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Scanning electron microscopy of *Clinostomum* metacercaria from *Oreochromis niloticus* from Assiut, Egypt.

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ABSTRACT
The topography of the tegument of *Clinostomum* metacercaria showed variable features according to the different sites of the surface. At the ventral side, the rims of the oral and ventral suckers had minor differences, while their floor showed great variation in topography: the floor of the oral sucker had longitudinal wrinkles with nodule-like structures and their upper margins had minute sensory papillae, while the ventral sucker had a rugose surface possessing some smooth dome-like papillae. The area between the two suckers had a corrugated surface. The post-acetabular region was highly folded with sponge-like margins, while the inner area was smooth. The rest of the body surface had two forms of polygonal units of villi each surrounding one or two sensory papillae. The dorsal side had plenty of pores and apertures: several crescent slits each had nail-like covers distributed marginally at the anterior region; a narrow aperture with a dentate margin was seen at both postero-lateral sides; behind each lay a large aperture with single operculum; and finally there was the excretory pore at the end of the body. Micropores were distributed in many sites of the dorsal side. The surface was like cobblestones at the anterior region of the body, but flattened, smooth, and polygonal-like on most of the median area, and papillated around the excretory pore. The possible function of each structure was discussed.

KEYWORDS: Scanning, topography, *Clinostomum*, *oreochromis*, *metacercaria*.

INTRODUCTION

Clinostomum worms are digenetic trematodes which naturally parasitize the throat and oesophagus of piscivorous birds (Yamaguti 1958), fish- and frog-eating reptiles and mammals (Beaver *et al*. 1984). Many species of freshwater fish have been recorded as the secondary intermediate hosts of *Clinostomum* spp.(Aohagi; *et al*. 1992 & 1993). The life cycle of some *Clinostomum* species has been studied (Dias *et al*. 2003). Some human cases of laryngitis were recorded by Kamo *et al.* (1962), Chung *et al.* (1995) and Shirai *et al.* (1998), and Tiewchaloern *et al*. (1999) recorded a *Clinostomum* case in a human eye. To the best of our knowledge, no report exists on the topography of the tegument of either the metacercaria or the adult worm of *Clinostomum.* Several Digenea have been studied by SEM: *Crypytocotyle lingua* and *Fasciola hepatica* (Koie 1977; Koie *et al*. 1977); *Gastrodiscoides hominis* (Brennan *et al*. 1991), *Neoerilepturus aegyptensis* (Shalaby & Hassanine 1996). and *Fasciola gigantica* (Dangprasert *et al*. 2001).

The tegument is the interfacing layer that helps the parasite to maintain homeostasis and evade hostile environments including the host's immune attacks. It also plays a key role in the absorption and exchange of nutritive and waste molecules, the regulation of ionic equilibrium between the interior of the parasite and the surrounding host fluids, and in protecting the flukes from the immune responses of the host. The present study is concerned with the topography of the tegument of the metacercaria of *Clinostomum* sp. obtained from the fish *Oreochromis niloticus* and discusses the relation between the different structures and probable function of each.

