ZOOLOGY SECTION

Morphology of adult and juvenile *Acanthostomum absconditum* (Looss, 1901) (Cryptogonimidae: Acanthostominae) from *Bagrus bayad* at El-Minia, Egypt

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Abstract: Morphological features of adult and juvenile stages of the trematode *Acanthostomum absconditum* examined from the Nile catfish *Bagrus bayad* were studied using light and scanning electron microscopy. The present study showed that the oral sucker in the adult stage is equipped with a mean number of 19 (range 17 - 23) spines, numerous pits and secretory granules that appeared on the tegument covering the bases of spines. The body is covered by scale-like tegumental spines, which are numerous on the anterior and middle portions of the body and gradually become scarce towards the posterior extremity. In the early juvenile stage, the whole body is covered by tegumental spines, while the circumoral tegument is completely devoid of spines. As the parasite grows up, the density of tegumental spines at the posterior extremity gradually reduces and, at the same time, a collar of spines gradually protrudes from the circumoral tegument. The entire oral sucker of the juvenile and adult stages along with its circumoral spines is retractable and can be partially or completely invaginated into the anterior end of the body. In addition, new morphological features were found in the juvenile stages such as the presence of type-I (uni-ciliated) papillae on the tegument above the developing circumoral spines and type-II (roundly swollen) papillae on the body surface. The possible function of spines and their distribution pattern, the sensory papillae, and the retractable oral sucker are discussed.

Keywords: Trematode, parasites*, Acanthostomum absconditum*, *Bagrus bayad*, fish, El-Minia, Egypt.

Introduction

Trematodes of the subfamily Acanthostominae Poche, 1926 comprise a group of parasites infecting the intestine of fish and reptiles [1]. The *acanthostomine Acanthostomum absconditum* belongs to the genus *Acanthostomum* [2] which was erected by Looss [2] and has been first described from *Bagrus bayad* and B. docmac from the River Nile at Cairo, Egypt [3]. This species seems to be common in the Egyptian freshwater fish as it has been detected several times from the same fish hosts by many authors [4, 5]. Also, it has been recorded from the same fish hosts in Sudan [6, 7] and from *B. filamentosus* and *Chrysichthys furcatus* in Ghana [8]. The adult stage is characterized by the presence of a single crown of spines (19 - 24) around the oral sucker and a spinose tegument [9]. Although the surface ultrastructure of the adult stage of *A. absconditum* has been previously studied, little information is available concerning the morphology of the juvenile stage. So, the present study was conducted to describe the morphological changes occurring during growth and maturation of the juvenile stages of the trematode *A. absconditum* using both the light and scanning electron microscopy.

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Material and Methods

Alive caught samples of *Bagrus bayad* (Forsskål 1775) fish were collected by fishermen or from local fish markets at El-Minia. The collected fish were transported to the laboratory using portable aquarium fitted with an air pump, where they were immediately dissected.

After dissection, the intestinal contents were discharged in 0.75% physiological saline solution, examined under a stereomicroscope and worms were isolated and washed gently with the saline solution. Afterwards, worms were fixed in 4% neutral formaldehyde solution followed by washing in distilled water for 5 min. Worms were subsequently stained in acetic alum carmine, dehydrated in an ascending grades of ethanol, cleared in xylene, and then, mounted in DPX for light microscopic examination. Morphometric measurements were taken on the stained flattened worms.

For scanning electron microscopy, some worms were fixed in 2.5% buffered glutaraldehyde (pH 7.4) made in 0.1 M sodium cacodylate at 4ºC. Post fixation treatment was carried out using 1% osmium tetroxide made in the same buffer at 4ºC for 1 - 2 hours. Later on, worms were dehydrated in ascending grades of ethanol, and then, critical point dried using carbon dioxide as a drying medium. Worms were then mounted on metallic stubs and coated with gold under vacuum conditions, using a JEOL JFC-1100E ion sputtering device. Examination was carried out with a JEOL JSM-5400 LV stereo scan device at 15 kV.

