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Ultrastructure of the digestive system and experimental study of feeding in the monogenean skin and fin parasite *Macrogyrodactylus congolensis*

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Abstract

In the present study, transmission electron microscopy (TEM) has been used to study the ultrastructure of the digestive system, namely the pharynx, oesophageal glands and intestine, of the monogenean skin and fin parasite *Macrogyrodactylus congolensis*. The pharynx consists of an anterior highly muscular region and a posterior mainly glandular syncytial region. The anterior region is provided with six pharyngeal papillae, the centre of each of which is occupied by electron dense secretory bodies, identical with those in the posterior region of the pharynx. The intestine has an uninterrupted syncytial gastrodermis and the luminal surface is provided with many unbranched lamellae. The intestine of living specimens contains large and small granules which give it a reddish brown colour. Large particles, presumed to be lipid droplets, and small granules, presumed to be melanin granules, were found in the gastrodermis and in the intestinal lumen. Parasites were induced to feed and then preserved for TEM at the following intervals: just after feeding, 30 min after feeding, 1 h 30 min after feeding and 2 h after feeding. The specimens were then processed for TEM and sections cut through the intestine of each specimen were examined with the transmission electron microscope. Three types of vacuoles (V1, V2, V3) were detected in the gastrodermis.Vacuoles V1 have thick walls and are likely to be endocytotic, enclosing luminal contents at the surface of the gastrodermis. V2 vacuoles may be lysosomes that fuse with V1 vacuoles. V3 vacuoles may serve to dispose of residual digestive material into the lumen.

Keywords

Monogenea, *Macrogyrodactylus congolensis*, ultrastructure, digestive system, feeding, digestion.

Introduction

The freshwater North African catfish, *Clarias gariepinus* (Burchell, 1822) is a potential candidate for aquaculture. However, parasites can do serious damage in crowded fish farms. The gills of *C. gariepinus* are infected by a monogenean (platyhelminth) parasite, *Macrogyrodactylus clarii* Gusev, 1961 and the skin and fins by a relative, *M. congolensis* (Prudhoe, 1957) Yamaguti, 1963. The latter was recorded for the first time in Egypt by El-Naggar *et al.* (1999). Since then, there have been several studies of *M. congolensis* (see, for example, El-Naggar *et al.* 2001a,b; Arafa *et al.* 2003; El-Naggar *et al.* 2007; Arafa 2011). None of these studies has focussed on food, feeding and digestion in *M. congolensis*. However, histology of the gut and feeding of monopisthocotyleans have been previously studied by many authors (see for example, Halton and Stranock 1976 a,b; Kearn 1979; Kritsky *et al*. 1994; Cable *et al.* 1997, 2002). With the possible commercial importance of this parasite in mind and given the accessibility of skin parasites for experimentation and observation, it was decided to study feeding behaviour and, at the ultrastructural level, the anatomy of the digestive system. Ultrastructural changes in the gut during the process of digestion were also explored, providing a more detailed account of the digestive cycle of a monogenean than previous studies.

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Materials and Methods

Collecting the parasites

Specimens of *Clarias gariepinus* (syn: *C. lazera*) were obtained from the Demietta branch of the River Nile, near Mansoura, Dakahlia province, Egypt. Fishes were kept alive until required in tanks containing river water. Fishes were killed by a blow on the head and the skin was scraped using a scalpel. The scrapings were placed in Petri dishes containing filtered river water and searched for *M. congolensis* with a stereomicroscope. Fins were also removed, immersed in river water in separate dishes and searched for parasites with a stereomicroscope. Living specimens of *M. clarii* were collected from the gills of *C. gariepinus*.

Observations and experiments on feeding

To study the ultrastructure of the digestive system of *M. congolensis*, some specimens were processed for TEM according to Arafa (2011) and sections through different regions of the digestive system were examined. To induce parasites to feed, they were isolated from the host for 30 min – 2 h at about 25°C in dishes containing river water. Pieces of fins or skin were removed from the freshly killed fish and starved parasites were then placed on the host skin surface and kept under constant observation with a stereomicroscope. Whole living fishes were not suitable for feeding experiments because the movements of the fish created difficulties during the observation period. Specimens which were observed to feed successfully were preserved for TEM as described by Arafa (2011) at the following intervals: just after feeding, 30 min after feeding, 1 h 30 min after feeding and 2 h after feeding. Specimens were processed for TEM, sectioned at different levels through the intestine and observed with the transmission electron microscope as described by Arafa (2011). Part of the pelvic fin of a freshly killed fish was processed for TEM and sectioned and observed in a similar way. A piece of host skin on which *M. congolensis* had recently fed was preserved for scanning electron microscopy (SEM) according to Arafa *et al.* (2003). The transmission and scanning electron microscopy were conducted at the School of Biological Sciences, University of East Anglia, Norwich, England.

An attempt was made to induce the skin parasite *M. congolensis* to feed on the gills of its host and the gill parasite *M. clarii* to feed on the fin.

It should be mentioned that the feeding status (whether recently fed or not) is unknown for the specimens of *M. congolensis* that were sectioned for studying the ultrastructure of the digestive system.

Results

The main internal organs of *Macrogyrodactylus congolensis* are shown in Figure 1A. At the light microscope level, the digestive system of *M. congolensis* consists of mouth, buccal tube, pharynx, post-pharyngeal sphincter, oesophagus, oesophageal glands and intestine. The pharynx consists of an anterior highly muscular region and a posterior mainly glandular region (Fig. 1B). The anterior region is provided with six pharyngeal papillae. The intestine is composed of a single median tube which branches to two intestinal limbs. For more details, see El-Naggar *et al.* (1999).

Ultrastructure of the digestive system

The pharynx

TEM observations revealed that the anterior region of the pharynx is lined with a thick layer of tegument (Fig. 2) which is different from the tegument covering the general body surface. The pharyngeal tegument is highly folded and supported by a well developed basal lamina (Fig. 2). The tegumental cytoplasm is fibrous, moderately electron-dense and contains scattered highly electron-dense bodies (Fig. 2). There is no evidence of Golgi bodies, ribosomes or endoplasmic reticulum in the tegumental cytoplasm and there are no microvilli projecting from the surface.

The muscular wall of the anterior region of the pharynx is composed of rings of muscles (Fig. 1B). At the TEM level, each ring consists of circular and radial muscle fibres (Figs 2, 3). Many mitochondria and large nuclei are associated with the muscle fibres (Fig. 3). The anterior region of the pharynx is enclosed by a thick fibrous layer containing relatively small muscle fibres and resting on a relatively thin basal lamina (Fig. 3). Sarcodesmosomes occur beneath the tegument of the pharyngeal lumen (Fig. 2) and adjacent to the outer fibrous layer (Figs 2, 3). The basal plasma membrane of the pharyngeal tegument protrudes into the tegumental layer forming membranous folds (Fig. 2).

Each pharyngeal papilla is covered with the pharyngeal tegument (Fig. 4). Beneath the tegument is a well developed basal lamina and beneath this is a thick layer of longitudinal muscle fibres (Fig. 4). Only the outer regions of the longitudinal muscle cells are contractile. The non-contractile inner regions of these cells lack thick and thin filaments and contain numerous mitochondria (Fig. 4). The centre of each papilla is occupied by electron-dense secretory bodies, identical with those produced in the posterior region of the pharynx (Fig. 4). The arrangement of these secretory bodies in each papilla is not random. They are arranged in rows each of which consists of a single line of secretory bodies (Fig. 4). The arrangement of secretory bodies in the papillae is imposed by membranous partitions running roughly in a radial direction (Fig. 4). Each partition appears in some regions to be composed of a double membrane enclosing translucent material (Fig. 5). The membrane lining the pharyngeal papillae extends to form cytoplasmic finger-like processes projecting into a sac near the apex of each papilla (Fig. 5). Extensions of the membranous partitions pass centrally through each finger-like process (Fig. 5). The cytoplasm lining the sac is highly electron-dense (Fig. 6). Each sac has a single aperture at the apex of the papilla, but finger-like processes and secretory bodies were not seen close to the apex (Fig. 6).

