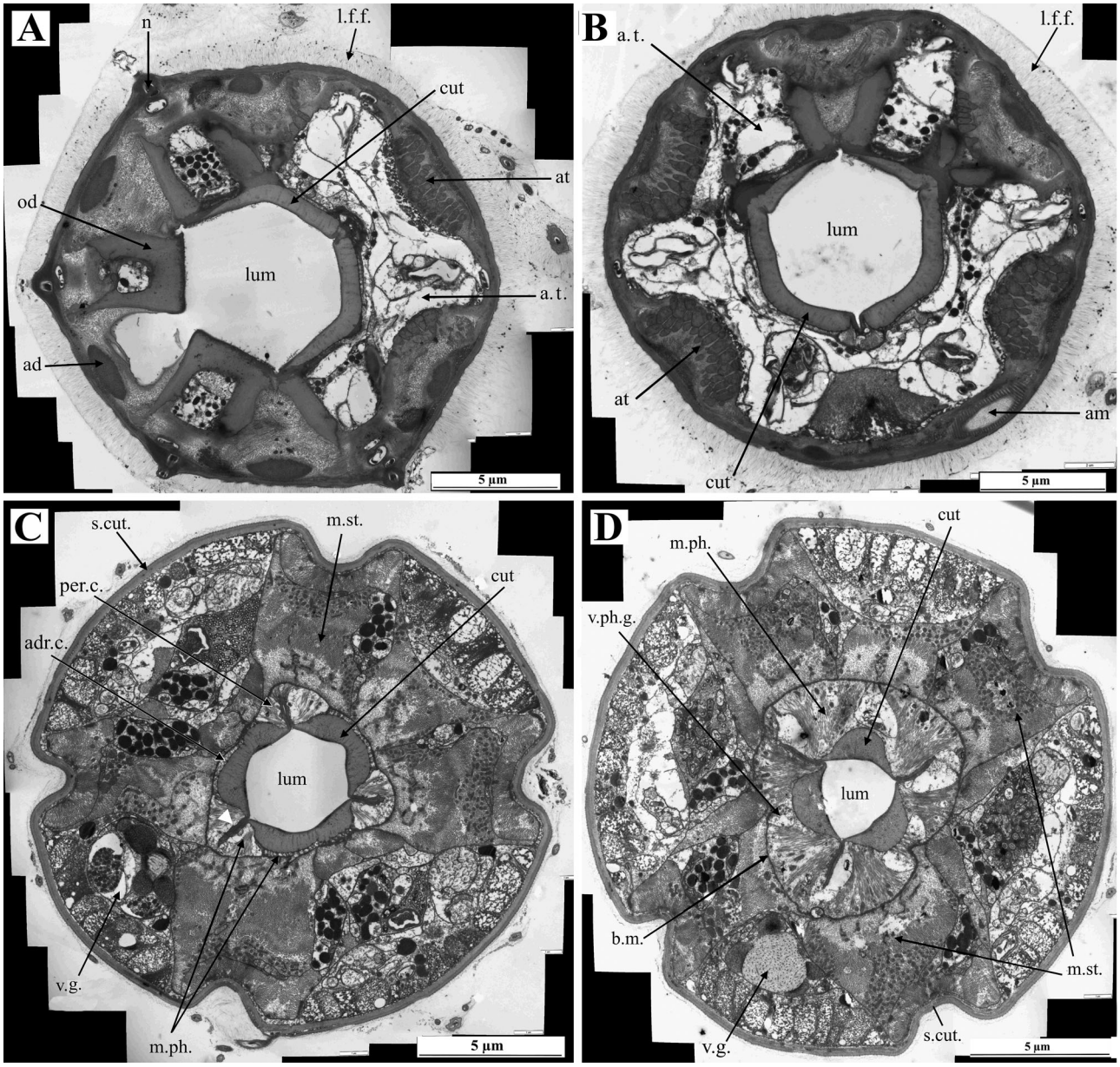

Fig. 6. Transverse section of posterior part of stoma of *Odontophora deconincki*, TEM. A: Level of posterior part of odontia; B: Just posterior to level of odontia; C: Middle of posterior part of the stoma; D: Posteriormost region of stoma. Abbreviations: a.t. = arcade tissue; ad = armilloids; adr.c. = adradial cells; am = amphid; at = armilliths; b.m. = basal membrane; cut = cuticle; l.f.f. = layer of the dense finest filaments; lum = lumen; m.ph. = muscles of pharynx; m.st. = muscles of stoma; n = nerve; od = odontia; per.c. = perradial cells; s.cut. = somatic cuticle; v.g. = ventral gland; v.ph.g. = ventrosublateral pharyngeal gland.

cells contain sarco-plasmatic regions with mitochondria, apart from zones of myofibrilles. Some local interspaces between myocytes are occupied by bunches of nerve processes.

Four body wall myocytes run tightly adpressed to the body cuticle in a submedian position. The cuticle in the region of the body wall myocytes is invaginated inwards, making four longitudinal furrows myocyte (Fig. 7A, B).