Results

I. Adults

Light microscopy

The body of adult worms is elongated in shape. The oral sucker is terminal and nearly cup shaped; it is armed with a crown of 19 - 24 peribuccal spines (Fig. 1a). The entire oral sucker along with its circumoral spines appeared to be retractable and can be partially or completely invaginated into the anterior end of the body (Fig. 1b). The ventral sucker is somewhat rounded. The oral sucker leads to a muscular, cup shaped buccal cavity that, in turn, leads to a short pre-pharynx followed by a large oval to rounded strong muscular pharynx. The esophagus is short and bifurcates into two intestinal caeca or crura that extend laterally towards the posterior extremity of the body (Fig. 1c). The caeca open posteriorly by two independent dorso-lateral anal pores locating just anterior o the excretory opening. The testes are irregularly rounded or oval in shape, and are situated at the posterior end of the body (Fig. 1d). The anterior testis is slightly smaller than the posterior one. The

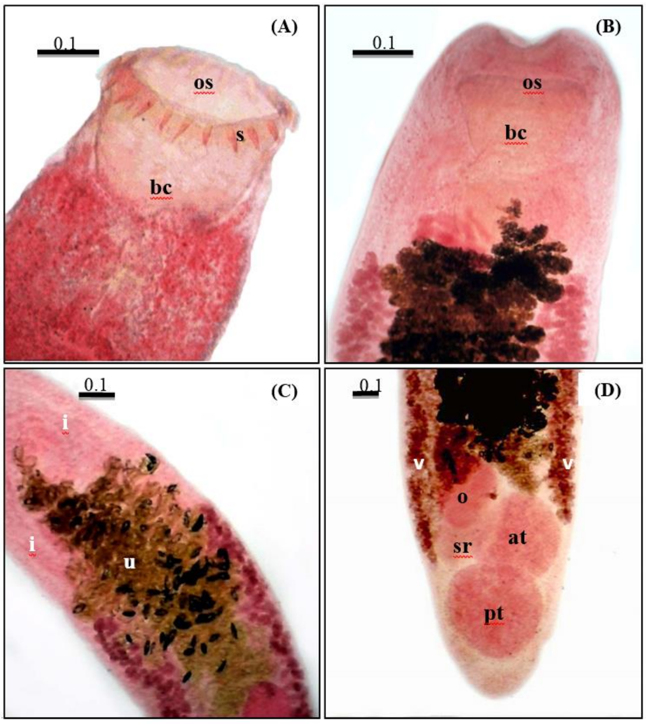

Fig. 1. Light micrographs showing whole mounts of adult *Acanthostomum absconditum* stained with acetic alum carmine. **a** Anterior end of the body showing a semispherical buccal cavity and the collar of spines surrounding the oral sucker (ventral view); **b** Anterior end showing that the entire oral sucker and its circumoral spines are completely withdrawn into the anterior part of the body (ventral view); **c** Middle portion of the body showing the two intestinal caecae and the uterus filled with ova (ventral view); **d** Posterior extremity showing vitellaria, ovary, seminal receptacle, anterior testis and posterior testis (ventral view); *at* anterior testis; *bc* buccal cavity; *i* intestinal caeca; *o* ova; *os* oral sucker; *pt* posterior testis; *s* spines; *sr* seminal receptacle; *u* uterus; *v* vitellaria; *vs* ventral sucker.

Table 1. Comparison between the morphometric measurements of both adult and juvenile stages of *A. absconditum* from *B. bayad* at El-Minia Province, Egypt.

Morphometric measurements	Adult stage			Juvenile stage		
	Range	±SD	n	Range	±SD	n
Total body length	1.34-2.8	0.422	14	0.85-.25	0.125	8
Maximum width	0.34-0.6	0.092	14	0.20-0.31	0.041	8
Oral sucker length	0.15-0.26	0.046	14	0.11-0.17	0.025	8
Oral sucker width	0.11-0.38	0.074	14	0.10-0.21	0.036	8
Peribuccal spines length	0.039-0.07	0.016	14	0.02-0.035	0.006	7
Peribuccal spines width	0.01-0.019	0.001	14	0.006-0.01	0.001	7
Ventral sucker length	0.05-0.19	0.036	13	0.06-0.09	0.013	6
Ventral sucker width	0.05-0.19	0.035	13	0.07-0.09	0.008	6
Anterior testis length	0.05-0.19	0.045	14	0.04-0.12	0.035	5
Anterior testis width	0.05-0.25	0.055	14	0.06-0.15	0.036	5
Posterior testis length	0.06-0.24	0.055	14	0.05-0.14	0.037	5
Posterior testis width	0.05-0.26	0.054	14	0.07-0.13	0.025	5
Ovary length	0.04-0.19	0.039	11	-	-	-
Ovary width	0.04-0.17	0.036	11	-	-	-
Egg length	0.032-0.035	0.001	14	-	-	-
Egg width	0.01-0.016	0.002	14	-	-	-