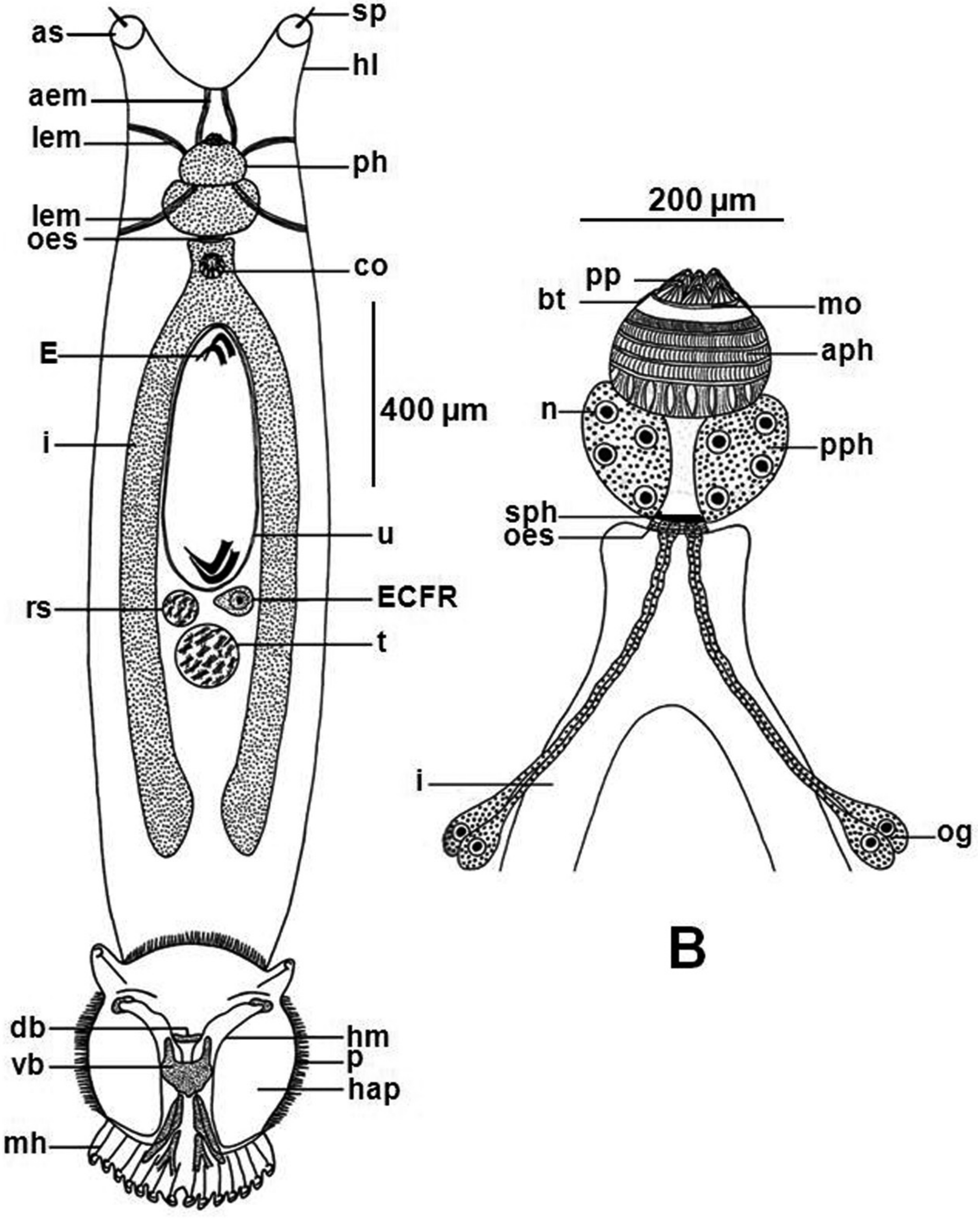


Fig. 1 A. Diagram showing the main internal organs of *Macrogyrodactylus congolensis* in ventral view and the extrinsic muscles attached to the anterior region of the pharynx. **B.** Diagram showing the pharynx, oesophagus and oesophageal glands; aem – anterior extrinsic muscles; aph – anterior region of the pharynx; as – anterior adhesive sac; bt – buccal tube; co – male copulatory organ; db – dorsal bar; E – embryo; ECFR – egg–cell–forming region; hap – haptor; hl – head lobe; hm – hamulus; I – intestine; lem – lateral extrinsic muscles; mh – marginal hooklet; mo mouth; n – nucleus; oes – oesophagus; og – oesophageal glands; p – haptoral papillae; ph – pharynx; pp – pharyngeal papillae; pph – posterior region of the pharynx; rs – receptaculum seminis; sp – spike sensillum; sph – sphincter t – testis; u – uterus; vb ventral bar

The posterior region of the pharynx is mainly a glandular syncytium (Fig. 7). The luminal surface of the posterior region of the pharynx is lined with tegument which is similar to that lining the luminal surface of the anterior region (Fig. 8). The posterior region of the pharynx contains variously orientated muscle fibres, the most prominent of which are muscle fibres which run in a radial direction (Fig. 8). The glandular syncytium appears to be divided into regions (inner, middle and outer) by thick fibrous partitions to which are attached the terminal portions of the radial muscles (Fig. 7). The luminal surface of the posterior region of the pharynx is highly folded. Beneath the tegument lining this region of the pharynx are longitudinal, radial and circular muscle fibres (Figs 7, 8). The glandular cytoplasm contains large nuclei with conspicuous nucleoli and abundant granular endoplasmic reticulum with dilated cisternae, as well as secretory bodies which vary in size and have an irregular or roughly spherical shape (Figs 7, 8). These secretory bodies in the posterior region of the pharynx and secretory bodies in the pharyngeal papillae are highly electron-dense whatever their shapes and sizes and filled with granular material (Figs 4 INSET, 8).

Oesophageal glands

The oesophageal glands are relatively small cells. Each gland cell has an irregularly-shaped nucleus with a conspicuous nucleolus (Fig. 9). The cytoplasm of the oesophageal glands contains abundant, small, electron-dense secretory bodies, in addition to granular and smooth endoplasmic reticulum and mitochondria.

The intestine

TEM sections revealed that the anterior median tube of the intestine and the two intestinal limbs have the same structure. They consist of an uninterrupted syncytial gastrodermis resting on a fibrous basal matrix in which widely spaced muscle fibres occur. The syncytial gastrodermis contains roughly spherical nuclei each with a conspicuous nucleolus (Fig. 10). The luminal surface of the intestine is provided with many lamellae projecting into the lumen (Figs 10-12). These lamellae are numerous in all regions of the intestine. The lamellae are mostly unbranched. In a few cases, the tips (distal extremities) of these lamellae were seen to be swollen, containing material similar to that present in the intestinal lumen. In other cases, some of these lamellae were seen to recurve and rejoin the gastrodermal surface.

The syncytial gastrodermis contains rod-shaped mitochondria with tubular cristae and a small amount of granular endoplasmic reticulum (Fig. 11). The cisternae of the granular endoplasmic reticulum are not conspicuous. The gastrodermis also contains different kinds of vacuoles (V1, V2 and V3) and many tubular structures (Fig. 11). In some sections, a great number of V2 vacuoles was detected (Fig. 12). For detailed descriptions of V1, V2 and V3 vacuoles, see below.

Large particles, presumed to be lipid droplets, and small granules, presumed to be melanin, were found in the gastrodermis and in the intestinal lumen (Fig. 12). The numbers of lipid droplets and melanin granules vary from one specimen to another and even from one region to another within the intestine. Lipid droplets were also found in tissues other than those of the intestine, such as parenchymal tissues and the haptor, while the melanin granules were restricted to the intestine.