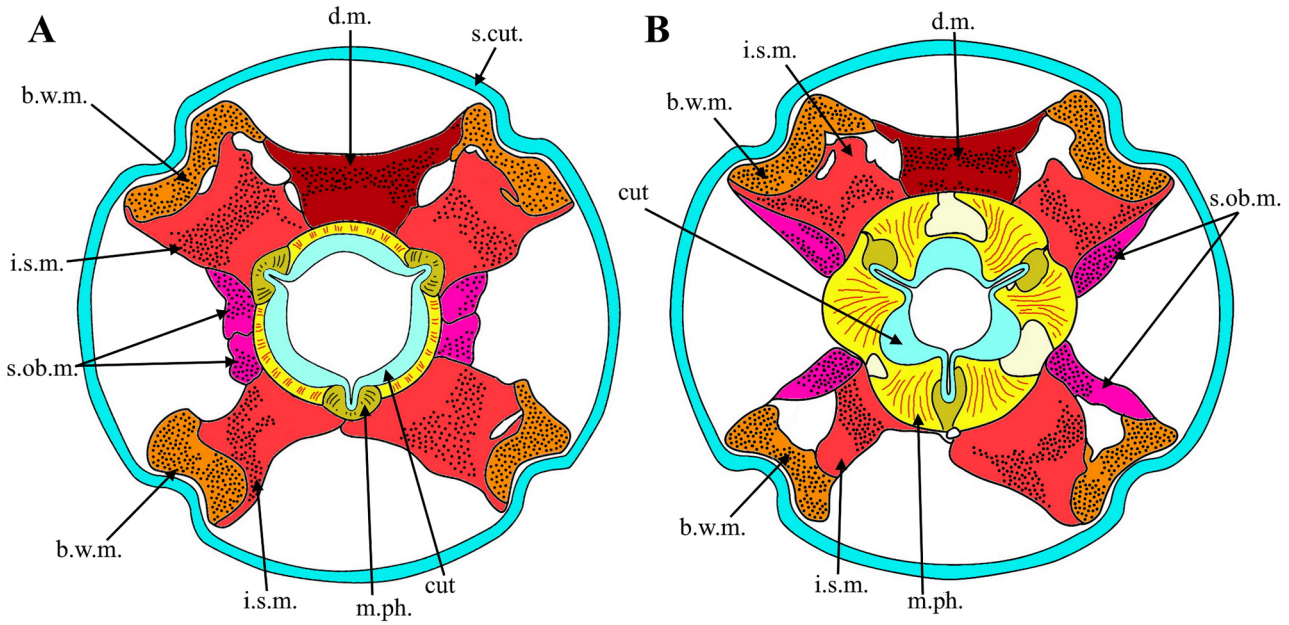

Fig. 7. *Odontophora deconincki*, schematic representation of muscles surrounding stoma in transverse section of posterior part of stoma. A: More anterior level; B: More posterior level. Abbreviations: b.w.m. = body wall myocytes; cut = cuticle of pharynx; d.m. = dorsal myocyte; i.s.m. = inner submedian myocytes; m.ph. = muscles of pharynx; s.cut. = somatic cuticle; s.ob.m. = sublateral oblique myocytes.

Each body wall myocyte is coupled to an inner submedian myocyte (Fig. 7A, B). The latter extend radially between the body wall submedian myocyte and the pharynx. In the posterior stoma compartment, the inner submedian myocyte forms a thin process to the body cuticle at the lateral side of the dorsosublateral body wall myocyte (Fig. 7A, B, i.s.m.). The inner submedian myocytes are attached by hemidesmosomes (Fig. 4F) both exteriorly to the body cuticle and interiorly to the basal lamina of the pharyngeal tissue envelope. The attachment sites are marked interiorly by hemidesmosomes and exteriorly by tonofilaments stitching through the subcuticle.

The third set of muscles present two pairs of sublateral oblique myocytes (one pair of laterodorsal myocytes and a pair of lateroventral myocytes) with their anterior ends attached by hemidesmosomes to the basal lamina of the pharyngeal tissue enveloping the anterior part of the posterior stoma compartment (Fig. 7A). At this anterior level, the posterior stoma compartment is almost entirely encircled by extrapharyngeal longitudinal musculature. Slightly more posteriorly, the sublateral oblique myocytes of each couple diverge, leaving a free space between them (Fig. 7B); the sublateral oblique myocytes here are adjoined to the inner submedian myocytes and partly to the pharyngeal basal lamina but are not attached by hemidesmosomes.

Finally, an unpaired dorsal myocyte is attached by hemidesmosomes to the pharyngeal tissue basal lamina in between the two dorsosublateral inner myocytes (Fig. 7A, B). The dorsal myocyte forms two lateral processes running along the inner myocytes and tapering towards the body wall, the processes clearly reaching the body wall posterior to stoma level.

The 3D structure of these muscles can be visualised under a confocal microscope. We cannot directly compare confocal microscope and TEM data and therefore provide a description of muscle location below. There are two groups of muscles in the anterior body: *i*) body wall longitudinal muscles positioned along the longitudinal body axis adjoining to the body wall along their entire length. They are four myocytes in submedian positions (right and left subdorsal, right and left subventral myocytes). The myocytes contact the epidermis by tonofilaments penetrating the cytoplasm of the epidermis (subcuticle) which is very thin in these areas (1.7-2.7 μm thinner than the somatic cuticle). Anteriorly, the myocytes are attached from within to the perioral somatic cuticle by strong tonofilament bunches (Fig. 4F); and *ii*) oblique muscles

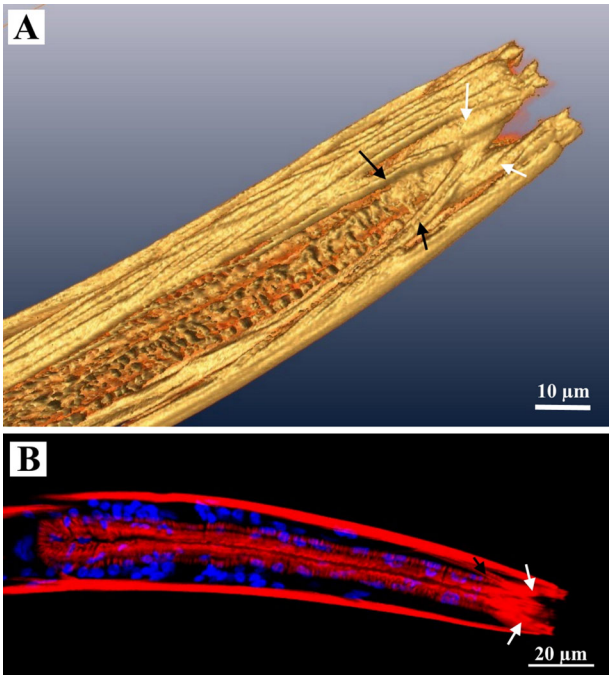

Fig. 8. Stoma and pharynx of *Odontophora deconincki*, laser confocal microscopy. A: Head muscles, 3D reconstruction. B: Head and pharynx (nuclei are blue; muscles are red). White arrows in both images point to a group of muscles connecting the anterior part of the stoma and basal membrane of the pharyngeal muscles; black arrows point to the second muscle group that connects the posterior part of the stoma and the epidermis.

extend from the body wall to the buccal cavity. One group of muscles connects the anterior part of the stoma and basal membrane of the pharyngeal muscles (Fig. 8A, B, white arrows). The second group connects the posterior part of the stoma and the epidermis (Fig. 8A, B, black arrows). This group begins with one muscle bunch from the stoma cuticle and bifurcates to two bunches posteriorly (Fig. 8A). A second group of muscles extends far anterior to the posterior part of the stoma. These muscles are probably involved with odontia operation.

