The ultrastructure of the buccal cavity of the monhysterid nematodes

Geomonhystera disjuncta and Diplolaimella dievengatensis

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SUMMARY

This study aims to elucidate the ultrastructure of the buccal cavity of two monhysterid nematodes, Geomonhystera disjuncta and Diplolaimella dievengatensis. The cuticular differentiations, as well as the identity of the surrounding structures or tissues is used to identify the consecutive buccal regions. The resemblance in configuration between G. disjuncta and D. dievengatensis is immediately noticeable, despite the apparent differences in general form. The configuration in the buccal region can be summarized as follow: (1) the cheilostom is surrounded by head cuticle, (2) the prostom is surrounded by epidermal “arcade” tissue, (3) the mesostom is surrounded by the tip of the pharynx that contains electron-dense filaments, (4) on the metastom insert myofilaments of the anterior most pharyngeal muscle m1 and (5) on the telostom insert myofilaments of m2.

RéSUMÉ

Ultrastructure de la cavité buccale des nématodes Monhystérides Geomonhystera disjuncta et Diplolaimella dievengatensis

Le but de la présente étude est d’élucider l’ultrastructure de la cavité buccale de deux nématodes Monhysterides, Geomonhystera disjuncta et Diplolaimella dievengatensis. Les différenciations cuticulaires, de même que la nature des tissus les entourant, sont utilisées pour identifier les zones successives de la cavité buccale. Les ressemblances de configuration chez G. disjuncta et D. dievengatensis sont immédiatement perceptibles en dépit de différences dans la forme générale. La configuration de la région buccale peut être résumée ainsi : (i) le cheilostome est entouré par la cuticule de la tête; (ii) le prostome est entouré par un tissu épidermique « en arcades »; (iii) le mésostome est entouré par l’extrémité du pharynx contenant des filaments; (iv) sur le métastome s’insèrent des myofilaments du muscle le plus antérieur du pharynx, m1; (v) sur le télostome s’insèrent des myofilaments provenant de m2.

The buccal cavity is the anterior differentiation of the alimentary tract, and hence plays a function in the initial uptake of food. The variety in buccal structures is used extensively in taxonomic and phylogenetic considerations. Historically, the division of the buccal cavity is based on rhabditid nematodes, in which three consecutive regions, the cheilostom, the protostom and the telostom are distinguished (Steiner, 1933). The prostostom region has later been subdivided in prostom, mesostom and metastom. The cuticular differentiations on the wall of a region are designated with the suffix -rhabdia. Since the classical division of the buccal cavity of nematodes is historically based on a rhabditid buccal cavity (Steiner, 1933), Wright and Thomson (1981) could easily adopt the same terminology and division to Caenorhabditis elegans. But it has also been used in different major taxa, however without any evidence for homologies. This gave rise to the actual rather confused situation in which the homology concepts presently in use are only applicable within each major taxon (see also Coomans, De Coninck & Help, 1978).

In most nematodes the study of the buccal cavity is based on light microscopical observations, which have an inherently limited resolution. As a consequence, the exact nature, significance and relative importance of different features cannot easily be evaluated.

Moreover, no general agreement has been achieved concerning the precise delimitation of the different buccal regions. Several suggestions have already been formulated for e.g. the delimitation of the anteriormost part, the cheilostom. According to De Coninck (1965), the cheilostom is the cavity which is limited by the inside of the lips. Inglis (1966) considers the cheilostom to be the secondary invagination (or elongation, or overgrowth) of the edge of the mouth. Belogurov (1985) describes the separation of the cuticle in the head into an external layer and an internal layer, the latter named the “endocupola”, with a cavity between them. He defines the cheilosom as the region between the oral opening and the endocupola.

In monhysterids especially the morphology of the buccal cavity is considered to be crucial for taxonomy.
and phylogeny. But their small size makes it extremely difficult to discriminate between the different buccal regions. Jacobs et al. (1990) give a light microscopical description of the bipartite buccal cavity of Diplolaimella dievengatensis Jacobs et al., 1990, while the buccal cavity of Geomohystera disjuncta (Bastian, 1865) Jacobs, 1987 can be characterized as being U-shaped. This study aims to elucidate the ultrastructure of the buccal cavity of *G. disjuncta* and *D. dievengatensis*, and tries to make a comparison of our results with the hitherto ultrastructurally described buccal cavities in the literature.