Feeding in living specimens

Starved parasites readily attached themselves to the host skin by the haptor and sometimes made leech-like movements. Feeding usually commenced within a few minutes after placing the parasite on the host skin surface. At the commencement of feeding, the parasite attached itself to the host's surface by the mouth region. Because the mouth is ventral, it was not possible to see whether the pharynx was protruded during feeding. However, it was possible to see that the anterior adhesive zones on the head region moved freely and were not attached to the host tissue.

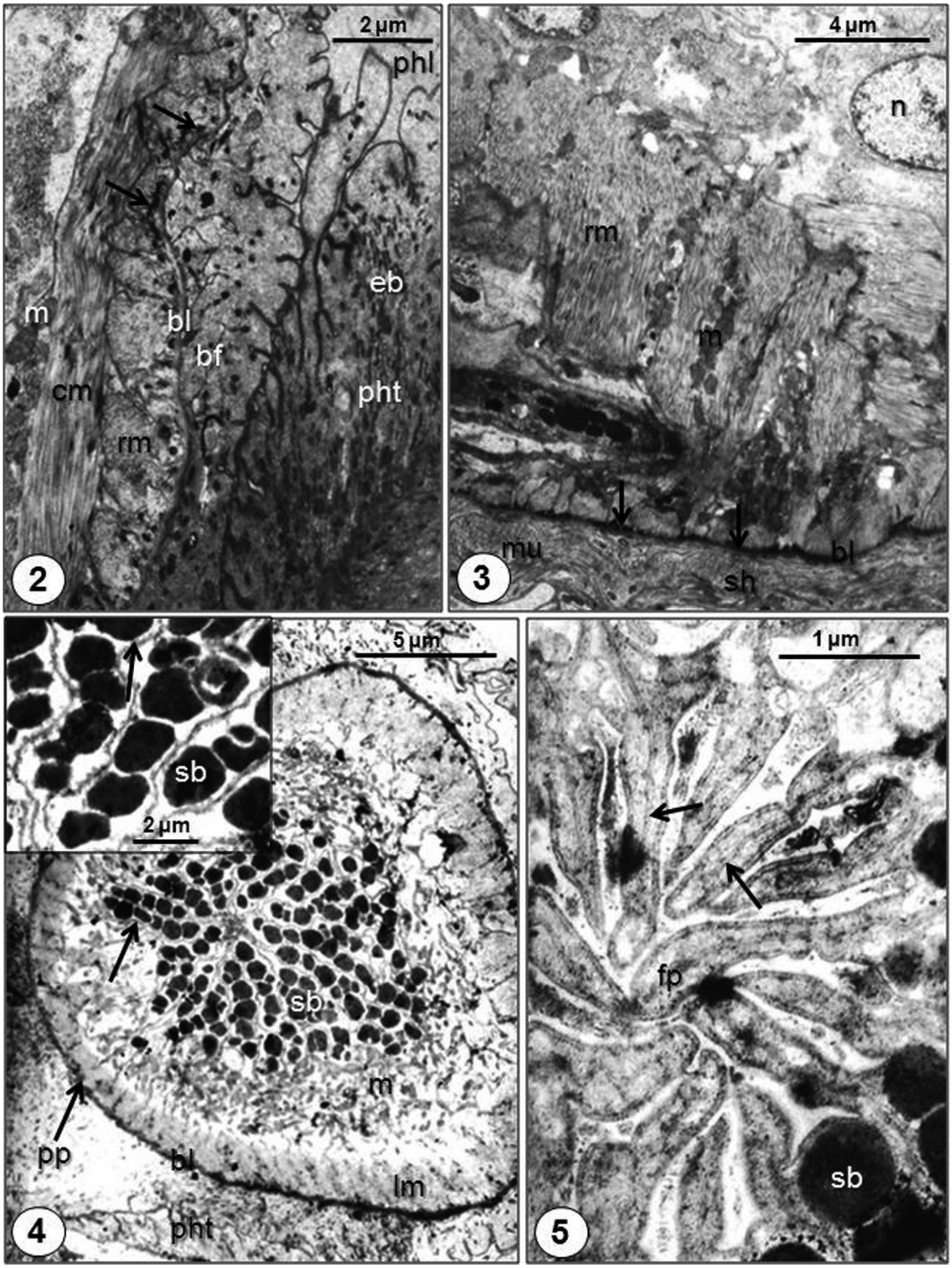
During feeding, the intestinal contents were pushed vigorously backwards and forwards, perhaps propelled by contraction and relaxation of muscles around the intestine or by contraction of the pharyngeal muscles. Waves of body movement were also observed during feeding, presumably produced by contraction and relaxation of the muscles beneath the tegument.

At a temperature of about 25 °C, feeding lasted for about 5 (2–8) min. At the end of feeding, the mouth region detached itself from the host surface. The contraction and relaxation of the body and intestine then ceased for a few seconds. Sometimes, the parasite started feeding at a new site nearby, without moving the haptor to a new position. In other cases, the parasite moved in a leech-like manner and, after reattaching the haptor, repeated the feeding process at another site. It was noticed that when the parasite spent a long time feeding (about 8 min), there was evidence of erosion at the feeding site, which appeared paler in colour when viewed with the light microscope than the neighbouring black skin. In an SEM view, a feeding wound appeared as a dark pit (Fig. 13), suggesting that epidermal tissue and possibly some dermal tissue had been removed. On one occasion egestion was observed. Turbid whitish liquid was ejected from the mouth of the parasite.

When living, starved specimens of *M. congolensis* were placed on the gill filaments, they were unable to attach themselves by the haptor and did not feed. Also, when starved living specimens of the gill parasite *M. clarii* were placed on the fin, they failed to attach themselves successfully by the haptor and also failed to feed.

TEM of the fin of Clarias gariepinus

Examination of TEM sections of the pelvic fin of *C. gariepinus* revealed that there are small pigment granules in the epidermal



Figs 2–5. Electron micrographs of TEM sections through different regions of the digestive system of *M. congolensis*. **Fig. 2.** Section through the anterior region of the pharynx showing the tegument of the pharyngeal lumen (pht), radial muscles (rm) and circular muscles (cm). Note the sarcodesmosomes (arrows) between the radial muscles and the basal lamina (bl). bf – basal folds of the plasma membrane; eb, electron–dense bodies; m – mitochondrion; phl – pharyngeal lumen. **Fig. 3.** Section through the anterior region of the pharynx showing radial muscles (rm) and fibrous sheath (sh) with small muscle fibre (mu). Arrows indicate sarcodesmosomes; as in Figs 1, 2. **Fig. 4.** Transverse section through the middle part of a pharyngeal papilla (pp). Note that its wall consists of longitudinal muscle fibres (lm) with a well developed basal lamina (bl) and an outer covering of pharyngeal tegument (pht). Note also the membranous partitions (arrows) running between the secretory bodies (sb) and mitochondria (m). **INSET:** The central region of the middle part of a pharyngeal papilla showing the secretory bodies (sb) separated by membranous partitions (arrows). **Fig. 5.** The central region of a pharyngeal papilla near the apex showing finger-like processes (fp). Note that the cytoplasmic partitions (arrows) are composed of two membranes enclosing translucent material. sb – secretory bodies

tissue (Fig. 14). These pigment granules are few in number and have a roughly circular profile. The diameter of these granules varies from about 400 nm to about 2.4 mm. Most of these granules are located inside lacunae (Fig. 14). The epidermal cells of the fin which contain these granules were connected to each other by junctional complexes (Fig. 14). It seems likely that these epidermal pigment granules are melanin, although tests to confirm this have not been conducted.

