Ultrastructure of the anterior adhesive apparatus of the gill parasite *Macrogyrodactylus clarii* and skin parasite *M. congolensis* (Monogenea; Gyrodactylidae) from the catfish *Clarias gariepinus*

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Abstract

Transmission electron microscopy (TEM) was used to study the anterior adhesive apparatus of the gill parasite *Macrogyrodactylus clarii* Gussev, 1961 and skin parasite *M. congolensis* (Prudhoe, 1957) Yamaguti, 1963. Despite the different microhabitats occupied by these parasites, they have a similar anterior adhesive system. In both parasites, this consists of three types of gland cells: G1 cells that produce rod-shaped bodies (S1), G2 cells manufacture irregularly shaped bodies (S2) and G3 cells form mucoid-like secretions (S3). In the cytoplasm of G1 cells, a single layer of microtubules encloses each developing rod-shaped body. Some fully developed S1 bodies are attached to each other, forming large condensed globules. S1 bodies are extruded through multiple apertures whereas S2 and S3 bodies are released through ducts each with a single opening. The adhesive sacs are lined with two types of tegument (st1 and st2). A third tegument type (st3) connects the st2 tegument with the general body tegument. Only st1 has microvilli. Each adhesive sac is

provided with a spike-like sensillum and single uniciliated sense organ. The possible functions of microvilli in increasing the surface area and assistance in spreading and mixing of the adhesive secretion and the role of sense organs associated with the adhesive sacs are discussed.

Key words: Platyhelminthes, Monogenea, fish ectoparasite, temporary adhesion, adhesive apparatus, ultrastructure

1. Introduction

Monogenean ectoparasites attach to their hosts primarily with their posterior attachment organ (haptor), which is equipped with hamuli and marginal hooklets [1], but in order to move from one position to another they rely on their anterior adhesive apparatus [2]. Typically, monogeneans move on the host or artificial substrates by stretching out their bodies and attaching with head lobes to the host tissue, releasing and moving the haptor to attach close to the adhesive areas of the head lobes, and then they detach the head lobes to move anteriorly where they attach again to a new site. Some can move in a similar leech-like manner upside down, using the water surface tension [3].

The anterior adhesive apparatus has been studied with transmission electron microscopy (TEM) and/or scanning electron microscopy (SEM) in many monogenean parasites, including the gyrodactylids [4,5,6], dactylogyrids [7,8], entobdellids [2,9], acanthocotylids [10], monocotylids [11.12] and ancyrocephalids [13]. They have various kinds of gland cells that open either into the outer syncytial tegumental layer [7,14], or onto the specialized haptoral [13,15,16,17] or ventrally-located head regions (see for example, El-Naggar and Khidr [8], Wong et al. [13]). The monogenean anterior adhesive apparatus produces one to three types of secretion bodies. Species with rod-shaped bodies (S1), spherical bodies (S2)

and irregularly-shaped, electron-lucent vesicles (S3) include *Gyrodactylus eucaliae* (see Kritsky, 1978) [4], *G. sprostonae* (see Yuan and Long [18]), *Dactylogyrus amphibothrium* and *D. hemiamphibothrium* (see El-Naggar and Kearn [7]), *D. aristichthys* (see Yuan and Long [19]), *Cichlidogyrus halli* (see El-Naggar and Khidr [8]) and *Merizocotyle icopae* (see Cribb et al. [20]). Two types of secretion, rod-shaped bodies (S1) and spherical bodies (S2), were reported in *Entobdella soleae* (see Kearn and Evans-Gowing [9]), *Acanthocotyle lobianchi* (see Rees and Kearn [10]) and *Caballeria liewi* (see Wong et al. [13]). Only one kind of secretion, rods, is produced in the anterior adhesive apparatus of *Monocotyle spiremae* (see Cribb et al. [11]) and spherical bodies in *Enterogyrus cichlidarum* (see Khidr et al. [21]).