**Materials and methods**

*G. disjuncta* was isolated from the “Sluice Dock” of Ostend, a man-made lagoon near the Belgian coast. The other species, *D. dievengatensis*, was collected from the sediment of the “Dievengat”, a brakish water pond near Knokke (Belgium). The latter population was previously erroneously identified as *Monhystera microphthalma*, but this will be rectified in Jacobs et al. (1990).

Vranken et al. (1984) developed a culture method for both species. The nematodes were grown in small vented Petri-dishes filled with bacto-agar, using a monoaxenic bacterial culture, belonging to the Alteromonas haloplanktis rRNA branch, as a food organism.

Young adults of both species were picked from the Petri-dishes, cooled in an ice-bath to stretch and then killed and fixed in an ice-cooled fixative. The fixative for *G. disjuncta* was made up of 1.5% acrolein, 3% glutaraldehyde and 1.5% paraformaldehyde in 0.2 M sodium cacodylate buffer. The fixation of *D. dievengatensis* took place in 1:1 fixative diluted with distilled water.

After fixation and rinsing the specimens were post-fixed in 2% osmium tetroxide, followed by an en bloc staining in 2% uranyl acetate. Finally they were dehydrated in graded ethanols and embedded in Spurr's resin (Spurr, 1969).

Ultrathin sections were cut on a Reichert OMU-2 Ultramicrotome and picked up on Formvar filmsupported slot grids. The sections were stained in 2% uranyl acetate for 45 min and in lead citrate according to Reynolds (1963) for 15 min.

**Results**

**Geomohystera disjuncta**

The cuticle

The importance of the cuticular structure in the delineation of the different buccal regions underlines the necessity of a detailed description of both body and head cuticle.

The body cuticle (Fig. 1 A-C) is relatively thin, about 0.1 µm near the head region, gradually increasing to 0.15 µm in the more posterior part of the body. The outermost layer, the epicuticle (ec), is clearly delineated from the rest of the cuticle and only 20 nm thick (Fig. 1 A, B). A trilamellar constitution is recognizable: a thin electron-dense outer layer, a broader moderate electron-dense middle layer, and again a thin electron-dense inner layer (Fig. 1 A). At regular distances circularly around the animal, the epicuticle invaginates so deep that it reaches the median layer (see below), causing transverse striae on the cuticle (Fig. 1 A). The resulting cuticular rings (annuli) between the invaginations are about 0.25 µm broad (Fig. 1 A, C).

The cortical layer (cl), situated just underneath the epicuticle, is moderately electron-dense and about 40 nm thick. It fills the space between consecutive invaginations of the epicuticle. On transverse sections (Fig. 1 B) this layer seems to be amorphous, but a fine, regular striation is visible in longitudinal sections (Fig. 1 A).

The median layer (ml) is generally rather electron-dense and of variable thickness, from about 20 nm anteriorly in the pharyngeal region to 40-50 nm near the beginning of the intestine. The median layer seems rather amorphous in both longitudinal and transverse sections (Fig. 1 A, respectively 1 B), but shows a regular broad striation in oblique sections (Fig. 1 C).

The innermost layer, the basal layer (bl) is moderately electron-dense (Fig. 1 A-D). It occupies the space between the median layer of the cuticle and the outer cell membrane of the epidermis. Since the latter is rather uneven (see e.g. Fig. 1 B, C) the basal layer is variable in thickness. This layer has a very fine-grained appearance in all examined sections.

About 2 µm posterior to the anterior tip of the head, the space between the cortical and the basal layer widens (arrowheads in Fig. 1 E). It is filled with the continuation of the median layer, which has a moderate to more electron-dense appearance without any striation. Cellular structures of the anterior sensilla (as) of the head are embedded in this median layer (Fig. 1 D).

In the head, the continuation of the basal layer becomes somewhat broader than in the body region, but it keeps the same appearance (Fig. 1 D). As in the body region, the epicuticle covers the cortical layer of the head. The cortical layer gradually widens in the head, and clearly shows a fine periodic striation orientated almost perpendicular to the head's outer surface. The striation consists of an alternation of thin electron-dense bands and broader less electron-dense bands (Fig. 1 D).

The cheilostom (Figs 1 E, 2 A, 3 A)

The cheilostom (C) is the hexaradiate space between the oral opening and the place where the basal layer of the head cuticle is in contact with the pharyngeal cuticle.
It also comprises the space under the vaulted lips. The cheilostom is lined with head cuticle and is surrounded by the lips (Fig. 3 A). It is the broadest part of the buccal cavity, with a diameter of about 2.5 μm in cross section, and a length of 0.5 μm.