Pharynx

The pharynx is a uniformly, mainly muscular cylindroid organ gradually widening posteriorly until the poorly visible terminal expansion (Fig. 9). In cross section, the pharynx consists of a triradiate internal lumen (Fig. 9) lined with ultrastructurally simple cuticle 0.12-0.19 μm thick without any thickenings or marginal tubes (Fig. 9). The pharynx includes several types of cells in cross section: *i*) three large marginal (radial) cells; *ii*) two muscle cells or cell units in each sector, which contain few interradial contractile myofilaments; and *iii*) three gland ducts, one in each sector (Fig. 9).

Midgut

The midgut or intestine is seemingly uniform throughout its entire length, being cylindrical and occupying almost the entire inner body space, although locally compressed against the body wall by the reproductive organs (Fig. 10B, C). The intestine consists of 5-7 enterocytes in transverse section. The borders between the cells are very distinct. Throughout the intestine, all the cells look similar with dark cytoplasm of similar density and similar distribution of various inclusions and organelles. The basal lamina is 0.1 μm wide (Fig. 11A, D) and slightly folded, but does not invaginate deeply. All three layers of the basal membrane are of equal thickness (*ca* 0.03 μm). The apical parts of the cells are connected by tight (adherens) junctions (Fig. 11B), whereas the basal parts are connected by cell lockers (interdigitations) (Fig. 11A). In the middle part of the cells, the membranes of adjacent cells simply run parallel without any thickenings or folds.

Organelles are distributed evenly along the apical-basal cell axis and include a nucleus, mitochondria, Golgi apparatus, endoplasmic reticulum, digestive vacuoles, dense granules, and spherocrystals (Fig. 11A-E). Spherocrystals are spherical or irregularly rounded-shaped inclusions 1-2 μm in diam. (Fig. 11C, D) and have very distinct concentric circles visible internally. There may be from 2-10 circular zones within the spherocrystals, the zones differing in electron density and width. The quantity of spherocrystals in a cell is not related to the presence or absence of food in the gut lumen. Numerous dense granules with moderate to dark electron density (Figs 10D; 11B) were also present.

The apical area forms regular cylindrical microvilli 0.3 μm long and 0.06-0.1 μm diam. and with truncate to rounded apices. The microvilli are located at an equal distance from each other (0.1 μm) and have neither apical modifications nor axial structures (Fig. 12A-D). The layer of apical cytoplasm just beneath the microvillar brush is 0.1 μm thick, does not contain any organelles and conspicuously differs from the deeper cytoplasm in other parts of the cells (Fig. 11A-D). This layer is homogenous and finely granulated whereas the underlying cytoplasm is transparent and contains ribosomes. However, this layer does not contain an obvious terminal web.

The microvillar brush is covered by extracellular matter of the glycocalyx (Figs 11B, E; 12A-D). Its structure