Two gyrodactylid monogeneans of the Nile catfish, *Clarias gariepinus*, *Macrogyrodactylus clarii* [22] and *M. congolensis* [23,24] infect the gills [25], and the skin and fins [26], respectively. Although the haptors of *M. clarii* and *M. congolensis* show the same basic structure, there are some differences [25,26] possibly reflecting the different habitats of the parasites. The haptor of *M. clarii* possesses two lateral rows of tegumental papillae, whilst that of *M. congolensis* has three rows (two lateral and one anterior). The dorsal bar consists of two articulating sclerites in *M. clarii* and just one in *M. congolensis*. Moreover, the ventral bar of *M. clarii* is posteriorly associated with three long accessory sclerites, while that of *M. congolensis* has two long horns and possesses two posterior accessory sclerites [25,26].

Light microscopy of the anterior adhesive apparatus of *M. clarii* (see El-Naggar and Serag [25] and *M. congolensis* (see El-Naggar et al. [26]) revealed two kinds of gland cells, one producing two types of secretion (rod-shaped bodies and spherical bodies) and the other manufacturing irregularly-shaped bodies. With the exception of Kritsky [4], no ultrastructural studies have been conducted on the anterior adhesive apparatus of gyrodactylid parasites.

However, SEM has been used to study the head lobes of *Gyrodactylus groschafti* (see El-Naggar [5]), *M. clarii* (see El-Naggar [6]) and *M. congolensis* (see Arafa et al. [27]). In these three gyrodactylids, each head lobe bears a single, ventrally-located adhesive sac provided with emergent papillae which are densely covered with microvilli and perforated by gland duct openings [5,6,27].

The present study assesses whether there are any adaptive differences in the anterior adhesive apparatus of *M. clarii* and *M. congolensis* based on their microhabitat. *M. clarii* lives on the gill filaments of *Clarias gariepinus* and are exposed to strong gill ventilating water currents, while *M. congolensis* parasitizes the skin and fins of the same host.

2. Materials and Methods

Specimens of the Nile catfish *Clarias gariepinus* (Burchell, 1822) were caught from the Demietta branch of the River Nile near Mansoura City, Daqahlia Province, Egypt and transported alive to the Faculty of Sciences, Mansoura University. Here, fish were maintained for a few days in an aquarium containing aerated river water at room temperature (25 ± 5 °C) with natural daylight. The catfish (n = 50) were killed by pithing and severing the spinal cord. The gills, fins and scrapings of the skin were removed and placed in Petri dishes containing filtered river water. Gills were searched for *Macrogyrodactylus clarii*, while fins and scrapings of the skin were searched for *M. congolensis* using a dissecting microscope. Some living specimens of both species were flattened between a glass slide and a coverslip and stained with light green and eosin according to El-Naggar et al. [23]. Living and stained flattened specimens (N=10) were examined using light and phase-contrast microscopy with oil immersion, and the different kinds of gland cells were counted.

For TEM, specimens of *M. clarii* and *M. congolensis* were washed in distilled water and then fixed in 2.5% glutaraldehyde buffered to pH 7.3 with 0.1 M sodium cacodylate-HCl

buffer at 4 °C for 2 h. They were then washed for at least 1 h in several changes of cold buffer (0.1 M sodium cacodylate-HCl containing 3% sucrose and 0.1 M $CaCl_2$), post-fixed in 1% osmium tetroxide in sodium cacodylate buffer at 4 °C for 1 h, washed overnight in the same buffer, then dehydrated using an ascending series of ethanol solutions before transfer to a 1 : 1 mixture of propylene oxide and Spurr resin. Specimens were transferred into gelatin capsules containing pure resin and placed in an oven overnight at 60 °C. Ultrathin sections were cut at 70–90 nm using an LKB NOVA ultramicrotome and glass knives. The sections were mounted on single-hole and 75 mesh coated grids and stained in a solution of 1-2% aqueous or alcoholic uranyl acetate for about 30 min followed by 2-3% lead citrate for 5 min. The sections were examined using a JEOL 100SX transmission electron microscope operating at 80 kV. Measurements of different secretory bodies are based on >10 organelles from electron micrographs.