*n, number of worms

ovary is nearly spherical in shape (Fig. 1d) and situated above the anterior testis. The seminal receptacle is spherical or oval in shape, situated between the ovary and the anterior testis and sometimes overlaps either the testis or the ovary. Vitellaria are follicular in shape and extend on both sides of the body, approximately from the level of the mid-way between the ventral sucker and the ovary. They almost reach the mid-level of the anterior testis (Fig. 1d). The uterus is large, crowded by eggs and extends from the posterior margin of the ventral sucker to the ovary and is surrounded laterally by the vitellaria (Fig. 1c). The genital opening is ventral and situated just anterior to the ventral sucker. The egg is elongated and yellowish in color and non operculated. The excretory opening is terminal and lies at the posterior end of the body.

Scanning electron microscopy

Ultrastructural observations on the surface topography of the adult stage showed that the oral sucker has a rounded opening armed with a crown of 19 long pointed circumoral spines (Fig. 2a). Each spine is ensheathed from the base up to its second third by the tegument. This part of tegument is covered by numerous pits and secretory granules (Fig. 2b). The body is covered dorsally by tegumental spines, which are dense, tongue-shaped on the anterior portion and become less numerous on the mid-dorsal surface, and then they gradually diminish towards the posterior extremity (Figs. 2c- e). On the ventral surface of the body, just posterior to the ventral sucker, the spines gradually decrease in size and density; becoming more digitated at the posterior extremity. The latter is corrugated and covered with sparse spines on both its dorsal and ventral surface. The excretory opening is terminal, and the two slit-like anal openings are situated on a dorsolateral position slightly anterior to the excretory opening (Fig. 3a).The mature egg is oval in shape (Fig. 3b). The egg shell is rough in appearance and shows some sort of perforation.

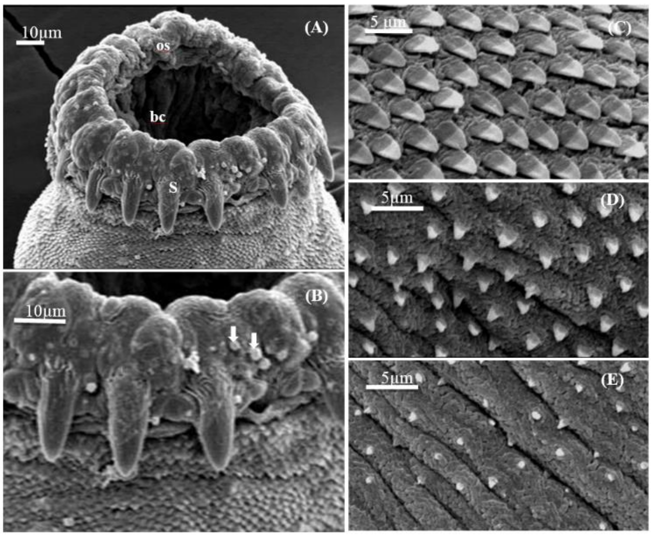

Fig. 2. Scanning electron micrographs (SEM) of an adult *A. absconditum*. **a** Anterior end showing the buccal cavity and the oral sucker surrounded by a crown of circum oral spines; **b** Enlarged portin of Fig. 2a showing numerous pits and secretory granules on the tegument covering the bases of spines; **c** Tongue shaped tegumental spines on the anterior dorsal surface; **d** Tegumental spines of the mid-dorsal surface; **e** Tegumental spines on the posterior dorsal surface of the body; *bc* buccal cavity; *os* oral sucker; *s* spines; *arrows* numerous pits and secretory granules.