TEM observations at intervals after feeding

Lipid droplets and melanin granules

In all specimens of *M. congolensis* preserved after feeding, the content in the intestinal lumen (Fig. 15) and the gastrodermis (Figs 17, 20–22) contained large and small granules presumed to be lipid droplets and melanin granules respectively. The lipid droplets are mainly circular in shape with a diameter varying from about 1.7 mm to about 3 mm. They have an electron density higher than that of the surrounding cytoplasm. The melanin granules were highly electron-dense, circular in shape, with diameters ranging from about 250 nm to about 450 nm. These granules are similar to the pigment granules in the fin epidermis (Fig. 14), although most of the granules from the host tissue are larger.

Parasite preserved immediately after feeding (Figs 15, 18)

TEM sections through the intestine of *M. congolensis* preserved just after feeding revealed that the anterior regions of the two intestinal limbs were collapsed with reduced intestinal lumen. On the other hand, the cavities of the middle and posterior regions of the intestinal limbs were considerably expanded. The food material present inside the intestinal lumen was found adjacent to the inner surface of the intestinal wall. The food material present in the intestinal lumen was partially digested. Mitochondria were identifiable in the gut contents (Fig. 15). In some TEM sections, the food material contained a few electron-dense circular structures (Fig. 16). Such structures were not detected in the gastrodermis. Melanin granules were present in the intestinal lumen (Fig. 15) and in the gastrodermis (Fig. 17).

The gastrodermis contained a variety of inclusions. Three kinds of vacuoles (V1, V2 and V3) were identified. Particularly conspicuous were large vacuoles (V3) containing heterogeneous granular material, some of which was more electron-dense than the rest (Fig. 17). Melanin granules were sometimes present in these vacuoles (Fig. 17). More V3 vacuoles were observed near the luminal surface than in the deeper parts of the gastrodermis (Fig. 17). V2 vacuoles contained a more uniform, homogeneous granular material, in which melanin granules were occasionally embedded (Fig. 18). Near the luminal surface were small vacuoles (V1) with relatively thick walls. There is evidence that these are food vacuoles formed by endocytosis, involving invagination of the gastrodermal surface between the gastrodermal lamellae. It was found that the number of V2 vacuoles differed from one region of the gastrodermis to another in the intestine of the same specimen. In some regions there are relatively few V2 vacuoles, while in other regions, V2 vacuoles are abundant (Fig. 18).

Parasite preserved 30 min after feeding (Fig. 19)

The anterior and middle regions of the intestine of this specimen were collapsed, reducing the volume of the intestinal lumen. In contrast, the lumina of the posterior region of the intestine were greatly enlarged. However, the amount of food material present in the intestinal lumen was less than in the specimen preserved just after feeding. In the specimen preserved 30 min after feeding, the food material present in the intestinal lumen seemed to be partially digested, with lipid droplets and melanin granules free in the lumen. However, in one section, an intact cell was detected in the intestinal lumen. Melanin granules and lipid droplets were also detected in the gastrodermis. Endocytotic food vacuoles (V1) are more abundant in the gastrodermis of this specimen than in the previous specimen which was preserved just after feeding (Fig. 19). Some of these V1 vacuoles are united with tubular structures. Some small heterogeneous vacuoles (V3) were found in the gastrodermis but these were relatively uncommon compared with the parasite which had just fed.

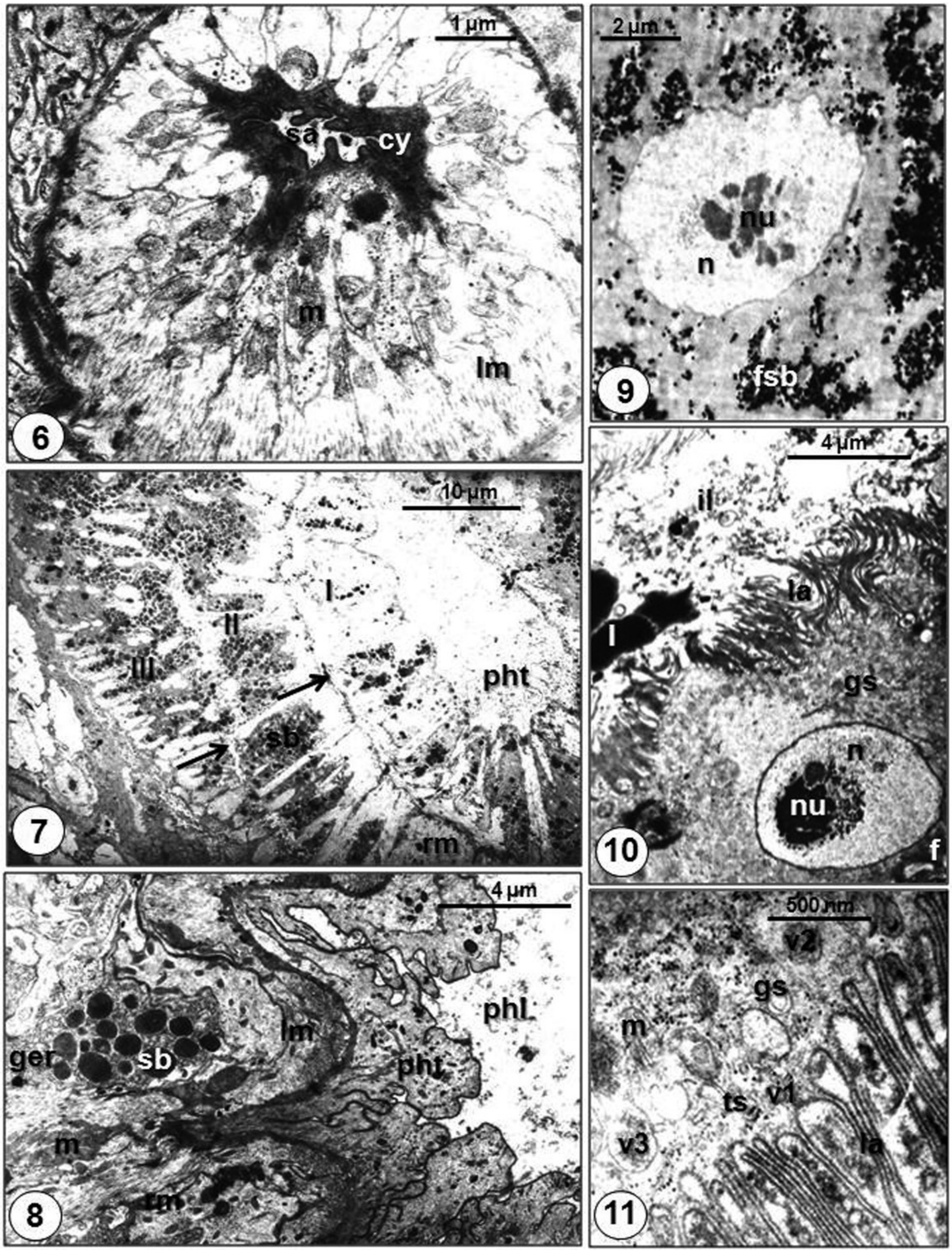
Parasite preserved 1h 30 min after feeding (Figs 20–22)

The luminal surface of the intestine of this specimen was folded and the intestinal lumen was reduced. Lipid droplets and melanin granules were present in the intestinal lumen and in the gastrodermis. Endocytotic food vacuoles (V1) were abundant (Fig. 20). The most characteristic feature of the gastrodermis of this specimen was the presence of tubular structures connecting some of the food vacuoles (V1) forming a conspicuous reticular system. Homogeneous vacuoles (V2) were abundant in some regions. Large heterogeneous vacuoles (V3) were detected in the gastrodermis, especially near the lamellar surface (Fig. 20).