At the posterior end of the cheilostom, the central part of the lumen is nearly circular in cross section, with six outward pointing protrusions in between the lips (Fig. 3 A). Three of these protrusions, the perradial ones, are posteriorly continuous with the three prostomial radii, the anterior parts of the pharyngeal radii. The three other protrusions, the interradial ones alternating with the perradials, are in contact with the corner points of the prostom (see below).

The prostom (Figs 1 E, 2, 3 B)

The prostom (P) immediately follows the cheilostom, has about the same length and is only slightly narrower than the latter (Fig. 2). The essential difference resides in the fact that the prostom is not surrounded by head cuticle, but is limited by a thin electron-transparent amorphous cuticle (Fig. 4). The prostomial radii (**in Fig. 3 B) are about 1 μm long and limited by a rather moderately electron-dense amorphous cuticle (Fig. 3 B). The prorhabdia are angular structures, with a corner in the middle, so that the two flat sides are situated at an angle of about 120° from one another (Fig. 3 B). This configuration results in a rather hexagonal central part of the lumen (Fig. 3 B).

The mesostom (Figs 1 E, 2, 4)

At the mesostom (M), the buccal cavity gradually becomes narrower. The mesostom itself is about 1 μm long. This region is limited by a thick pharyngeal cuticle of up to 300-400 nm. The cuticle consists of a rather electron-transparent layer, in which radial, electron-dense bands are situated, and the whole is covered with an electron-dense layer (Figs 1 E, 4). The mesorhabdia are rather strongly curved (Fig. 4). The pharyngeal radii gradually increase in length posteriorly. They are covered with a cuticle (Fig. 4 B) similar to the one found in the prostomial radii, although thinner (50-100 nm thick).

The cuticle of the mesostom is surrounded by an electron-dense layer, of which the electron-density is caused by the abundance of packets of electron-dense filaments (Figs 1 E, 2, 4). This electron-dense layer forms the tip of the pharynx and partly encapsulates the ring of anteriormost pharyngeal muscles (m1) (Figs 1 E, 2). Towards the posterior end of the mesostom, the tips of the m1 muscles appear in the corners between the mesorhabdia and the pharyngeal radii (Fig. 4 B).

The metastom (Figs 1 E, 2, 5)

At the metastom (M'), the buccal cavity narrows further down and is about 1.5 μm long (Figs 1 E, 2). The cuticular lining of the metarhabdia strongly resembles the mesostom cuticle, but is somewhat thinner. The metarhabdia are strongly curved. The cuticle of the pharyngeal radii is thin, amorphous and moderately electron-dense (Fig. 5).

The essential distinction between meso- and metastom consists of the fact that the myofilaments of the m1 muscles are attached to the metarhabdia. Anteriorly in the metastom region, the m1 muscle in each pharyngeal sector is composed of two fan-like bundles of myofilaments (Fig. 5 A). Towards the cuticular lining, these myofilaments insert in the corners between the metarhabdia and the radii, from where they diverge to an evenly distributed attachment to the outer surface of the pharynx (Fig. 5 A).

More posteriorly in the metastom, each sector contains one broad fan-like packet of myofilaments of the m1 muscle, inserting in an even distribution on both the metarhabdion and the outer side of the pharynx (Fig. 5 B). At the most posterior end of the metastom, the m1 muscle in each sector consists of a bundle of almost parallel myofilaments, and a wedge-like tip of the m2 muscle is situated at each side of the m1 bundle (Fig. 5 C). The myofilaments of the second set of pharyngeal muscles (m2) do not have an insertion on the metarhabdia. In this configuration the m1 muscles are in an interradial position, and the m2 muscles in an adradial position. However, at this level only the tips of the m2 muscles are visible, which obscure the fact that the m2 muscles in their entirety are actually interradial (see below).

The telostom (Fig. 6 A)

The telostom (T) is about 1.8 μm long, has a rather narrow lumen, of which the central part is triangular in cross section (Fig. 6 A). The cuticular lining is thin, moderately electron-dense and amorphous. The myofilaments of muscle m2 insert on the telorhabdia throughout the entire length of the telostom. Each m2 is composed of two broad packets of almost parallel myofilaments per sector (Fig. 6 A). Towards the periphery of the pharynx, the three most posterior tips of m2 are each present interradially between two myofilament packets of m2 (Fig. 6 A). Towards the posterior end of the telostom, wedge-formed tips of m3 appear beside m2, but their myofilaments do not insert on the telorhabdia (Fig. 7 shows the muscular arrangement in the anterior part of the pharynx in longitudinal sections).