3. Results

The head region of both *Macrogyrodactylus clarii* and *M. congolensis* consists of two head lobes. Each bears a single adhesive sac located ventrally at its distal extremity and terminates in a single spike-like sensillum (Fig. 1). The lateral regions of the head contain numerous unicellular glands with their ducts converging on and opening into the two adhesive sacs (Fig. 1). Three kinds of gland cells (G1, G2 and G3) are present in both *M. clarii* and *M. congolensis*. Generally, the anterior adhesive apparatus of *M. congolensis* resembles that of *M. clarii* with just minor differences in the number of G2 gland cells. In *M. congolensis*, the G2 glands comprise at least 10 cells while in *M. clarii* they constitute only seven cells. The Gl gland cells produce rod-shaped bodies (Sla) and relatively large spherical globules (S1b) (Fig. 1). On each lateral side of the head of both *M. clarii* and *M. congolensis*, there are sixteen G1 cells that are arranged in three groups, one lies lateral to the cerebral region and comprises five cells and the second consists of six cells and lies lateral to the

anterior region of the pharynx, while the third comprises five cells and lies lateral to the anterior unbranched region of the intestine. In both *M. clarii* and *M. congolensis*, the G2 cells are found in a single group lying lateral to the posterior region of the pharynx and the anterior unbranched region of the intestine. The G2 cells are larger than the G1 cells and produce irregularly-shaped secretory bodies (S2). In both *M. clarii* and *M. congolensis*, the G3 cells are three in number located lateral to the cerebral region and produce translucent mucoid secretory bodies (S3) (Fig. 1).

3.1 Gland cells

TEM of both *M. clarii* and *M. congolensis* revealed that each G1 gland cell has a nearly spherical nucleus with granular nucleoplasm, conspicuous nucleolus and condensed chromatin (Fig. 2). The cytoplasm is moderately electron-dense and contains abundant granular endoplasmic reticulum (GER), numerous ribosomes (Figs. 2-5), a few Golgi bodies, small electron-lucent vesicles and mitochondria. The dilated cisternae of the GER enclose an amorphous, finely granular material with an electron density slightly higher than that of the basal cytoplasm (Fig. 2). Generally, each fully-developed, rod-shaped S1 body is of high electron density and measures 0.3-0.5 (average 0.4) µm in diameter. The maximum length measured in sections is 4- 6.5 (average 6) µm. These bodies are membrane bounded and contain a finely granular dense matrix in which small particles are embedded within higher electron-dense material (Figs. 2-5). In sections, immature S1 bodies have a greater diameter than that of the fully developed ones (Figs. 4, 6). They measure 0.4-0.7 (average 0.6) µm in *M. congolensis* and *M. clarii* and contain granular material, with electron-density lower than that of the smaller S1 bodies (Figs. 4, 6). Each of the large, immature, less electron-dense bodies and some of the small highly electron-dense bodies are enclosed by a single layer of microtubules, which appear to be parallel with each other and with the long axis of the rod (Figs. 4-6). Some of the less electron-dense S1 bodies contain a peripheral layer of small electron-dense granules and have no

bounding membrane (Fig. 4). In cross sections, some fully developed S1 bodies attach to each other, forming large condensed globules with various sizes and shapes (Figs. 2, 4, 5). Their number varies from 3-7 S1 bodies in each globule. In a few sections of *M. congolensis*, some fully formed S1 secretory bodies with peripheral translucent vesicles were detected (Fig. 7).

Each G2 cell is enclosed by a layer of fibrous interstitial material. They have an irregularly shaped nucleus with a relatively large, conspicuous nucleolus, granular nucleoplasm, small chromatin patches and nuclear membrane with characteristic nuclear pores (Fig. 8). The cytoplasm is moderately electron-dense, but it is slightly darker than that of the G1 cells. It contains abundant GER, free ribosomes, and numerous Golgi complexes, which in many sections are aggregated (in groups of 2-4) in close proximity to the nuclear membrane (Fig. 9). Each Golgi complex consists of 3-5 narrow parallel cisternae terminating with small and large vesicles (Fig. 9). Both cisternae and vesicles are filled with homogeneous, highly electron-dense material. In sections, the irregularly shaped bodies (S2) have different sizes ranging from 0.7-1.5 (average 1.2) µm in diameter. They are abundant and contain granular, highly electron-dense material (Figs. 8, 9). However, in *M. clarii* with higher magnification, each S2 body contains tubular structures with lower electron-density, which are embedded in highly electron-dense ground substance (Fig. 10). In most regions of the G2 cells, fully developed S2 bodies are surrounded by cytoplasm characterized by translucent ground substance (Figs. 8, 10).