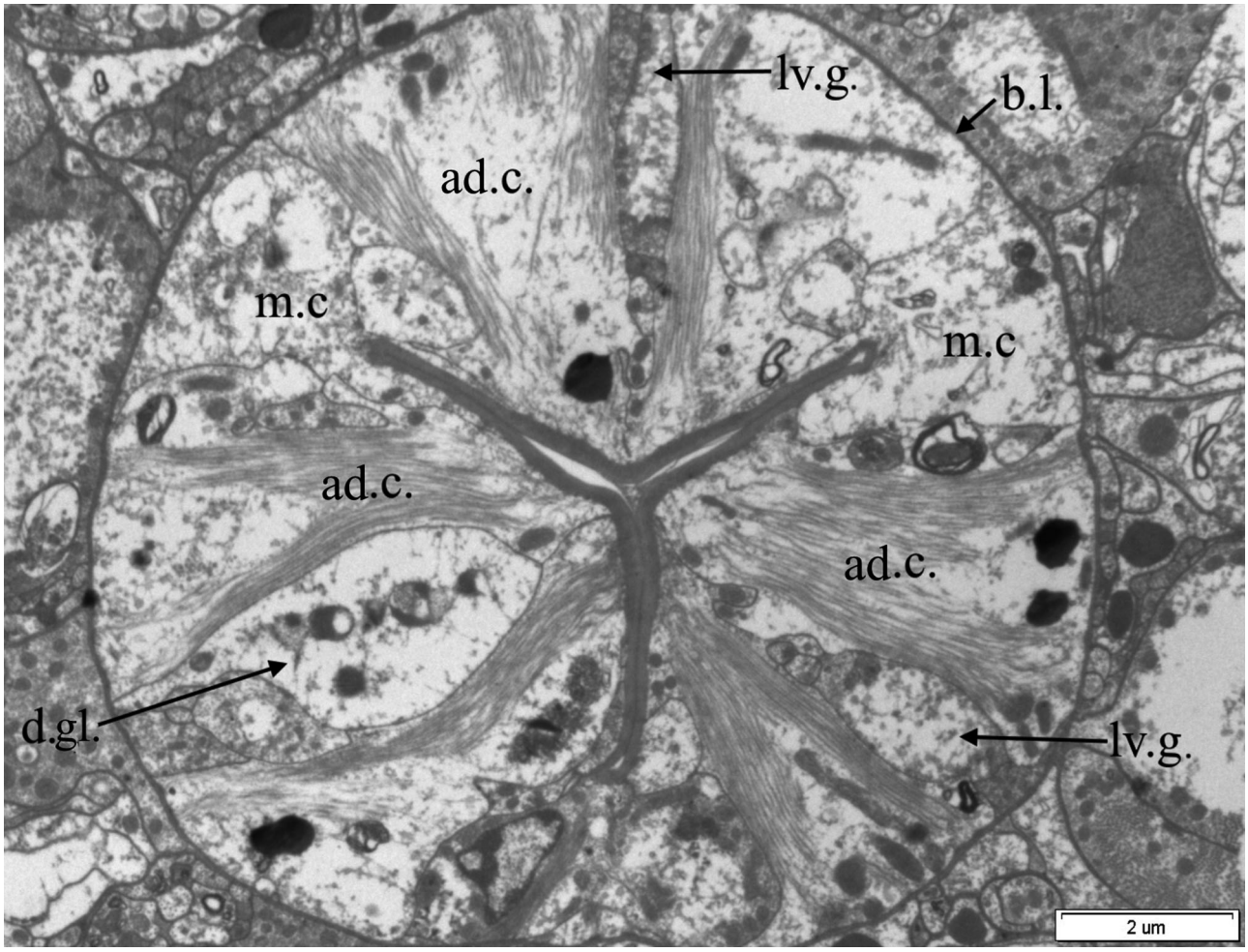

Fig. 9. *Odontophora deconincki*, transverse section at level of anterior part of pharynx. TEM. Abbreviations: ad.c. = adradial cell; b.l. = basal lamina; d.gl. = dorsal gland; lv.g. = latero-ventral glands; m.c = marginal (perradial) cell.

varies slightly depending on presence or absence of food content in the gut lumen. In the absence of food in the lumen, the glycocalyx is thin (0.1-0.5 μm), amorphous or with unclear lamellae (Fig. 12D). In the presence of food the glycocalyx becomes thicker, *ca* 2.0-2.5 μm wide, and more complicated in structure. The inner glycocalyx zone (between and just over the microvilli) is amorphous whereas the outer glycocalyx zone consists of long lamellae. They are thin, dense and fibrous, and lack a specific structure. Such lamellae can be located separately quite far from the microvillar layer. The lamellae envelop food particles. In the posterior part of the midgut, microvilli and glycocalyx disappear. Several layers of lamellae are visible, which tighten the lumen of the intestine (Figs 10D; 11F – the membrane is indicated by arrows).

Gut content

The midgut was full in most of the examined specimens, the content being compressed and appearing like a long dense sausage. Under the light microscope, the content may appear as a black, brown, orange or colourless transparent mass (Fig. 13A-F). Usually, the content is finely granular (Fig. 13B-D); sometimes large drops and/or separate solid rounded or oblong particles can be observed (Fig. 13A, D). Rarely, small dense particles can be seen in the gut lumen (Fig. 13E, F). TEM pictures may demonstrate discrete bacterial cells in the buccal cavity