Posterior to the telostom

Behind the telostom, myofilaments of m3, m4 and subsequent muscles overlap and have their insertion on the pharyngeal cuticle. While m1 and m2 are actually

interradial muscles, with only one muscle per sector, the more posterior muscles are always present as a pair of muscles per sector (Figs 5, 6, 7). In the latter case, the bundles of myofilaments are situated adradially, most of the time with a narrow interradial zone of nerve fibers in between them. In all pharyngeal muscles, the distance from the pharyngeal lumen to the pharynx outer surface is exactly one sarcomere long. Thus the typical muscle sarcomere striation is recognizable: an I-band against the outer surface of the pharynx followed by an A-band with an H-band situated in its middle, again followed by an I-band near the lumen (indicated on Fig. 6 A).

Almost throughout the entire length of the pharynx, the interradial zone of the dorsal sector contains the gland duct of the dorsal pharyngeal gland (Fig. 7 A). This gland duct widens to an ampulla, which is connected to a short, narrow, cuticle lined canal that opens into the pharyngeal lumen (Fig. 6 B). The opening of the canal is situated at about 1 μm behind the posterior end of the telostom (Fig. 2). At the level of the opening of the dorsal gland, each pharyngeal radius has, about halfway, a rounded dilatation (Fig. 6 B).

**DIPLOLAIMELLA DIEVENGATENSIS**

**Cuticle**

The cuticle of *D. dievengatensis* is a lot thicker than the cuticle of *G. disjuncta*. In the pharyngeal region, it is about 0.5 μm thick, gradually narrowing down to 0.25 μm near the head (Fig. 8 A-C). The epicuticle of both species is similar: about 20 nm thick and composed of three layers (Fig. 8 A). The invaginations of the epicuticle again give rise to the transverse striation of the cuticle, with cuticular rings about 0.3 μm wide (Fig. 8 A). In *D. dievengatensis* the invaginations them-
Fig. 1. Legend: see p. 136.
Fig. 2. Legend: see p. 136.
Buccal cavity of monhysterid nematodes

Fig. 3. Legend: see p. 136.
Fig. 4. Legend: see p. 136.
Buccal cavity of monhysterid nematodes

Fig. 5. Legend: see p. 136.

Fig. 6. Legend: see p. 136.

Fig. 7. Legend: see p. 136.
Fig. 8. Legend: see p. 136.
Buccal cavity of monhysterid nematodes

Fig. 9. Legend: see p. 136.

selves are broader and hence more clearly visible than in G. disjuncta (Figs 8 A, respectively 1 A).

The cortical layer is moderately electron-dense, 80 nm thick, and shows a very fine striation in both longitudinal and transverse sections (Fig. 8 A, B). The median layer varies in thickness and is composed of two layers: (1) a rather electron-dense even layer of ± 80 nm closest to the cortical layer, and (2) a less electron-dense layer towards the interior. This layer is even at the contact surface with (1), but is serrated towards the basal layer (Fig. 8 A). The basal layer itself is about 100 nm thick, and has a rather electron-dense, fibroid appearance (Fig. 8 A, B). The layer as a whole is serrated, with invaginations corresponding to the transverse striations in the upper layers (Fig. 8 A).

As in G. disjuncta, the cortical and the basal layer of the cuticle diverge near the head. In D. dievengatensis this starts at about 1.5 μm posterior to the insertion of the cephalic setae (Fig. 8 C). The interstitial space is again filled with the continuation of the median layer. Anteriorly, this median layer is up to 0.5 μm thick and is traversed by the dendrites of the setae (Fig. 8 C).

The buccal cavity

The cheilostom is situated between the oral opening and the place where the head cuticle is in contact with the pharyngeal cuticle (Fig. 8 C). It is about 1.5 μm in diameter and 1 μm in length. The cheilostom is completely surrounded by the head cuticle.

The prostom is somewhat broader than the cheilostom, but only 0.25 μm long (Fig. 8 C, D). The thin prostom cuticle is surrounded by a narrow layer of epidermal tissue of the head, the arcade tissue (Fig. 8 C, D).

The mesostom is only slightly longer than the prostom, but narrows the buccal cavity down from ± 1.6 μm in diameter anteriorly to ± 1 μm in diameter posteriorly (Fig. 8 D). The cuticle of the mesorhabdia is relatively thick, about 250 nm (Fig. 8 C, D). The mesostom is surrounded by the rather electron-dense tip of the pharynx.