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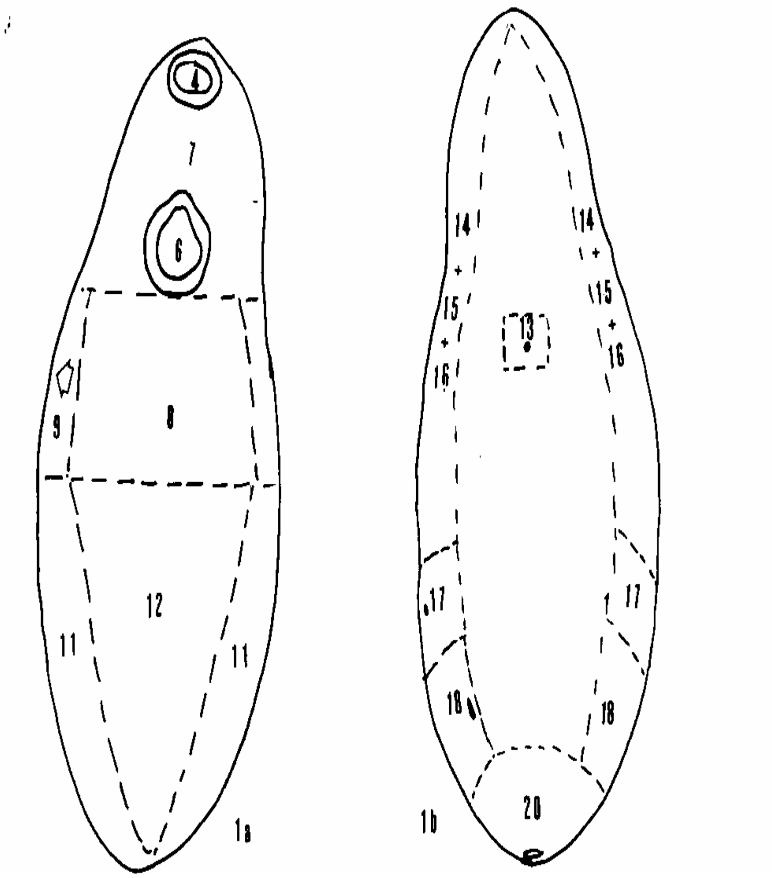
MATERIALS AND METHODS

Specimens of *Clinostomum* metacercaria were collected from the tissues around the gills and body cavity of the Nile Perch *Oreochromis niloticus*. The fish samples were obtained from the market. The encysted metacercariae were deposited in normal saline for about 30 min: excysted metacercariae were gently pressed between two slides and fixed in 4% glutaraldehyde for 24 h, washed in cacodylate buffer, post fixed in1% osmium, dehydrated in graded ethanol solution, coated with gold on a special holder and then examined by a Jeol JS T220 A Scanning Electron Microscope.

RESULTS

The metacercariae were recovered mainly from tissues around gills and rarely from the body cavity. The excysted metacercariae were tongue-shaped, attenuated at the acetabular level. Metacercariae were about 12.9 mm (range 11.5-13.5) in length, and 3.8 mm (3.5-4.3) in width (Figs. 1a & 1b).



Figures 1a &1b: Camera lucida drawings showing a map of the outlines of the metacercaria and the distribution of different features. 1a = ventral view; 1b = dorsal view.

The ventral surface: The topography of the oral sucker showed that the outer rim was thick and smooth, while its floor had deep wavy wrinkles extended at antero-posterior direction. The wrinkles were connected to each other by fine septa. In some sites these wrinkles formed nodule-like structures; the upper margins of the wrinkles and the nodules possessed minute sensory papillae (Figs 2-4).

Fig. 2: SEM photograph showing the anterior part of the fluke.

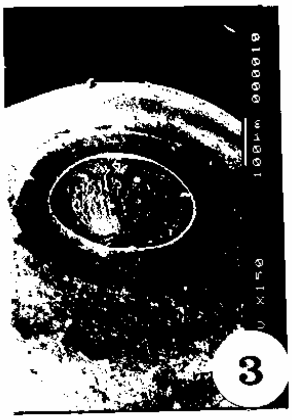


Fig. 3: Magnified SEM photograph of Fig. 2 showing the anterior sucker with its smooth rim.

Fig. 4: Magnified SEM micrograph of Fig. 3 showing the wrinkles at the floor of the oral sucker and the minute sensory papillae.

The rim of the posterior (ventral) sucker was thick and cutaneous with irregular protrusions. The floor of the same sucker had a rugose surface possessing scattered minute smooth dome-like papillae; it also possessed micro-ridges separated by micropores. Some droplets of secretions were seen (Figs 5&6).