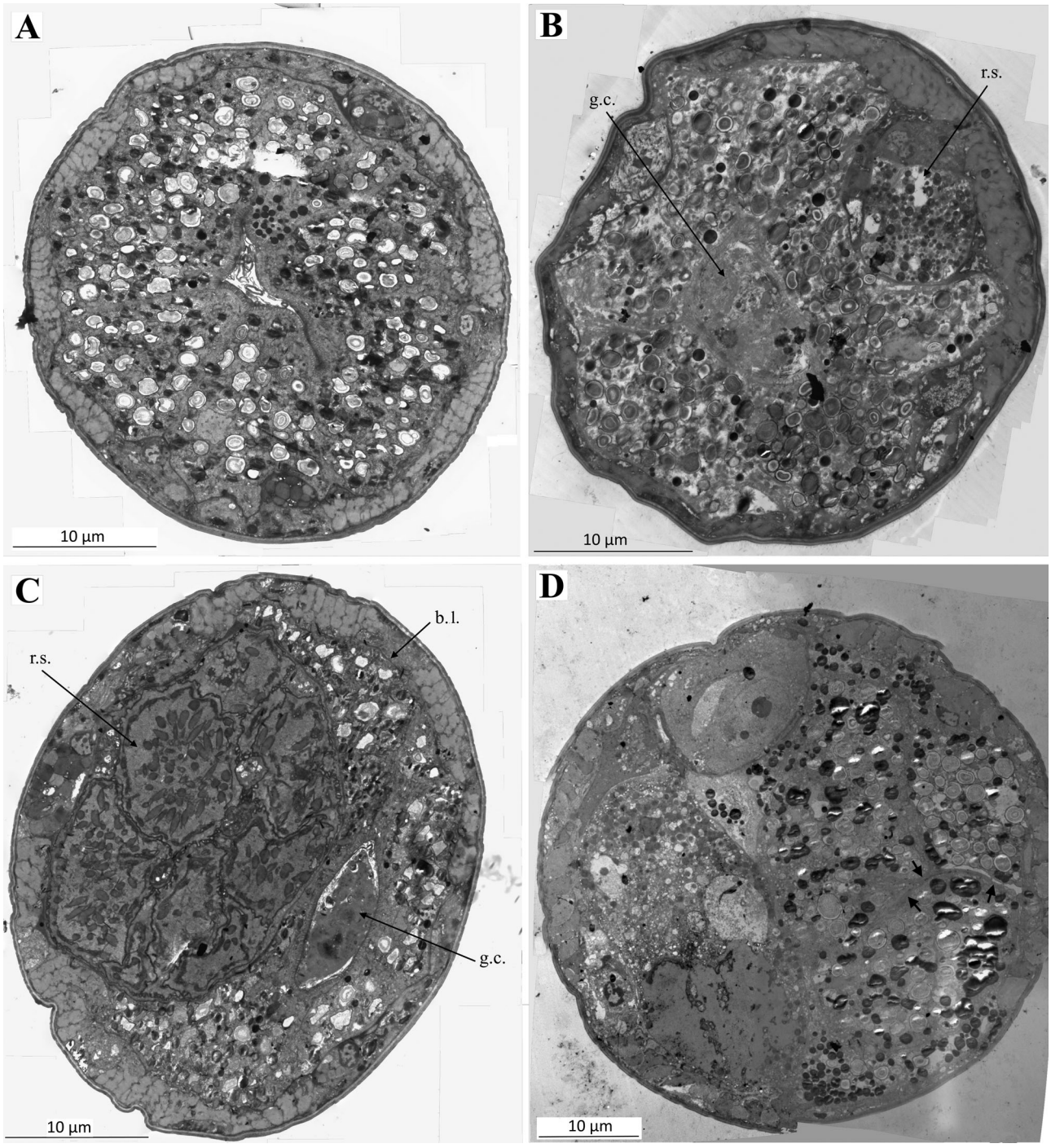

Fig. 10. Transverse midgut sections of *Odontophora deconincki*. A, B, D = female, C = male. TEM. A: Anterior part of midgut; B: Level of anterior part of reproductive organs (ovaries); C: Mid-body level; D: Posterior midgut. Abbreviations: b.l. = basal lamina; g.c. = gut content; r.s. = reproductive system.

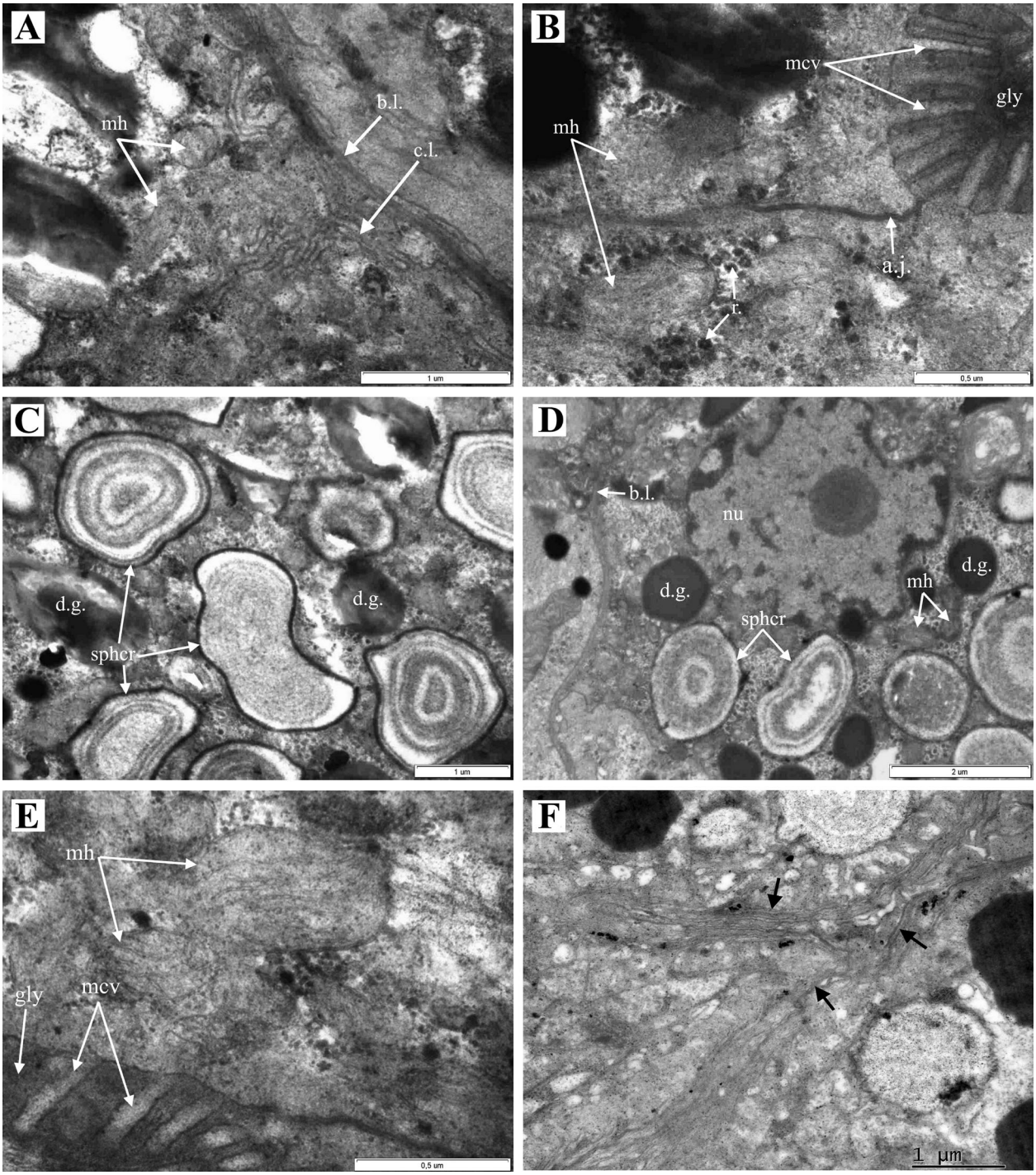

Fig. 11. Detail of midgut cells of *Odontophora deconincki*, TEM. A: Cell locks; B: Adherens junction; C: Spherocrystals; D: Organelles; E: Mitochondria; F: Membranes in posterior part of gut (black arrows). Abbreviations: a.j. = adherens junction; b.l. = basal lamina; c.l. = cell locks; d.g. = dense granule; gly = glycocalyx; mcv = microvilli; mh = mitochondria; nu = nuclei; r = ribosome; sphcr = spherocrystal.