Each G3 gland cell has a nearly oval nucleus with granular nucleoplasm, conspicuous nucleolus and condensed chromatin patches (Fig. 11). Some GER have dilated cisternae. The mucoid secretory bodies (S3) are abundant, irregularly shaped (1-1.9, average 1.4, µm) and contain granular moderately electron-dense material (Fig. 11).

3.2 Gland ducts and adhesive areas

Ducts of the G1, G2 and G3 gland cells carrying the secretory bodies S1, S2 and S3, respectively, extend anteriorly as cytoplasmic processes where they converge on adhesive papillae through which they open into the adhesive sacs (Figs. 1, 12-21). As the gland ducts approach the adhesive sac, they dilate and become closely packed (Figs. 12,19). At this point, some of the gland ducts are associated with muscle fibers that are present beneath the tegument lining the adhesive sac (Fig. 13). Most ducts of the G1 cells are filled with completely formed rod-shaped bodies (Figs. 12,13,14), but in some sections, a few condensed globules of attached rods are found beside S1 bodies (Fig. 15). There are no microtubules in any of the gland ducts. Each G1 gland duct opens to the exterior via multiple apertures (Figs. 14-16). At the openings of the G1 ducts, five layers, three electron-dense and two electron-lucent (Fig. 16), bound each aperture. The outer layer membrane connects with the surrounding tegument by means of septate desmosomes (Fig. 14). Each one of the multiple apertures allows passage of a single rod (Fig. 14). Although large globules were detected in the terminal portion of the G1 ducts just beneath the multiple apertures, none of them were seen passing through the openings or outside the body (Fig.15). Each of the G2 and G3 gland ducts opens to the exterior by a single aperture (Figs.17-20).

The adhesive sac is lined with three types of tegumental layer (st1, st2 and st3) (Figs. 14, 15, 17, 22, 24). The first (st1) represents the outer tegumental layer covering the ventral surface of the adhesive papillae surrounding the gland duct openings (Figs. 12, 14, 15), while st2 represents the outer tegumental layer covering the lateral surfaces of the adhesive papillae (Figs. 12, 14, 22). The third type (st3) is the outer tegumental layer of the inner rim of the adhesive sac and connects st2 and the tegumental layer of the general body surface (i.e. the outer surface of the head lobe) (Figs. 12, 22). Comparing the three tegumental layers, the st1 layer is relatively thin, electron-dense and has numerous microvilli but lacks secretory bodies (Figs. 14, 15). The st2 layer is highly electron-dense and contains abundant electron-dense

198 bodies (Figs. 14, 22). No cytoplasmic organelles like mitochondria, Golgi bodies, GER or free
199 ribosomes were found in st1 or st2 tegument. The st3 tegumental layer connects with the st2
200 tegument by means of junctional complexes (Fig. 22) and contains a few translucent vesicles
201 containing moderately electron-dense particles (Fig. 24). These vesicles are restricted to the
202 outer region of the tegument. Some electron-dense granular bodies, abundant rod-shaped,
203 electron-dense bodies and a few mitochondria were also seen (Fig. 22 inset). The general body
204 tegument contains abundant translucent vesicles and some electron-dense granular secretory
205 bodies, but no rod-shaped bodies (Fig. 22).

206 No experimental work was performed to study the mechanism of attachment and
207 detachment of the head lobes of *Macrogyrodactylus* species. However, in most sections the
208 terminal portions of G1 ducts, homogeneous particulate material was detected around the S1
209 bodies (Figs. 14, 15) while sections of the terminal portions of G2 and G3 ducts revealed
210 considerable change in appearance of the secretory bodies particularly S2 and S3. The S3 bodies
211 lose their membranes and their secretory components form homogeneous particulate material
212 (Figs. 18,19), while S2 bodies become slightly smaller in size and their particulate components
213 diffuse into the lumen of the duct in-between bodies that are still membrane-bounded (Figs.
214 19,21). Moreover, in the same region, these sections show a network of homogeneous material
215 covering the surface of the adhesive papilla (Figs. 19, 21).