The surface of the area between the two suckers was puckered with a corrugated surface and apparent grooves: the main structure of this region is the papillae (Fig. 7).

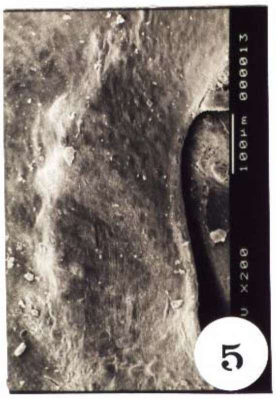


Fig. 5: Magnified SEM micrograph of Fig. 2 showing the rim of the ventral sucker: note the minute protrusions.

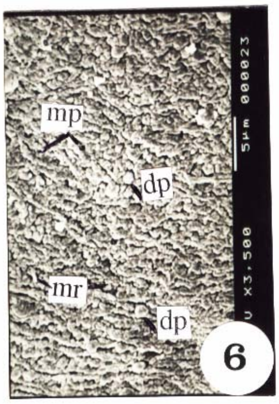
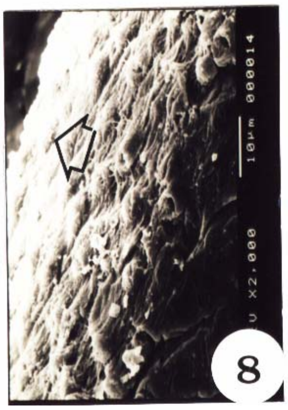


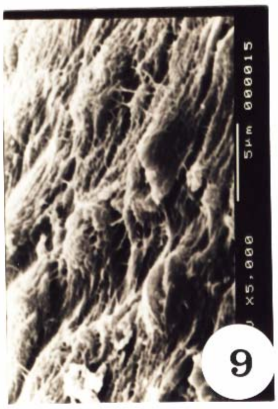
Fig. 6: Magnified SEM micrograph of Fig. 2 showing the floor of the ventral sucker: note the minute dome-like papillae (dp), micro-ridges (mr), and micropores (mp).

Fig. 7: SEM micrograph showing papillae covering the area between the two suckers.

The topography of the post-acetabular region showed two areas, one highly folded to give the appearance of a natural sponge with longitudinal wavy protrusions at the outer sides, while the other median area had a smooth surface divided by fine irregular deep grooves (Figs 8 & 9).

The posterior region of the ventral surface is covered with two forms of polygonal tegumental structure; each structure contains either single or double sensory papillae (Fig. 10). The unites surrounding the double sensory papillae were restricted mainly at the outer side of the body surface, while the other form was at the median area (Figs. 11 &12).





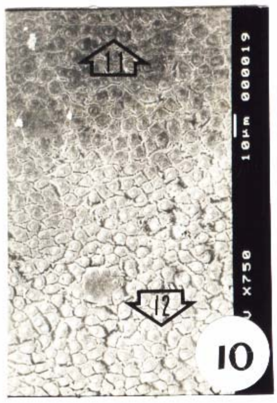


Fig. 8: SEM micrograph showing the post acetabular region: note the outer spongy surface and inner smooth one.

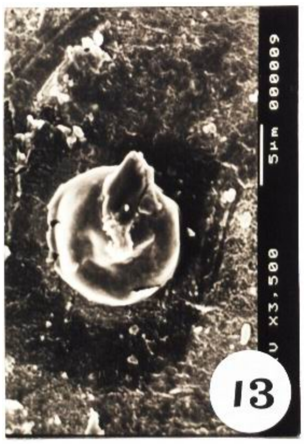
Fig. 9: Magnified SEM micrograph of Fig. 8 showing the spongy surface.

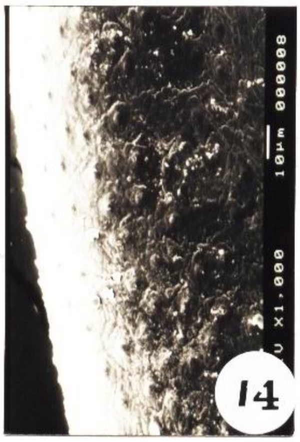
Fig. 10: SEM micrograph showing the posterior half of the fluke: the darker part is the outer margin.