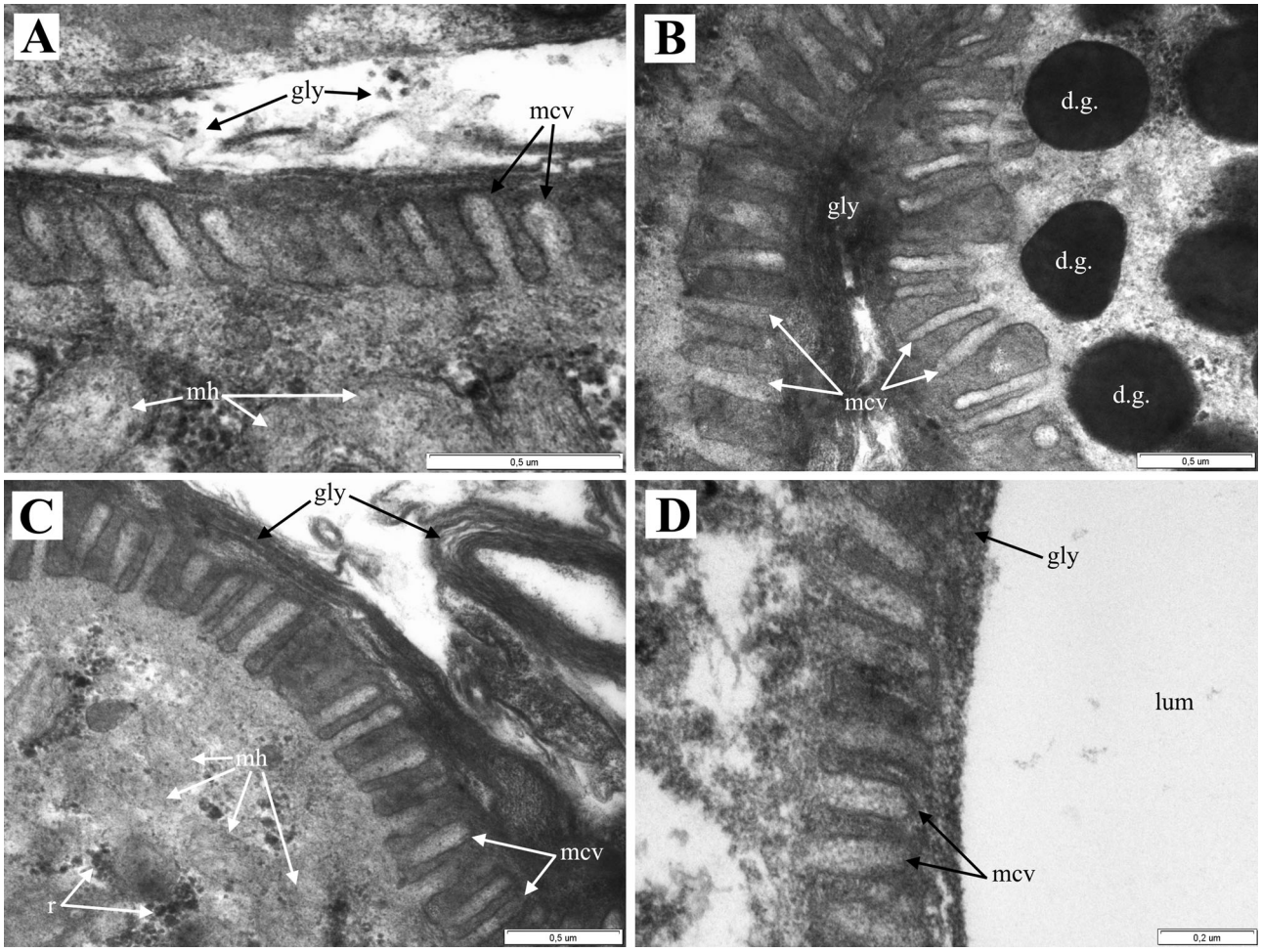

Fig. 12. Microvilli and glycocalyx on transverse midgut sections of *Odontophora deconincki*, TEM. A-C: Semi-full gut; D: Empty gut. Abbreviations: d.g. = dense granule; gly = glycocalyx; lum = lumen; mcv = microvilli; mh = mitochondria; r = ribosome.

(Fig. 5, arrows). The gut content in transverse sections is not clearly understandable. It consists of homogeneous to densely compressed flocculent matrix with roundish inclusions (Fig. 10B).

Discussion

Stoma construction and its musculature

The nematode buccal cavity consists of two principal parts: the anterior cheilostome formed by thickened or invaginated somatic cuticle, and the posterior pharyngostome formed by, to some extent, modified internal cuticular lining of the pharynx (*e.g.*, Decraemer *et al*., 2014). The pharyngostome is further subdivided into a gymnostome enveloped by arcade tissue and a stegostome surrounded by pharyngeal myoepithelium (De Ley *et al*., 1995). In the case of *O. deconincki*, the inner peripheral layer of the cheilostome, cuticle of the odontia and the pharyngostome cuticle scarcely differ from each other in ultrastructure or even in thickness. The borders between the pharyngostome, gymnostome and stegostome are not identified by a cuticular structure. The borders between all three stoma subdivisions are not simply flat and circular but are complex in three-dimensional space.