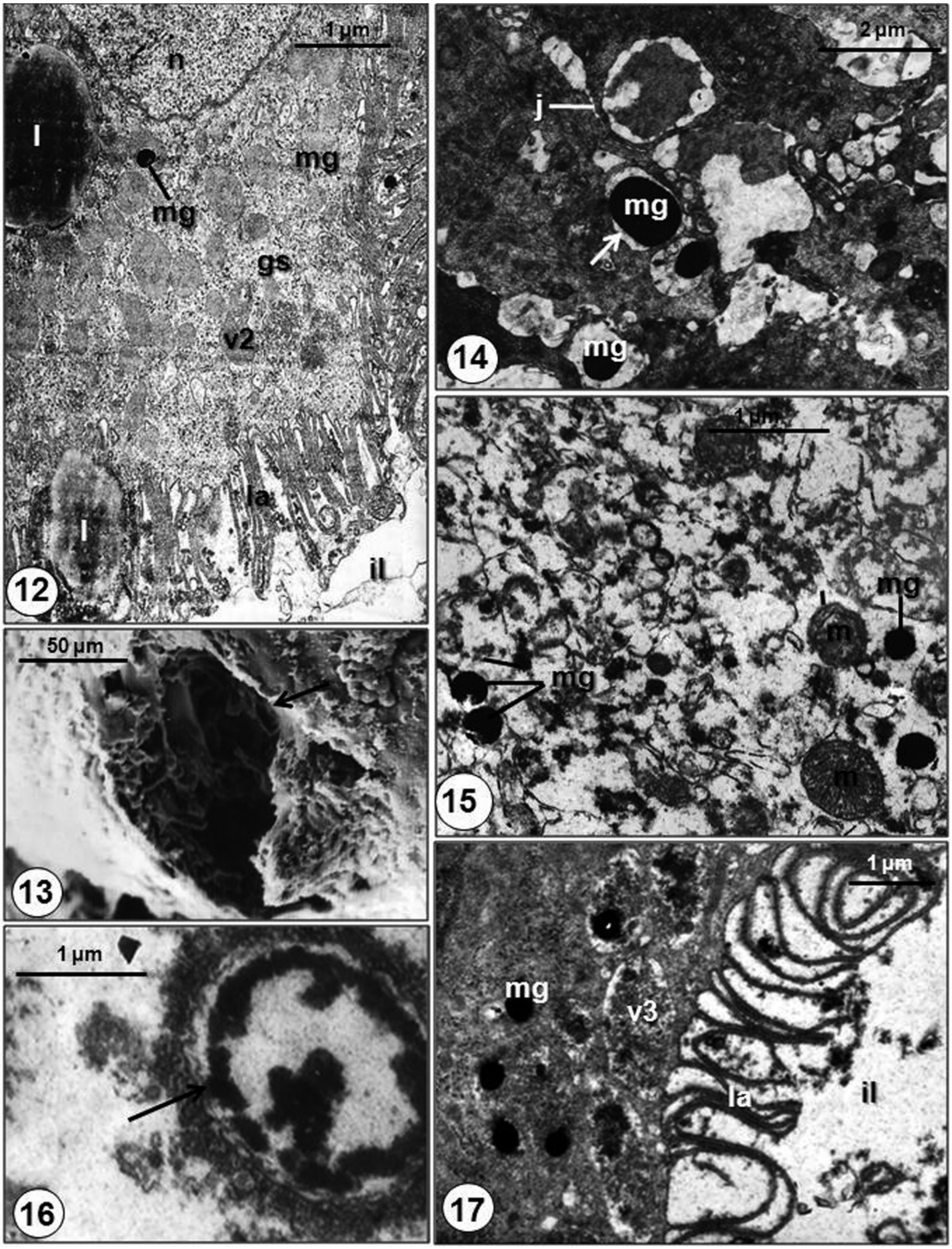
The granular endoplasmic reticulum was more conspicuous in this specimen than in specimens preserved immediately after feeding and 30 min after feeding. Granular endoplasmic reticulum was located adjacent to the highly folded basal lamina (Fig. 21) and in isolated regions distributed throughout the gastrodermis (Fig. 22). Some highly folded membranous structures were found (Fig. 22). An unidentified micro-organism (mo) was found in the gastrodermis (Figs 21, 22).

Parasite preserved 2 h after feeding

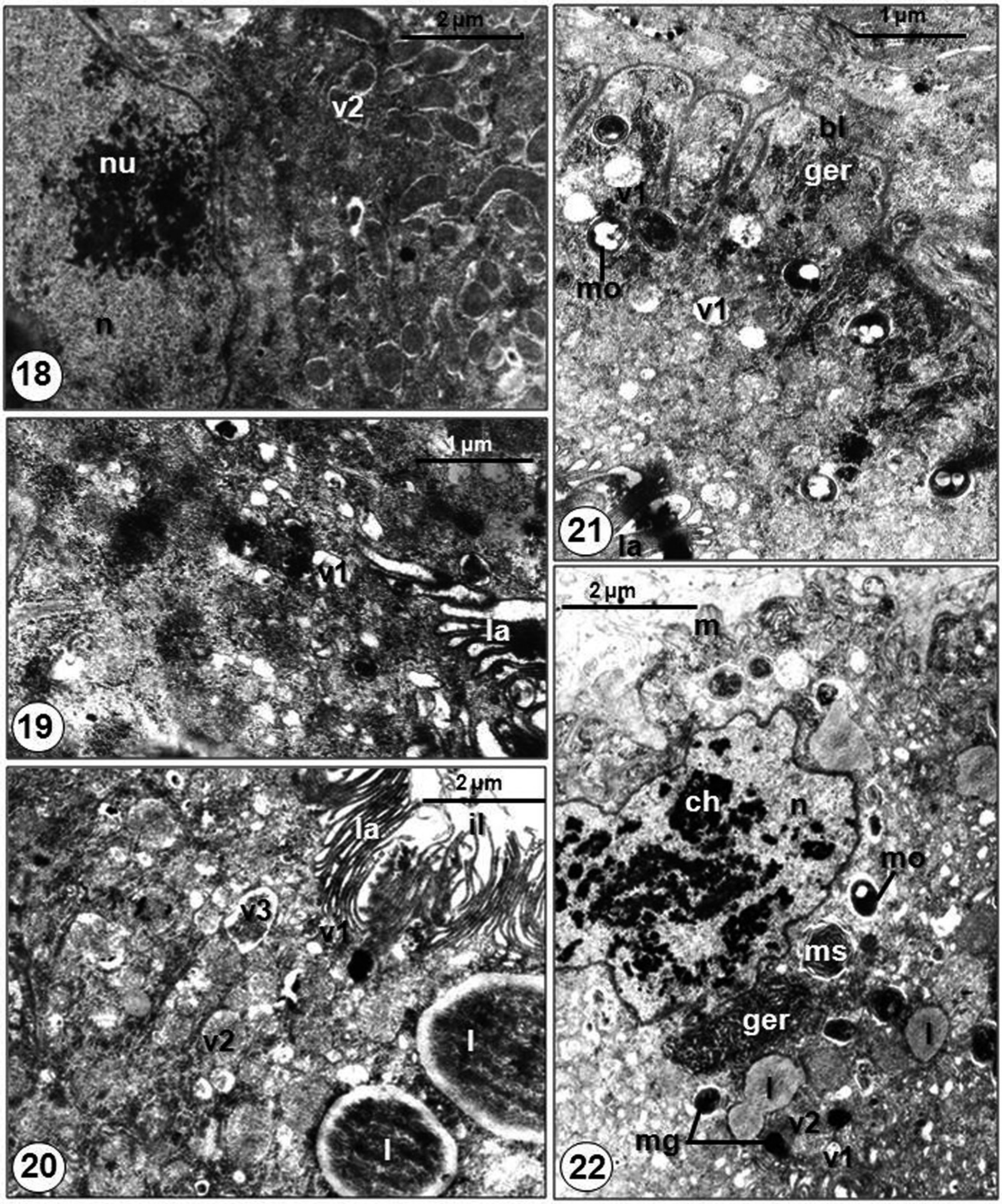
The intestine of the specimen was very much collapsed leading to much reduction of the intestinal lumen. Very little food material was found in the intestinal lumen. The gastrodermis