Fig. 11: Magnified SEM micrograph of Fig. 10 showing the polygonal villi surrounding the double sensory papillae.

Fig. 12: Magnified SEM micrograph of Fig. 10 showing the polygonal villi surrounding the single sensory papillae.

The dorsal surface: Plenty of pores and apertures were found to be distributed all over the dorsal surface side by side with the other features. At the end of the median line of the anterior third of the body lies a special type of sensory papilla-like structure similar to the styloconic sensilla of insects (Fig. 13).





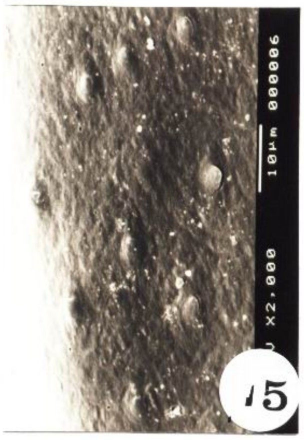


Fig. 13: SEM micrograph showing the styloconic-like sensory papilla.

Fig. 14 showing the slits with their nail-like covers.

Fig. 15: Magnified SEM micrograph of

The surface of the lateral sides of the anterior region of the body is covered with cobblestone-like protrusions which are regularly distributed (Fig 14). In magnified microphotographs of this region, each protrusion was seen as nail-like covering a crescent slit (Fig.15). In more magnified microphotographs, more details of these slits were seen. The area around these apertures possesses small protrusions and irregularly distributed micropores; the median part of the body surface was flattened, smooth, and polygonal-like (Figs. 15 & 16).

The body surface of the posterior half of the metacercaria possessed two other types of apertures lying marginally; each of these is larger than those present at the anterior half. The first type was narrow and surrounded with a dentate margin: the body surface around this aperture is corrugated (Fig. 17). The second type is the largest: it is crescent-like with a single operculum, with a surrounding flattened, smooth, and polygonal-like surface. Secretory droplets were seen scattered around the aperture (Fig. 18).

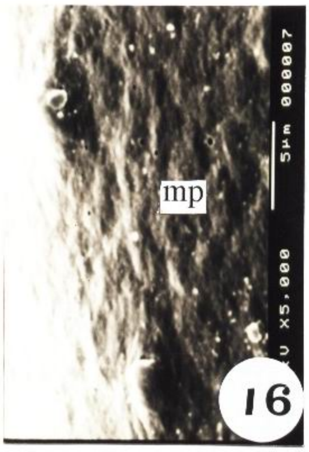


Fig. 16: Magnified SEM micrograph of fig. 15 showing another view of the same structure and micro-pores (mp).

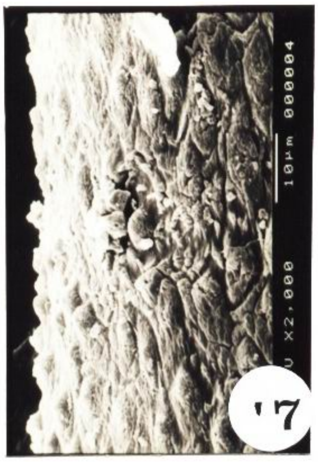


Fig. 17: SEM micrograph showing another type of apertures with dentate margin.

Fig. 18: SEM micrograph showing the largest aperture.

Near the end of the dorsal side of the body lies the excretory pore. Just anterior to this pore the body surface is papillated: each papilla had a pore at its top, and papillae are separated from each other with deep grooves (Figs. 19 & 20).

Fig. 19: SEM micrograph showing the excretory pore.