The cheilostome is complicated by odontia and accessory buccal structures (definition of Leduc & Zhao, 2016) (Fig. 1). The latter are represented by two kinds of structures: armilloids and armilliths. The latter terms were first used in the Russian Ph.D. thesis of Smolyanko (1994)

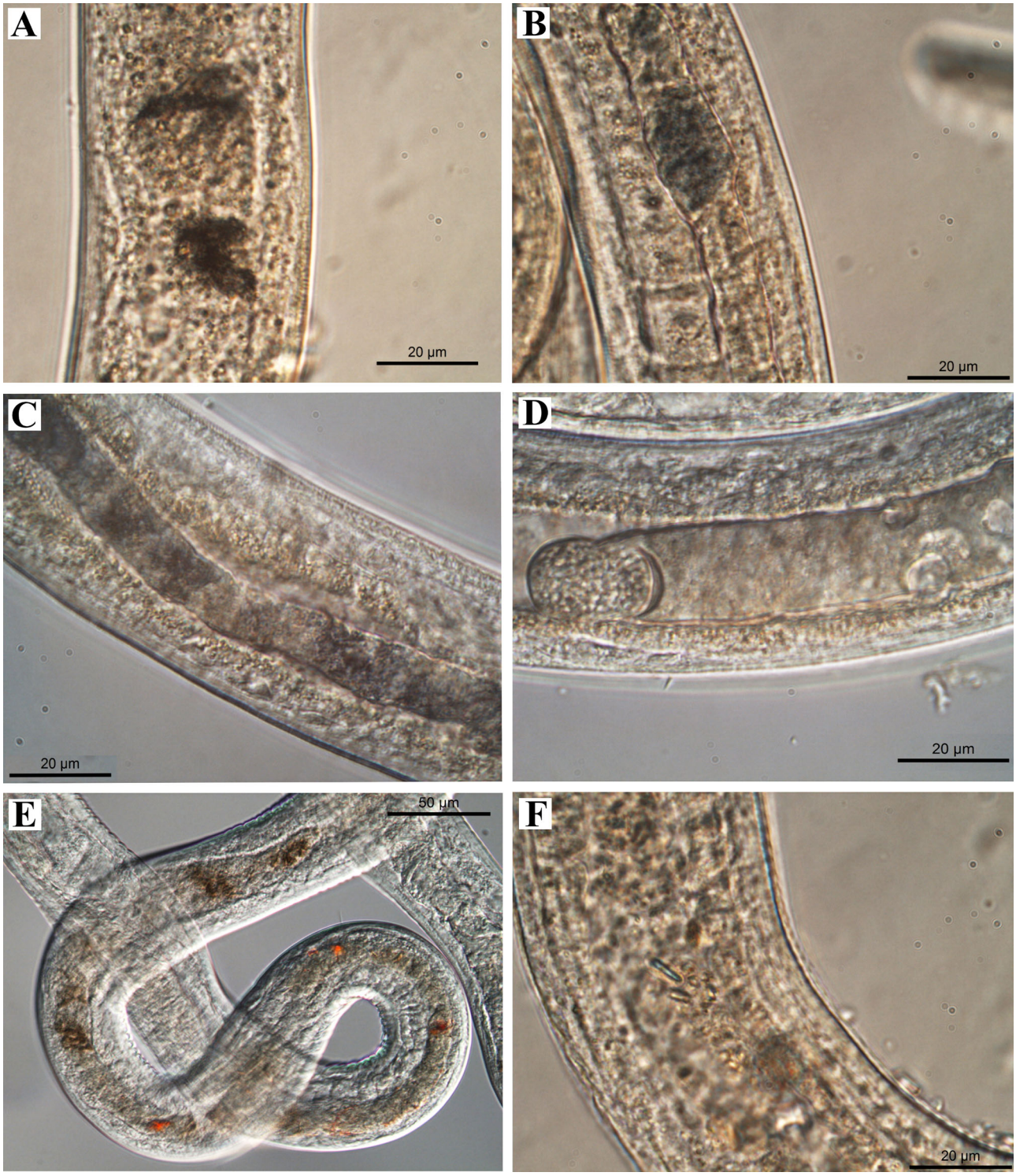

Fig. 13. Gut content of *Odontophora deconincki*, light microscope. A: Amorphous content with granules; B, C: Amorphous content; D: Amorphous content with drops; E, F: Content with dense coloured inclusions.

and then by Smolyanko & Belogurov (1995) for *Parodontophora*. Smolyanko (1994) mentioned the presence of the accessory buccal structures not only in *Parodontophora* species but also in several species of *Odontophora* and *Axonolaimus* (Axonolaimidae). Using only the light microscope, Smolyanko described armilloids and armilliths as light-refractive intracuticular grains having a supporting function. Similar or identical structures were previously depicted, although without interpretation, in taxonomic papers by Ditlevsen (1918) for *O. armata* (Ditlevsen, 1918) Allgén, 1929, Platt (1973) for *O. rectangula* Lorenzen, 1971 (as cuticular bodies), Warwick & Platt (1973) for *O. exharena* Warwick & Platt, 1973, and in more detail by Leduc & Zhao (2016) for *O. atrox* Leduc & Zhao, 2016. Both armilloids and armilliths are arranged within enlarged circumoral cuticle in pairs: an anterior armilloid and a posterior armillith, the armilloid-armillith pairs alternate with the odontia. The accessory buccal structures likely serve as supporting elements by active movement of the mouth, which is also confirmed by the presence of muscles attaching to the armilloid-armillith sites and not directly to the odontia. The six odontia are equal, strong claw-like teeth that evert and spread outwards during mouth opening and play a crucial role in gathering food particles. Supposedly, the nematode abrades a surface using the odontia and then ingests all the separated particles.

The musculature operating the movement of the mouth and stoma is rather complex. Usually, nematodes with a simple and unarmed buccal cavity do not possess specialised muscles apart from those of the myoepithelial cells of the pharynx and longitudinal myocytes of the body wall. Both types of stoma are scarcely differentiated from the following pharyngeal lumen (*e.g.*, in *Ceramonema*: Tchesunov, 1995) and large barrel-shaped stoma (*e.g.*, in *Sphaerolaimus*: Tchesunov & Fedyaeva, 2019) may be associated with simple unspecialised musculature. In such cases, the mouth is permanently open (if the mouth is small) or open whenever necessary by contraction of longitudinal body muscles (if the mouth is wide). In cases where the stoma is armed with teeth, special armaments, mandibles, spears, *etc*., the mouth apparatus is provided with additional specialised muscles (*e.g.*, in Enoplidae and Thoracostomopsidae (Inglis, 1964); Ironidae (Van der Heiden, 1974); Mononchidae (Grootaert & Wyss, 1979)).