216 TEM revealed the presence of a single sensillum on each adhesive papilla (Fig. 20), in the
217 intervening region between the adhesive sac and general body tegument (Fig. 23) and on the
218 anterior region, which is covered by general body tegument (Fig. 24). Each sensillum has an
219 elongated nerve bulb, which terminates in a single opening through which a single cilium
220 protrudes (Figs. 20, 23, 24). Close to the opening, there is an electron-dense thickening and the
221 lining of the opening is connected with the intervening tegument st3 via desmosomes (Figs. 23,

24). The nerve bulb contains neurotubules, electron-dense bodies and mitochondria (Figs. 23, 24).

4. Discussion

This is the first ultrastructural study of the anterior adhesive apparatus of the monogeneans *Macrogyrodactylus clarii* [22] from the gills of *Clarias gariepinus* and *M. congolensis* [23,24] from the skin and fins of the same host. TEM revealed that the anterior adhesive apparatus of both parasites consists of three types of gland cells (G1, G2 and G3). The G1 cells produce rod-shaped bodies (S1) and roughly spherical large globules, G2 cells secrete irregularly shaped, highly electron-dense bodies with tubular contents (S2) and G3 cells manufacture irregularly shaped, mucoid-like secretion (S3). These glands resemble those of the anterior adhesive apparatus of other monogenean parasites [28,29,30]. Previous studies illustrated that congeners in the same microhabitat tend to have similar types of anterior adhesive secretions [7,29,31]. In the present study, the anterior adhesive apparatus of *M. clarii* and *M. congolensis* have the similar morphological features, despite the differences in their microhabitat, with the exception of the number of G2 cells: 10 pairs in *M. congolensis* and 7 pairs in *M. clarii*. Morphological similarities, however, do not exclude the possibility of chemical and/or functional differences [30].

In *D. amphibothrium*, El-Naggar and Kearn [7] found that S1 bodies in the G1 ducts connect with each other by membrane-like structures and a similar feature of interlinking band-like structures was observed between S1 bodies and S2 bodies in *Bravohollisia gussevi* and *Caballeria liewi* (see Wong et al. [13,16], respectively). Also, the bounding membranes of S1 bodies in *Entobdella australis* and *Entobdella* spp. (see Whittington and Cribb [29]) showed periodic dense bandings. None of these structures, however, were observed in either *M. congolensis* or *M. clarii*. A unique feature of these parasites though is the presence of large globular bodies in the cytoplasm and ducts of the G1 cells, in addition to fully formed S1 bodies.

With TEM, it became evident that these globules are aggregations of S1 bodies. There was no evidence that S2 or S3 bodies in *M. clarii* and *M. congolensis* aggregate and coalesce in the cytoplasm of their cells but they become closely packed as they reach the terminal portions of the ducts. Another important feature of *M. clarii* and *M. congolensis* is that S2 bodies contain tubular structures, a feature not reported in any other monogeneans studied by TEM. In addition, the present study indicates that the fully formed rods in *M. clarii* and *M. congolensis* are considerably larger than the S1 bodies in *Entobella* spp. (see Whittington and Cribb [29]).

During the early stage of assembly, the large, less electron-dense rods, and some of the smaller highly electron-dense rods of *M. clarii* and *M. congolensis* are enclosed by microtubules. The microtubules disappear when the rods are fully formed and become bounded by membrane. Microtubules have been reported in most other monogeneans studied (see for example Wong et al. [13]) except for monocotylids [11,12] and *Benedenia* spp. [32]. Moreover, the rods of *Monocotyle spiremae* have no bounding membrane and possess an outer electron-dense cortex and a more electron-lucent core [11]. El-Naggar and Kearn [7] suggested that encircling microtubules may play a role in transporting products from different parts of the cell prior to assembly of the secretory bodies. In addition, the microtubules may orientate the rods during their passage from within the gland cells to the lumen of their gland ducts, and help to maintain the parallel arrangement of rods into bundles [7].