Figs 6–11. Electron microgrographs of TEM sections through different regions of the digestive system of *M. congolensis*. **Fig. 6.** Transverse section of the sac near the apex of a pharyngeal papilla. Note that the cytoplasm (cy) lining the sac (sa) is highly electron-dense. lm – longitudinal muscles; m – mitochondrion. **Fig. 7.** Section through the posterior region of the pharynx showing the pharyngeal tegument (pht), radial muscles (rm) and secretory bodies (sb). Note that the glandular syncytium is divided into regions (inner I, middle II and outer III) by thick fibrous partitions (arrows). **Fig. 8.** Section through one of the luminal folds of the posterior region of the pharynx. ger, Granular endoplasmic reticulum; lm, longitudinal muscles; m, mitochondrion; phl, pharyngeal lumen; pht, pharyngeal tegument; rm, radial muscles; sb, secretory bodies. **Fig. 9.** Section through one of the oesophageal gland cells showing the irregularly-shaped nucleus (n) with nucleolus (nu) and fine secretory bodies (fsb). **Fig. 10.** Section through the intestine showing the gastrodermis (gs) resting on a fibrous layer (f). Note large numbers of lamellae (la) projecting from the luminal surface of the gastrodermis into the intestinal lumen (il). l– lipid droplet; n – nucleus; nu nucleolus. **Fig. 11.** The gastrodermis (gs) containing mitochondria (m), different kinds of vacuoles (V1, V2 and V3) and tubular structures (ts). la – intestinal lamellae



Figs 12–17. Fig. 12. The gastrodermis (gs) containing many V2 vacuoles. il – intestinal lumen; l – lipid droplet; la – intestinal lamellae; mg – melanin granule; n – nucleus. **Fig. 13.** Scanning electron micrograph of the skin of *Clarias gariepinus* showing a site on which *M. congolensis* had previously fed (arrow). **Fig. 14.** Electron micrograph of a TEM section through the epidermis covering the pelvic fin of *C. gariepinus*. j – junctional complex; mg – melanin granule inside lacuna (arrow). **Fig. 15.** Partially digested food material present in the intestinal lumen. m – mitochondrion; mg – melanin granule. **Fig. 16.** Electron-dense circular structures (arrows) found in partially digested food material in the intestinal lumen. **Fig. 17.** Gastrodermis containing heterogeneous vacuoles (V3). il – intestinal lumen; la – intestinal lamellae; mg – melanin granule



Figs 18–22. Electron micrographs of TEM sections through the intestine of a specimen of *M. congolensis* preserved at different intervals after feeding.**Fig. 18.** Gastrodermis containing abundant V2 vesicles. n– nucleus; nu – nucleolus. **Fig. 19.** Gastrodermis containing many V1 vacuoles and la – intestinal lamellae. **Figs 20–22.** Electron micrographs of TEM sections through the intestine of a specimen of *M. congolensis* preserved 1 h 30 min after feeding. **Fig. 20.** Gastrodermis containing lipid droplets (l), V1, V2 and V3 vacuoles. il – intestinal lumen; la – intestinal lamellae. **Fig. 21.** Gastrodermis containing V1 vacuoles, granular endoplasmic reticulum (ger) adjacent to the highly folded basal lamina (bl) and micro-organisms (mo). la – intestinal lamellae. **Fig. 22.** Gastrodermis containing groups of granular endoplasmic reticulum (ger), V1 vacuoles, V2 vacuoles containing melanin granules (mg), lipid droplets (l), mitochondria (m), membranous structures (ms) and micro-organisms (mo), ch – chromatin, n – nucleus

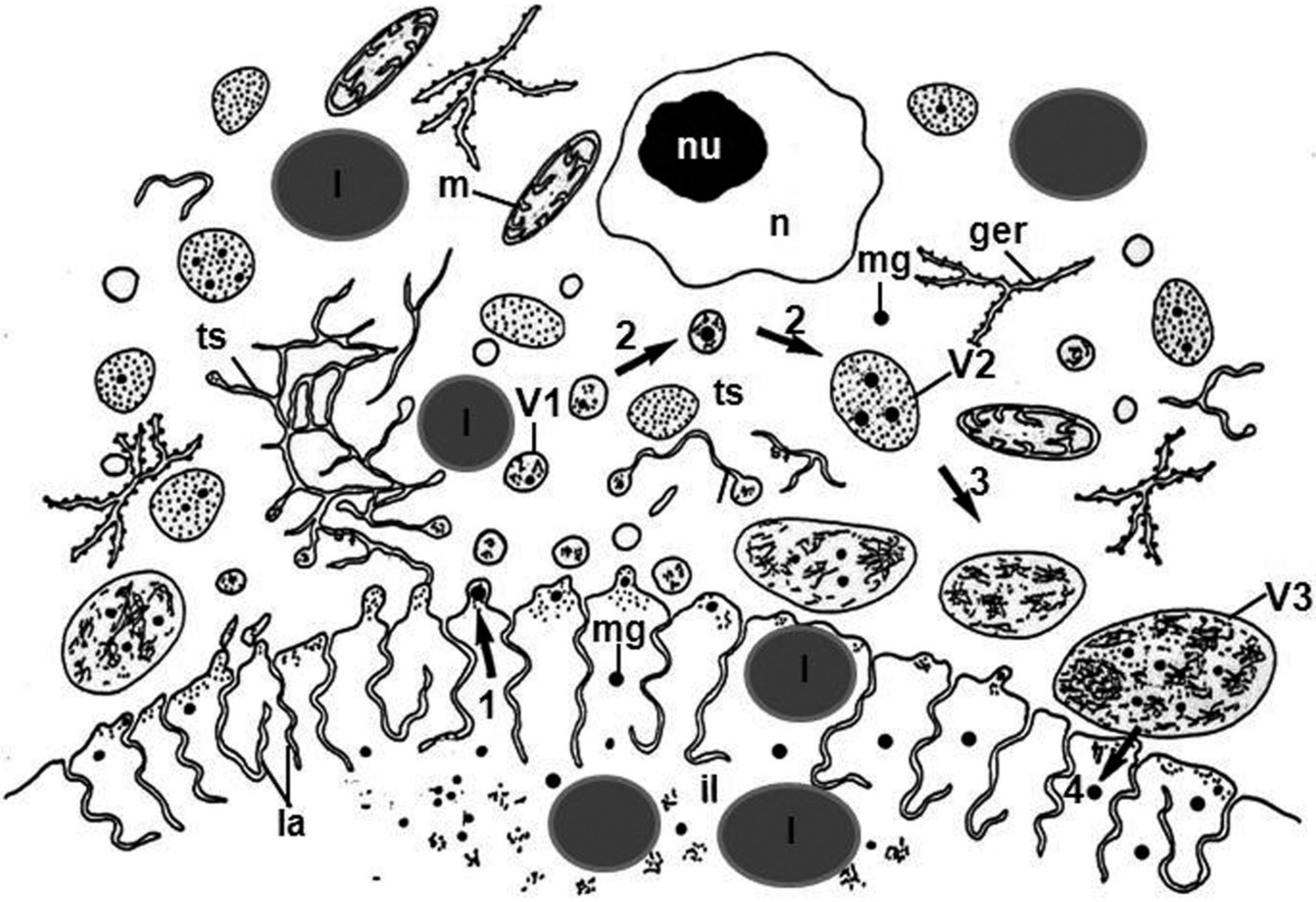


Fig. 23. Diagrammatic representation of the proposed pathway indicated by arrows for intracellular digestion in the syncytial gastrodermis of *Macrogyrodactylus congolensis*. 1) endocytosis of the food material creating V1 vacuoles united by a tubular reticular system (ts); 2) migration of V1 vacuoles in the gastrodermis and fusion with V2 vacuoles presumably containing digestive enzymes; 3) formation of residual vacuoles (V3) after digestion and absorption; 4) exocytosis of residual material into intestinal lumen (il). ger – granular endoplasmic reticulum; l – large granule (lipid droplet); la – intestinal lamellae; m – mitochondrion; mg – small granule (melanin); n – nucleus; nu – nucleolus

of this specimen had the same features as the specimen preserved 1h 30 min after feeding.