The metostom is about 1.5 μm long and has a funnel-shaped lumen (Fig. 8 D). Anteriorly the lumen is about 1 μm in diameter, and in most processed specimens it narrows down to become virtually closed. The cuticular lining of the metostom is about 100 nm thick, and is composed of a rather amorphous layer covered with a trilamellar layer (Fig. 8 C, D). It is surrounded by the anteriormost ring of pharyngeal muscles, the m1, of which the myofilaments insert on the metarhabdia (Fig. 8 C).

The telostom’s lumen is hemi-spherical, with the convex side directed to the anterior, and posteriorly measuring about 2 μm in diameter (Fig. 8 C). Its cuticular lining is rather electron transparent, amorphous, covered with a trilamellar layer, and in total only 80 nm thick (Fig. 8 C). Myofilaments of the second ring of pharyngeal muscles (m2) insert on its wall (Fig. 8 C).

Posterior to the buccal cavity

The telostom is followed by a transition zone, in which the lumen gradually narrows down from about 2 μm broad near the telostom to about 0.25 μm, as in the rest of the pharynx (Fig. 8 C). From this zone on, the cuticular lining is moderately electron-dense and about 150 nm thick (Fig. 8 C). The distance between the pharyngeal lumen and the outer surface of the pharynx is traversed by myofilaments of exactly one sarcomere long (I-, A- and H-bands are indicated in Fig. 8 C).

The opening of the dorsal pharyngeal gland is situated at the posterior end of the transition zone, i.e. at about 2.5 μm behind the telostom (Fig. 8 C).
Discussion

In this study we assumed that not only the cuticular differentiations, but also the identity of the surrounding structures or tissues can be used for identifying the buccal regions. The resemblance in configuration between \textit{G. disjuncta} and \textit{D. dienevagatensis} is then immediately noticeable, despite the apparent differences in general form. Moreover, the configuration is also similar to what Wright and Thomson (1981) described in \textit{C. elegans}. The mesostom of \textit{C. elegans} is surrounded by a collar of nine anteriormost epithelial cells belonging to the pharynx (Wright & Thomson, 1981). These cells contain a high amount of electron-dense filaments, and we assume that they are comparable with what we describe as “the electron-dense tip of the pharynx” in both \textit{G. disjuncta} and \textit{D. dienevagatensis}. These electron-dense filaments also have an appearance similar to the electron-dense periradial filaments that, in the rest of the pharynx, run from the pharyngeal radii to the outer surface of the pharynx.

The configuration in the buccal region can be summarized as follows: (1) the cheilostom is surrounded by head cuticle, (2) the prostom is surrounded by epidermal “arcade” tissue, (3) the mesostom is surrounded by a filament-containing tip of the pharynx, (4) the metastom is inserted by myofilaments of the anteriormost pharyngeal muscle \textit{m}_{1}; and (5) the telostom is inserted by myofilaments of \textit{m}_{2}. A schematic comparison between the buccal cavities of \textit{C. elegans}, \textit{G. disjuncta} and \textit{D. dienevagatensis} is given in Fig. 9. Although the species mentioned above are phylogenetically widely separated, the configuration of the buccal regions could represent true homologies, since they are based on several structural elements.

The connection between the body or head cuticle and the pharyngeal cuticle has frequently been considered in the past. The terminology “buccal ring” (introduced by Wieser, 1953) and “cephalic ring” (Inglis, 1964), is used to describe this connection in the enoplid head. Belogurov and Belogurova (1975) and Belogurov (1985) developed a clear terminology: the innermost layer of the head cuticle is called the endocupola, and the place where it meets the pharyngeal cuticle is called the buccal ring. This is a precise and valid definition of the term buccal ring. It implies that the buccal ring, defined as above, cannot undergo an elongation. Lorenzen (1978) however thought that the elongation of the buccal ring so that it becomes a cylinder is a tendency in the monhysterids. According to us, this so-called prolongation corresponds with an elongation of the pro- and/or mesostom.

The authors that have dealt with the separation of the cuticle in the head region assumed that this separation gives rise to either an empty space between the inner and outer cuticular layers (Belogurov & Belogurova, 1975) or to a fluid filled space (Inglis, 1964). Our observations in the monhysterids show the presence of a thick, rather electron-dense median cuticular layer, continuous with the median layer of the body cuticle.

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References


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