The monogeneans *M. clarii* and *M. congolensis* resemble other gyrodactylids in that the secretions of the anterior adhesive apparatus open into a single pair of adhesive sacs, one situated antero-ventrally on each of the two head lobes [4]. Other monogeneans, with the exception of gyrodactylids and some monocotylids, have three distinct zones on each side of the head for the release of secretions (see, for example, El-Naggar and Kearn [7]). Such organization into six separate points of contact presumably allows the parasite to be more resistant to detachment caused by water currents [10].

In the present study of *Macrogyrodactylus* spp., it has been established that the rod-shaped bodies and roughly spherical large globules produced by G1 gland cells are transported through ducts terminating with multiple apertures. Each aperture apparently permits the passage of only one rod but there is no evidence that the large globules pass through multiple apertures. Multiple apertures were reported in *G. eucaliae, Entobdella soleae* and *M. spiremae* (see Kritsky [4]). [2,11], respectively. However, in *D. amphibothrium* and *D. hemiamphibothrium* the rod-shaped bodies are released from ducts with single apertures (see El-Naggar and Kearn [7]). The unique feature of *M. clarii* and *M. congolensis* is that the ducts that carry the rod-shaped bodies also carry larger globules of the same secretion, but the globules were not seen passing through duct apertures. It is possible that the multilayered boundary of one of the small multiple openings dilate to permit passage of the larger globules. Alternatively, the large spherical globules may liquefy or fragment before passing through the multiple apertures. Presence of material similar to the contents of S1 bodies and large globules in the terminal portion of G1 ducts (Figs. 10, 11) supports the latter suggestion. There is also some evidence that the large globules in the G1 cells are composite structures, perhaps made by accumulation of rod-shaped bodies or components of them. If correct, then the globules might escape from the duct openings after disintegration into their small rod-like components. Moreover, in *M. clarii* and *M. congolensis*, the S2 and S3 bodies showed considerable change in their appearance inside the terminal portions of the ducts indicating that they are released from duct openings in a liquid form. A similar feature was reported by Kearn and Evans-Gowing [9] who found that the spheroidal secretory bodies associated with the anterior adhesive apparatus of *E. soleae* transform within the duct terminations immediately prior to attachment of the head region.

A characteristic feature of *M. clarii and M. congolensis* is that each adhesive sac is lined with three types of tegument (st1, st2, and st3) that are different from the general body surface. The first kind (st1) is thickly covered with microvilli, a feature that has been reported in the

297 adhesive areas of many monogeneans [2,4,5,7 ,9,11,33,34]. These specialized microvilli may be
298 important during attachment of the head lobes by increasing the surface area available for
299 binding the adhesive secretions to the head region. Lyons [34] suggested that these microvilli in
300 *Gyrodactylus* spp. may assist in spreading the adhesive secretion of the head glands over the
301 skin of the host into a thin "tacky" film. The microvilli may help to mix the products of different
302 gland cells, which might have to interact with each other or with water before the sticky
303 properties are developed.

304 Rod-shaped bodies are the most abundant component of the anterior adhesive apparatus of
305 *M. clarii* and *M. congolensis*. These bodies also represent the main component of the anterior
306 adhesive secretions of many monogeneans, which produce two or three types of secretory bodies
307 such as *D. amphibothrium* and *D. hemiamphibothrium* (see El-Naggar and Kearn [7]) and *E.*
308 *soleae* (see Kearn and Evans-Gowing [9]). Furthermore, rod-shaped bodies are the only
309 secretory body recorded in the anterior adhesive apparatus of the monocotylid, *Monocotyle*
310 *spiremae* (see Cribb et al. 11]).

311 The mechanism of attachment of *M. clarii* and *M. congolensis* may involve adhesion of
312 the adhesive sac rim to the host tissues, protrusion of the adhesive papillae by means of
313 associated muscles and release of secretory bodies through gland duct openings. The spike
314 sensillum and other ciliary structures may serve as chemoreceptors that control attachment of the
315 adhesive sacs. In monogeneans, it was suggested that stickiness could be a property of one type
316 of secretory body or could develop by mixing between two types of secretion [2,7,20,35].
317 Interaction between secretory bodies and water [2] or between secretory bodies and host mucus
318 [11] are possible alternative mechanisms.