Discussion

In the present work, the ultrastructure of the pharynx, oesophageal glands and intestine of *M. congolensis* were studied. Feeding is also described in *M. congolensis.* Examination of TEM sections confirmed the observations made on *M. congolensis* by Prudhoe (1957) and El-Naggar *et al.* (1999) that the pharynx consists of two regions: an anterior muscular and a posterior mainly glandular region. The anterior region is provided with six pharyngeal papillae; each one is ensheathed by a tegumental layer and longitudinal muscle fibres. The secretory bodies which are produced in the posterior region of the pharynx were found in the pharyngeal papillae arranged in radial rows separated by membranous partitions. There is a small sac near the tip of each pharyngeal papilla. The rows of secretory bodies terminate close to the lining of this sac and are presumably shed into the sac at some stage during feeding. Each sac has a single opening at the apex of each papilla. In *M. clarii* at the light microscope level, El-Naggar and Serag (1987) described 5-7 narrow gland ducts carrying secretory bodies in each pharyngeal papilla and stated that the ducts are separated from each other by longitudinal muscle fibres.

In the present study, TEM observations also confirmed the light microscope observations made by El-Naggar *et al.* (1999) that the posterior region of the pharynx of *M. congolensis* is a glandular syncytium. The posterior region of the pharynx of *M. clarii* was also recorded as syncytial at the light microscope level by El-Naggar and Serag (1987). Prudhoe (1957) recorded six large gland cells in the posterior region of the pharynx of *M. congolensis* and, according to Khalil (1970), *M. polypteri* has six large gland cells in the posterior region of its pharynx.

Like the monogenean skin parasite *Entobdella soleae* (see Kearn 1963), *M. congolensis* can be induced to feed experimentally by separating it from the host for 30 min – 2 h and replacing it on fresh skin. *M. congolensis* attaches itself to the skin surface by the mouth region during feeding, but it was not possible to determine whether the pharynx is everted as it is in *E. soleae* (see Kearn, 1963). However, El-Naggar *et al.* (1999) found that the tips of the pharyngeal papillae of *M. congolensis* often protrude in whole mount preparations, suggesting that protrusion of these papillae may occur during feeding. El-Naggar *et al.* (1999) also suggested that the extrinsic muscles connected to the anterior region of the pharynx may play a role in that protrusion. Mo (1994) stated that the glandular pharynx of gyrodactylids is everted through the mouth.

In *M. polypteri*, Malmberg (1957) found that the oesophagus and the anterior part of the gut diverticula are dark brown in colour, while the remaining part of each diverticulum is composed of three yellow and three brown alternating portions. Cable *et al.* (1997) found the same banding in *M. polypteri*. Banding of this kind was not recorded in *M. congolensis*, either in the present study or in the original description by Prudhoe (1957) and banding was not seen in *M. clarii* by El-Naggar and Serag (1987).

TEM sections revealed that the gastrodermis of *M. congolensis* consists of a syncytium as in other gyrodactylids, e.g. *Gyrodactylus* spp. (see Kritsky *et al.* 1994, Cable *et al.* 2002). In some monopisthocotyleans, e. g. in *Euzetrema knoepffleri* and *Calicotyle kröyeri*, the gastrodermis is cellular, and consists of a single cell type (see Halton and Stranock 1976a, Fournier 1978 respectively). The luminal surface of the intestine of *M. congolensis* is provided with many unbranched lamellae projecting into the intestinal lumen. Similar lamellae were recorded in *Gyrodactylus eucaliae* (see Kritsky *et al.* 1994) and in *M. polypteri* (see Cable *et al.* 1997). However, Kritsky *et al.* (1994) found that the lamellae of *G. eucaliae* are fewer in number and occasionally branched.

Harris (1982, in Sterud *et al.* 1998) recorded feeding every 15-30 min in *Gyrodactylus gasterostei* and described a feeding wound 20-30 mm in diameter which extended to the basement membrane. Malmberg (in Mo 1994) claimed that these parasites may penetrate the basement membrane into the dermis during feeding. *E. soleae* has a colourless gut and erodes only the epidermis of its host (*Solea solea*). It is the dermis not the epidermis that contains melanin in the upper skin of this fish and the feeding sites created by experimentally starved parasites appear darker than the surrounding undamaged skin. In contrast, *M. congolensis* has a pigmented gut and the feeding sites made by the parasite often appear paler than the surrounding skin, especially if feeding is prolonged. This does not necessarily mean that the dermis is eroded since melanin is located in the epidermis in *Clarias gariepinus*. Kearn (1979) observed that the ray *Amphotistius kuhlii* has epidermal pigment and that its skin parasite, the monocotylid *Dendromonocotyle kuhlii*, has a pigmented gut. *M. congolensis* failed to feed on host gill tissue, but it also failed to attach its haptor and firm attachment by the haptor seems likely to be an important prerequisite for feeding.

It is assumed that a circular patch of host skin is enclosed by the open mouth of *M. congolensis* or by the anterior margin of the protruded pharynx and that secretion originating in the posterior region of the pharynx travels anteriorly, enters the papillae and is expelled onto the host skin surface. Contraction of the radial muscles in the posterior region of the pharynx may help in pushing the secretion into the papillae. Adhesion between host epidermal cells may be destroyed by the pharyngeal secretion, permitting the parasite to ingest the loosened cells, but extracellular digestion may go further, leading to disruption of individual host cells. There is evidence that such disruption and extracellular digestion occur in the gut lumen of the parasite since cell debris, including mitochondria and melanin granules, was abundant in the lumen of the parasite preserved immediately after feeding. In this parasite, circular structures which may have been pyknotic host nuclei were observed in the intestinal lumen. Curiously, in a parasite preserved 30 min after feeding a single large apparently healthy cell was observed. As the gastrodermis of *M. congolensis* is syncytial, this cell may have been a host cell which, for some unknown reason, had resisted disruption.

The secretion of the oesophageal glands of the parasite, which probably enters the gut at the pharynx/oesophagus junction, together with possible persistent enzymatic activity in material exocytosed from the gastrodermal digestive vacuoles (V3), may contribute to extracellular digestion in the lumen. El-Naggar and Serag (1987) and Watson and Whittington (1996) claimed a similar function for the oesophageal gland secretion of *M. clarii* and *Concinnocotyla australensis* respectively.

TEM sections through the intestine of *M. congolensis* revealed that there are large and small granules in the intestinal lumen and the gastrodermis. The large granules were also detected outside the intestine, in the parenchymal tissue of the parasite and even in the haptor, while the small granules were restricted to the intestine. The large granules are presumed to be lipid droplets while the small granules are presumed to be melanin granules. Presumed lipid droplets were also found attached to and within the digestive cells of *Concinnocotyla australensis* by Watson and Whittington (1996). On the other hand, Halton and Stranock (1976a) identified lipid droplets in the intestinal lumen, in the gastrodermal cells and in the apical residual vacuoles in *Calicotyle kröyeri*. Since, according to Halton and Stranock (1976a), the contents of the residual vacuoles are destined to be expelled into the lumen, the lipid droplets in this animal seem destined for expulsion from the gastrodermis. In *M. congolensis*, lipid droplets in the gut lumen seem likely to be derived from the host tissue, but the relationship, if any, with lipid droplets in the gastrodermis and parenchyma is uncertain. The gastrodermal lipid may represent host lipid endocytosed from the intestinal lumen, but there is no evidence that the endocytotic vacuoles (V1) can engulf such large intact droplets and no evidence that the large droplets in the lumen are broken up and engulfed in small endocytotic vacuoles. It is even more difficult to imagine how large intact lipid droplets could leave the gastrodermis basally and gain access to the parenchyma. It remains a possibility that the lipid in the parenchyma is of parasite origin.