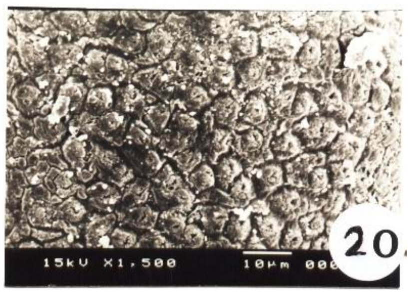


Fig. 20: SEM micrograph showing the papillar surface anterior to the excretory pore.

DISCUSSION

The metacercaria under study belongs to the genus *Clinostomum* Leidy, 1856. Most probably *Oreochromis niloticus* acts as a secondary intermediate host of more than one species of *Clinostomum* metacercariae, since the metacercaria described by Hassan (1993) in Qena was different from the present one in being covered with minute spines except for the posterior fourth of the body (under the light microscope) and was larger. The present study gives the first description of the topography of the metacercariae of this genus. The topography of its surface showed the presence of a variety of structures. It is known that the clinostomatid metacercariae are very similar to adult in most characters (Agrawal 1959; Ukoli 1966). The main observed features were sensory papillae, villi, spongy tissue, cobbled surface, flattened smooth polygonal-like areas, apertures and pores. The comparison between these features and those present in other related digenean flukes revealed many differences.

The rim of both the oral and ventral suckers in the present metacercaria is aspinus and non-papillated: the oral sucker is papillated in *Prohemistomum vivax* and the ventral sucker is covered with relatively smooth tegument (Khalil & Helal 1992). In *Orientocreadium batrachoides* the oral sucker has spines and sensory papillae (El-Naggar *et al*. 1993). The oral sucker of metacercariae and adults of *Gymnophalloides seoi* (Choi *et al*. 1995) has two sizes of papillae encircling its lip, while the ventral sucker is covered with cobblestone-like cytoplasmic processes and few papillae; in *Rhytidodes gelatinosus* the papillae are scattered around the latter (Noor El Din & Khalil 1999). The structure of the oral and ventral suckers is similar in *Neorilepturus aegyptensis* (Shalaby & Hassanine 1996) but in the present *Clinostomum* metacercaria it is completely different. The wrinkles present in the floor of the oral suckers are not present in any other trematode.

The spongy surface has also been found in the adhesive organ of *Cyathocotyle bushiensis* (Erasmus & Öhman 1965) and *Prohemistomum vivax* (Khalil & Helal 1992), while in the present *Clinostomum* metacercariae, this structure was present ventrolaterally on the median part of the surface. Erasmus & Öhman (1965) found that when the adhesive organ is attached to the host's mucosa the inner spongy surface was everted so as to create an intimate host-parasite interface. They suggested that this interface might have a secretory as well as an absorptive function. The spongy structure of the present parasite may have similar function. The cobblestone-like tegument reported in the present study is commonly observed in other trematodes (Khalil 1990; Khalil & Helal 1992). Wrinkles and villi have also been reported to be present in many of the closely related genera, but not from the same areas. In *Neorilepturus aegyptensis* (Shalaby & Hassanine 1996) the worm has villi-like appendages on the ventral side.

In the present work *Clinostomum* metacercaria showed both ventral and dorsal lateral sides lacking spines, which differs from many other related genera; for example *Prohemistomum vivax* (Khalil & Helal 1992), *Orientocreadium batrachoides*. (El Naggar *et al*. 1993), *Gymnophalloides seoi* (Choi *et al*. 1995) and adult *Fasciola gigantica* (Dangprasert *et al*. 2001) all possess spines of various sizes, densities and distributions over the body surface, whereas *Neorilepturus aegyptensis* (Shalaby & Hassanine 1996) is devoid of spines.

Various types of papillae were observed in many places on the worm. Papillae are abundant structures in digenean worms, present for example in *Prohemistomum vivax, Orientocreadium batrachoides,* and *Fasciola gigantica*. The function of the papillae is suggested to be tango-, mechano-, rheo-, and chemo- receptors. All such functions possibly help in feeding and assessment of the environment (Lee *et al*. 1987 & Fujino *et al*. 1989).