319 Detachment of the head lobes of *M. clarii* and *M. congolensis* may occur mechanically by
320 contraction of muscle fibres attached to the anterior region [11]. In *E. soleae*, tegument of the

adhesive pads may play a part in detachment of the head region, by release of secretory bodies, which are abundant in this layer, or by some other physical or chemical change mediated via the tegumentary membrane [9]. In *M. spiremae*, where only one type of secretion (rods) was found, detachment may involve additional glue, physical detachment by muscle contraction or extrusion of material surrounding the rods [11]. Experimental studies are still needed in this field to determine which secretion is responsible for attachment and how detachment takes place: a potentially lucrative area for industry in relation to binding agents in water.

Regarding parasite-host specificity, it has been reported that the epidermal mucous cells of specific fish hosts may influence parasite attachment (see review in Whittington et al. [31]). The anterior attachment region of *Gyrodactylus derjavini* contains mannose-rich glycoproteins, which are implicated in stimulating the alternative complement pathway in the host [36]. Specific differences in host fish epithelium and differences in monogenean anterior adhesive chemistry or in the chemistry of the specialized tegument of the anterior adhesive area may all contribute to host specificity amongst monogeneans [30].

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Figures

Fig. 1. Diagrammatic representation of the anterior adhesive apparatus and anterior region of the digestive system of *Macrogyrodactylus clarii* (ventral view). aph, Anterior region of the pharynx; as, adhesive sac; co, cerebral organ; G1, gland cell producing rod-shaped bodies (Sla) and roughly spherical bodies (S1b); G2, gland cell producing irregularly-shaped bodies (S2); G3, gland cell producing translucent secretory bodies (S3). gd, gland duct; hl, head lobe; it, intestine; mo, mouth opening; oes, oesophagus; pph, posterior region of the pharynx; sp, spike-like sensillum; ui, unbranched region of the intestine.

Fig. 2. G1 gland cell of *Macrogyrodactylus clarii* containing S1 rod-shaped bodies and large spherical globules (lg). dS1, Developing rod-shaped bodies; ch, chromatin; GER, granular endoplasmic reticulum; N, nucleus; Nu, nucleolus; r, ribosomes; S1, rod-shaped secretory bodies.

Fig. 3. Cytoplasm of the G1 gland cell of *Macrogyrodactylus clarii* containing fully formed S1 rod-shaped bodies and granular endoplasmic reticulum (GER). r, Ribosomes.

Fig. 4. G1 gland cell of *Macrogyrodactylus congolensis* containing fully formed rod-shaped bodies (S1), large globule (lg) and developing S1 (dS1) secretory bodies. Note that the developing rod-shaped bodies (ds1) have different sizes and are surrounded by microtubules (mt). Note also that some of the developing S1 bodies contain a peripheral layer of small electron-dense granules (arrows) and have no bounding membrane. GER, granular endoplasmic reticulum.

Fig. 5. G1 gland cell of *Macrogyrodactylus congolensis* containing fully formed rod-shaped bodies (S1) and large globules (lg) each containing many S1 bodies (arrow). Note the presence of small translucent vesicles (v) and developing rod-shaped bodies (dS1).

Fig. 6. G1 gland cell of *Macrogyrodactylys congolensis* showing longitudinal sections of the large developing rod-shaped bodies (dS1) and fully formed rod-shaped bodies (S1). Note the microtubules (mt) associated with dS1.

Fig. 7. Magnified S1 secretory bodies of *Macrogyrodactylus congolensis* with translucent vesicles (arrow heads).

Fig. 8. G2 gland cell of *Macrogyrodactylus clarii* surrounded by fibrous interstitial material (fm) and containing large nucleus (N) with conspicuous nucleolus (Nu) and irregularly shaped secretory bodies (S2) surrounded by translucent area (*).