In *O. deconincki*, there are only four submedian longitudinal somatic muscle fibres extended anteriad to the perioral cuticle. The body cuticle is slightly invaginated inwards along the attachment of the submedian muscles to the cuticle. The furrows are supposedly temporal and appear as a result of muscle contraction at the moment of fixation by glutaraldehyde. Another nine longitudinal and oblique muscles are attached by their anterior ends to the perioral body cuticle and posteriorly to the basal lamina of the pharyngeal cuff of the buccal capsule. Conspicuously, all these muscles originally belong to the body wall and not to the pharyngeal musculature since they are not enveloped by a pharyngeal basal lamina. Attachment points in areas of contacts of myocytes with cuticle and basal lamina of the pharynx present bundles of filaments attached to dense plaques, the structures being very similar to those described by Francis & Waterston (1991) for *Caenorhabditis elegans*. The symmetry of the anterior muscular system is formed by a combination of the triradiate or hexaradiate patterns of the pharyngeal fibres, the tetraradiate pattern of the extrapharyngeal longitudinal and oblique fibres and, notably, a single mid-dorsal fibre. The last element makes the entire pattern bilateral. The presence of the single mid-dorsal muscle does not correspond with any surface or stoma cuticular structures.

As judged by the position of muscles, contraction of the pharyngeal radial musculature leads to: *i*) opening of the mouth with eversion of the odontia at rightangles to the body axis; *ii*) expanding the anterior stoma compartment as performed by longitudinal extrapharyngeal muscles; and *iii*) expansion of the posterior stoma compartment and more posterior to the pharynx lumen.

Midgut

The construction of the cellular epithelium of the midgut or intestine is typical for 'normal feeding' free-living nematodes in all details such as basal lamina, basal interdigitations (cell locks), apical junctions, mitochondria, ribosomes, lipid globules, microvilli, and glycocalyx (*e.g.*, Nuss, 1985; Bird & Bird, 1991; McGhee, 2007). Enterocytes look mostly uniform throughout the midgut. Neither phagosomes nor digestive vacuoles or other indications on intracellular digestive processes were observed in the gut cells.

The midgut is characterised by rather short microvilli and complex glycocalyx. According to our observations, a thick and multilayered glycocalyx often occurs in the intestine of species swallowing large and coarse food items that could damage the brush border (*e.g.*, *Paramonhystera filamentosa* ingesting sediment with organic particles and angular sand grains or *Sphaerolaimus balticus* consuming live nematodes). The intestine of *O. decon-*

incki contains either a soft mass of torn and coarse particles or could be empty. In the latter case, the glycocalyx is very thin and amorphous. If the gut lumen is full, the glycocalyx becomes thick and dense and includes lamella-like structures. This observation supports a putative function of the glycocalyx as a protection for the delicate microvillar layer from damage by coarse food particles.

The cytoplasm of the enterocytes is densely filled with spherocrystals, which we assume to be a particular feature of *O. deconincki*. The spherocrystals have been observed in number of nematode species, mostly parasitic (Jenkins, 1973; Jenkins *et al*., 1977). Similarly, spherocrystals have been found in the tissues of parasitic flatworms. The spherocrystals usually contain compounds of calcium, phosphorus; rarely iron. Calcium is expected to stabilise phosphorus. According to Jenkins *et al*. (1977), the spherocrystals function as storage sites for phosphorus and mineral metabolic products.

Feeding

To our knowledge, only a brief direct observation exists on feeding of *Odontophora* species: an individual *O. longisetosus* sucked out "a piece of food" with peristaltic waves of the pharynx (Schuurmans-Stekhoven, 1931); the type of food is not specified. Taking into account the difficulty or impossibility of direct observations, Wieser (1953, 1959) divided the marine nematode genera into four trophic groups based on their type of buccal apparatus and anticipated diet. *Odontophora*, together with most other genera of the Axonolaimidae, was assigned to the group 1-B (non-selective deposit feeders). The group is characterised by having a buccal cavity without armature, food being obtained by the sucking power of the pharynx with additional help from movements of odontia and the anterior part of the buccal cavity itself. Later, Wieser (1960) described *Odontophora* spp. as epistrate feeders scraping food off sand grains. Recently, Moens & Wu (2019) identified *O. setosa* as a predator/omnivore using natural stable isotope ratios of carbon and nitrogen as well as fatty-acid profiles.

Our data add some valuable insights on the potential *Odontophora* diet. First, buccal structures, *i.e.*, movable claw-like odontia, indicate an ability to rasp away material from a hard surface. Such material may include attached bacteria, fungi, algae, *etc*. The sporadic presence of bacterial cells in the buccal cavity (Fig. 5) suggests that bacteria can comprise at least part of the diet of *O. deconincki*. The midgut of almost all examined specimens of *O. deconincki* was filled with a content that differentiates this species from some other nematode taxa whose intestine is usually empty, *e.g.*, Monhysteridae. The intestinal content of *O. deconincki* forms a rather dense sausage-like cylinder that often occupies the entire intestine and consists of heterogeneous objects pressed together (Figs 9, 13). The material mostly comprises crumbly bodies 2-4 μm in size and of uncertain origin, sometimes retaining residual shell, smaller grained lumps, vesicles, more or less evident remnants of bacterial cells, unidentified hard detritus, and/or concentric inclusions expelled from the cytoplasm of enterocytes. No refractive mineral particles, as occur in, *e.g.*, deposit-feeding *Daptonema* and *Paramonhystera* species (unpubl. obs.), were observed. Based on our observations, it is likely that *O. deconincki* is not predominantly bacterivorous, not a predator on metazoan prey and not a deposit feeder swallowing nutrient material with sediment particles. It is likely that the diet of this species comprises a wide range of objects that the nematode tears off and scrapes from the surface of sand grains. We can also conclude that the pabulum of *O. deconincki* likely contains a high portion of indigestible material that, based on the observation of the intestine being clogged by heterogeneous sausage-like lumps in most of the specimens, moves along the gut lumen rather slowly.

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