For the styloconic sensilla-like papilla we adopt the nomenclature proposed for insect chemosensilla by Zakaruk (1985).

The excretory pore lies near the posterior extremity of the body and opens dorsally, while it opens ventrally in *Orientocreadium batrachoides* (El-Naggar *et al*. 1993). To the best of our knowledge no author has described the apertures at the dorsal surface in any trematode fluke: in some insects there are similar structures (Zakaruk 1985; Zayed & Awad 2002) where they are identified as sensory papillae. The present apertures may have the same function, but further studies may be needed to confirm this function. The micropores present on the dorsal surface are similar to those seen by Shalaby & Hassanine (1996): they suggested that these pores represent the external opening of glandular cells.

Metacercariae of *Clinostomum* absorb glucose across the tegument by both active transport and facilitated diffusion (Uglem & Larson 1987). The same authors mentioned that the type of cutaneous transport system of glucose in digenean flukes is related to the parasite's location. Ling *et al.* (1981) suggested that the simultaneous operation of active transport and facilitated diffusion systems may play an important role in the transepithelial absorption of sugar by the small intestine of higher animals having luminal brush membranes: the glucose transport systems in *Clinostomum* resemble those found in higher animals (Stevens *et al.* 1984). This may explain the villar structures abundant in many parts of the ventral side of the metacercariae in the present study.

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الملخص العربى

دراسة بالميكروسكوب الإلكترونى الماسح لسطح الجسم فى ميتاسركاريا من نوع *كلينوستومم* من سمكة المياه العذبة *أوريوكروميس نيلوتيكس* من أسيوط مصر

عزيزة مروان و تركية أبو المجد محمد
قسم علم الحيوان-كلية العلوم-جامعة أسيوط

أظهرت دراسة سطح الجسم فى **ميتاسركاريا دودة *كلينوستومم*** العديد من المعالم، وقد تنوعت هذه المعالم تبعا لإختلاف مكانها على سطح الجسم. على السطح البطنى نجد إختلافا طفيفا بين الحافة الخارجية لكل من الممص الأمامى و الممص الخلفى. و يختلف قاع الممص الأمامى إختلافا كبيرا عن قاع الممص الخلفى، الأول به تموجات تحمل حافتها الخارجية حلمات حسية دقيقة، و الثانى مجعد وبه شقوق وثقوب وحلمات ذات قمم على شكل قباب. يغطى المنطقة بين الممصين طبقة من الخملات. يتميز سطح الجسم خلف الممص البطنى بوجود حافة خارجية إسفنجية المظهر و مساحة داخلية مسطحة ملساء تقطعها شقوق دقيقة. كما أن النهاية الخلفية للجسم تحتوى على شكلين من الوحدات عديدة الأضلاع من الخملات تحيط كل منها بحلمة حسية، الحلمات المزدوجة جهة الخارج و الأحادية جهة الداخل. على الناحية الظهرية توجد مجموعة من الفتحات و الثقوب الدقيقة بجانب بعض المعالم الأخرى. ففى نهاية الثلث الأمامى و على الخط المنصف للجسم توجد حلمة حسية ذات شكل خاص.أما على جانبى الجسم فتوجد مجموعة من الشقوق الهلالية ذات غطاء يشبه الظفر، خلف هذه الشقوق توجد فتحة ذات حافة سنية الشكل وخلف الأخيرة توجد أكبر الفتحات حجما وهى ذات غطاء غير مهندم، و فى نهاية الجسم توجد الفتحة الإخراجية. أما المنطقة الوسطى على إمتداد الجسم فهي مسطحة ملساء ذات شقوق فيما عدا المنطقة حول الفتحة الإخراجية فهى ذات سطح حلمى. وقد تم مناقشة الوظائف المحتملة لكل من هذه التراكيب.