Fig. 9. Magnified part of G2 gland cell of *Macrogyrodactylus clarii* with Golgi bodies (Go), granular endoplasmic reticulum (GER), ribosomes (r) and irregularly shaped secretory bodies (S2).

Fig. 10. Magnified S2 of *Macrogyrodactylus clarii* containing tubular structures with lower electron density and surrounded by a translucent area (*).

Fig. 11. G3 gland cell of *Macrogyrodactylus clarii* containing nucleus (N) with chromatin (ch), dilated cisternae of granular endoplasmic reticulum (dGER) and translucent mucoid secretory bodies (S3).

Fig. 12. Section through adhesive sac (as) of *Macrogyrodactylus clarii* showing the ventral surface of adhesive papillae (ap) covered with st1 tegument. S1, rod-shaped bodies.

Fig. 13. Section through adhesive sac (as) of *Macrogyrodactylus clarii* showing muscle fibres (mf) in between S1 ducts. S1, rod-shaped bodies.

Fig. 14. Adhesive papilla of *Macrogyrodactylus clarii* showing S1 body protruding from its aperture. Note the st1 tegument covering the ventral surface of the adhesive papillae and

st2 tegument covering the lateral surface of adhesive papillae. as, Adhesive sac; mi, microvilli; *, homogeneous material around S1 bodies.

Fig. 15. Duct of G1 gland cell of *Macrogyrodactylus congolensis* containing rod-shaped bodies (S1) and large globules (lg) close to the multiple apertures. Note that the membrane bounding the outer layer of the multiple apertures is connected to the adjacent tegument (st1) by means of septate desmosomes (d) and presence of homogeneous material (*) around S1 bodies. mi, Microvilli; st1, tegument covering the ventral surface of adhesive papillae.

Fig. 16. Cross section of the multiple apertures of a G1 gland duct of *Macrogyrodactylus congolensis* showing that each aperture is bounded by five layers (l), three electron-dense and two electron-lucent. S1, rod-shaped bodies.

Fig. 17. Terminal portion of G2 gland duct of *Macrogyrodactylus congolensis* carrying S2 secretory bodies. f, fibrous layer; mi, microvilli; st1, microvillous tegument.

Fig. 18. Section through adhesive sac of *Macrogyrodactylus clarii* showing terminal portions of gland ducts carrying S1, S2 and S3 bodies. Note that secretory bodies of S3 bodies form particulate material.

Fig. 19. Terminal portions of G1, G2 and G3 gland cells of *Macrogyrodactylus clarii* containing S1, S2 and S3 secretory bodies, respectively. Note that the component of S2 bodies (*) diffuse into the lumen of the duct. mf, muscle fibres.

Fig. 20. Duct of G2 gland cell carrying S2 secretory bodies of *Macrogyrodactylus clarii*. c, Cilium; nb, nerve bulb; nt, neurotubule; St1, tegument covering the ventral surface of adhesive papillae.

Fig. 21. The terminal portions of G2 gland ducts of *Macrogyrodactylus clarii* showing that the components of S2 bodies (*) diffuse into the duct lumen.

Fig. 22. Intervening tegument (st3) connecting the sac tegument of type st2 with the general body tegument (gt) of *Macrogyrodactylus congolensis*. Note that st3 tegument and st2 are connected by a junctional complex (j). gb, Electron-dense granular secretory bodies; rb, rod-shaped electron-dense bodies; v, translucent vesicle with electron-dense granule. **Inset:** magnified st3 with abundant electron-dense, rod-shaped bodies (rb), few electron-lucent vesicles with dark granules (v) and electron-dense granular bodies (gb), similar to those in the general body tegument.

Fig. 23. Section through the terminal part of ciliary sensillum of *Macrogyrodactylus clarii*. c, Cilium; edb, electron-dense bodies; et, electron-dense thickening; m, mitochondria; nb, nerve bulb; nt, neurotubules.

Fig. 24. Section through ciliary sensillum of *Macrogyrodactylus clarii*. c, cilium; d, desmosomes; et, electron-dense thickening; gt, general body tegument; m, mitochondria; r, root of the cilium; st3, intervening tegument.