Melanin granules present in the intestinal lumen and the gastrodermis of *M. congolensis* are similar in shape and electron-density to melanin granules present in the epidermal tissues covering the host's fins, although most of the latter are larger. However, large melanin granules in the epidermal tissue of the host fin may be broken into smaller granules during the feeding process. The ultrastructure of melanin granules is quite distinct and they differ from the irregular granules of haematin deposited in the gut of blood feeding monogeneans (see Tinsley 1973, Halton and Stranock 1976a, Fournier 1978). The presence of partially digested epidermal tissue in the intestinal lumen of *M. congolensis* and the absence of any haematin pigment in the lumen or gastrodermis confirm that *M. congolensis* is an epithelial browser, like other gyrodactylids and the majority of monopisthocotylean monogeneans.

TEM sections through the intestine of a specimen of *M. congolensis* preserved just after feeding revealed the presence of small V1 vacuoles near the luminal surface of the gastrodermis. Evidence was found to indicate that these vacuoles form by endocytosis involving invagination of the gastrodermal surface between the bases of the lamellae projecting into the lumen from the gastrodermis. During this process, partially digested and dispersed host cellular debris is taken into the vacuoles and transported into the gastrodermis of the parasite. A similar mechanism of endocytosis has been recorded in *Diclidophora merlangi*, *Calicotyle kröyeri*, *Euzetrema knoepffleri* and *Concinnocotyla australensis* (see Halton 1975, Halton and Stranock 1976a, Fournier 1978, Watson and Whittington 1996 respectively). However, Kritsky *et al.* (1994) could not find any evidence of endocytotic activity in the gastrodermis of *Gyrodactylus* spp. The number of endocytotic vacuoles (V1) in *M. congolensis* was found to be greater in the gastrodermis of the specimens preserved 30 min and 1h 30 min after feeding than in the specimen preserved just after feeding. This supports the idea that V1 vacuoles represent the first stage of the gastrodermal digestive cycle and suggests that endocytotic activity rises to a peak during the first 30 min after feeding. The observation that the food material present in the intestinal lumen preserved at later stages after feeding is much less than that present in the intestinal lumen of the specimen preserved just after feeding, supports the previous suggestion and underlines the rapidity with which food material is taken up from the lumen.

As melanin granules were found in some endocytotic vacuoles (V1) and in homogeneous vacuoles (V2), it seems likely that these two kinds of vacuoles fuse. In the pigmented region of the intestine of *M. polypteri*, Cable *et al.* (1997) also found many melanin granules in the gastrodermis. Most of these granules were incorporated into vacuoles similar to the vacuoles of *M. congolensis*. It is suggested that V2 vacuoles contain digestive enzymes; i. e. that they are lysosomes, which are responsible for an intracellular contribution to digestion of host material in the endocytosed V1 vacuoles; i.e. host material that has already been partially digested extracellularly in the gut lumen.

In a specimen of *M. congolensis* starved for 30 min after feeding, some tubular structures were detected. These tubular structures were better developed in the gastrodermis of a specimen preserved 1 h 30 min after feeding and appeared to form a reticular system connecting the V1 vacuoles. Such tubular structures were not detected in the gastrodermis of the specimen preserved just after feeding, which may indicate that it is a temporary system used during the digestive process. Halton and Stranock (1976a) found a tubular system which opens to the lumen at the bases of the intestinal lamellae of *Calicotyle kröyeri* and also communicates with the apical pockets (= V1 vacuoles). They suggested that this tubule system provides storage space in which food can accumulate prior to transfer to the digestive vacuoles, and, in this respect, it represents part of the endocytotic complex of the gastrodermal cells. Cable *et al.* (2002) found that when starved worms of *Gyrodactylus gasterostei* reattached to a fish, they began browsing on host epithelial cells within 1 min and ultrastructural evidence for phagocytic activity in the gastrodermis was detected after 5 min. Putative waste vacuoles increased in abundance after 5–30 min on the new host, coinciding with the reappearance of electron-dense vesicles and possibly indicating completion of the first intracellular cycle of digestion.

The origin of the digestive enzymes in the gastrodermis of *M. congolensis* is not known. Halton and Stranock (1976a) provided two possible sources in *Calicotyle kröyeri*: first lysosomes that fuse with the food vacuoles and secondly the structures that form part of the membranes delimiting the food vacuole system (tubules and related endocytotic vesicles). The large V2 vacuoles in *M. congolensis* seem likely to be lysosomes, but the endocytotic vacuoles V1, which appear to be equivalent to the food vacuole system of *C. kröyeri*, have thick walls and may also be a source of digestive enzymes.

V3 vacuoles are relatively large and contain condensed electron-dense material. These V3 vacuoles were particularly conspicuous in the specimens preserved just after feeding had taken place, following a 30 min – 2 h starvation period. Moreover, many of these V3 vacuoles were close to the luminal surface of the gastrodermis. V3 vacuoles appeared to be less abundant in specimens preserved 30 min and 1h 30 min after feeding. These observations support the suggestion that these V3 vacuoles represent the end of the gastrodermal digestive cycle and that their contents are residual material left over on completion of intracellular digestion. The contents of V3 vacuoles are presumably extruded into the intestinal lumen by exocytosis, involving fusion of the membrane of these vacuoles with the apical surface membrane of the gastrodermis. Some melanin granules were detected within these V3 vacuoles, indicating that these granules are indigestible. Presumably they are ejected into the intestinal lumen with the rest of the residual material in the V3 vacuoles. Thus, some melanin granules observed in the lumen may have been transported through the gastrodermis by the digestive cycle, while others may be freshly ingested and may not have entered the gastrodermis.

Halton and Stranock (1976a) found that in *Calicotyle kröyeri*, indigestible residues and lipid droplets accumulate in a large apical vacuole in each gastrodermal cell and are periodically released to the lumen by exocytosis. Each apical vacuole is formed by fusion of many smaller residual vacuoles. In *M. congolensis*, it is likely that each V3 vacuole releases its contents individually into the lumen, and there appears to be no fusion of V3 vacuoles to form large vacuoles equivalent to the apical vacuole of *C. kröyeri*.

It was found that the granular endoplasmic reticulum (ger) was very conspicuous in the gastrodermis of *M. congolensis* preserved 1 h 30 min after feeding. This ger lay adjacent to the basal lamina or was concentrated in small isolated areas throughout the gastrodermis. The ger was not prominent in the gastrodermis of the specimens of *M. congolensis* preserved just after feeding or 30 min after feeding. These observations suggest either that the ger is more abundant and conspicuous in some regions of the gastrodermis than it is in other regions of the same individual, or that the ger has an activity cycle which coincides with the intracellular digestive cycle.

On only one occasion, egestion was noticed in a living specimen of *M. congolensis*. It was not possible to see cells in the egested liquid. Kearn (1963) and Kearn *et al.* (1996) observed egestion of the gut contents through the pharynx and mouth in *Entobdella soleae*. The egested fluid appeared to contain cells but, if this is the case, these may be spent gastrodermal cells released by the parasite.

The proposed pathway for intracellular digestion in the syncytial gastrodermis of *M. congolensis* is shown diagrammatically in Figure 23. These events are likely to be as follows: (1) formation of V1 endocytotic vacuoles which may unite to form a tubular system; (2) migration of V1 vacuoles in the gastrodermis and fusion with V2 vacuoles, presumably containing digestive enzymes; (3) formation of residual vacuoles (V3); (4) exocytosis of residual material into the intestinal lumen. In conclusion, the observations on specimens preserved at known intervals after feeding permit a more detailed account of the events in the digestive cycle of a monogenean than previously possible.

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