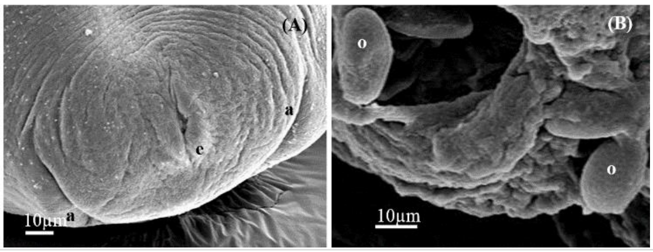

Fig. 3. Scanning electron micrographs (SEM) of an adult *A. absconditum*. **a** Posterior end showing the two dorso-lateral slit-like anal openings and the terminal excretory pore; **b** Three ova on the outer surface of the body; *a* anal openings; *e* excretory pore; *o* ova.

II. Juveniles

Light microscopy

The juveniles are divided into two developmental stages (I and II) based on the body size, state of development of the circumoral spines and state of development of the genital primordia. In early juveniles (stage-I), the body is small with a maximum length of about 0.8 mm. The oral sucker is terminal in position, circular in shape and completely devoid of spines and the genital primordia are absent or not formed yet. The two intestinal caecae are clearly observed and terminate posteriorly with two separate lateral anal openings.The excretory vesicle is swollen and y–shaped.

In older juveniles (stage-II), the tips of the spines appear at the periphery of the circumoral tegument. Testes primordia appear first as a group of massive cells near the posterior end of the body. Later on , the ovary premordia begin to appear as a small cluster of cells just above the anterior testis.

Scanning electron microscopy

In early juveniles (stage-I), the oral sucker is terminal and rounded. Although the musculature of the oral sucker is well developed, the circumoral spines are abscent (Fig. 4a). In older juveniles (stage-II), the tips of the spines project at the periphery of the circumoral tegument to form a collar-like structure (Fig. 4b). The oral sucker along with its circumoral tegumental spines is partially withdrawn into the anterior part of the body (Fig. 5a.) The circumoral tegument at the bases of developing spines has numerous ciliated papillae and openings of the glands are distributed near its periphery (Fig. 5b). The ventral sucker of stage II may be at the same length as that of stage I or may be slightly longer showing somewhat more capability of protrusion (Fig. 5a). The elevated tegument around the ventral sucker is completely covered with spines (Figs. 5 a, c). The entire body is covered dorsally and ventrally with small tongue-shaped spines, which are densely distributed on its anterior part; they are gradually converted into notched spines and become sparse on the posterior extremity of the body (Figs. 5 d - f).

Two types of sensory papillae, uni-ciliated (type-I) papillae and roundly swollen (type-II) papillae, are observed on the tegument of both stages of the juveniles. Type-I uni-ciliated papillae usually scatter on and around the oral sucker, while type-II papillae are sparsely distributed between the tegumental spines on the anterior part of the body. The excretory opening is situated at the posterior extremity of the body, slightly posterior to the anal pores (Fig. 5g).

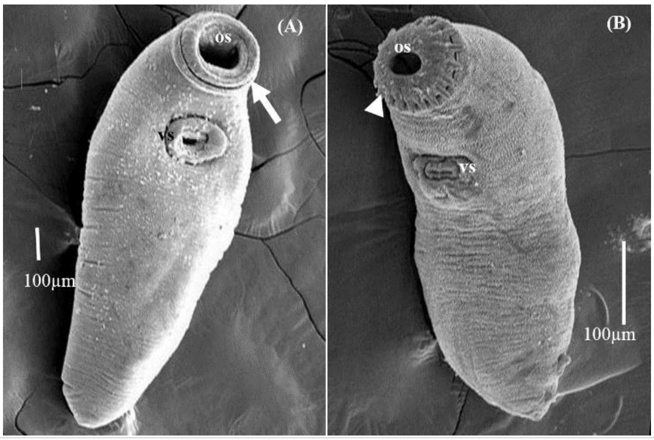

Fig. 4. Scanning electron micrographs (SEM) of *A. absconditum* juveniles. **a** Ventral view of a juvenile stage-I showing that the circum oral tegumental crown is devoid of spines and the ventral sucker is embedded inside the surrounding elevated circle of tegument; **b** Ventral view of a juvenile stage-II showing spines project at the periphery of the circumoral tegument. v ventral sucker; arrow the naked circum oral tegumental crown; head arrow projecting spines.

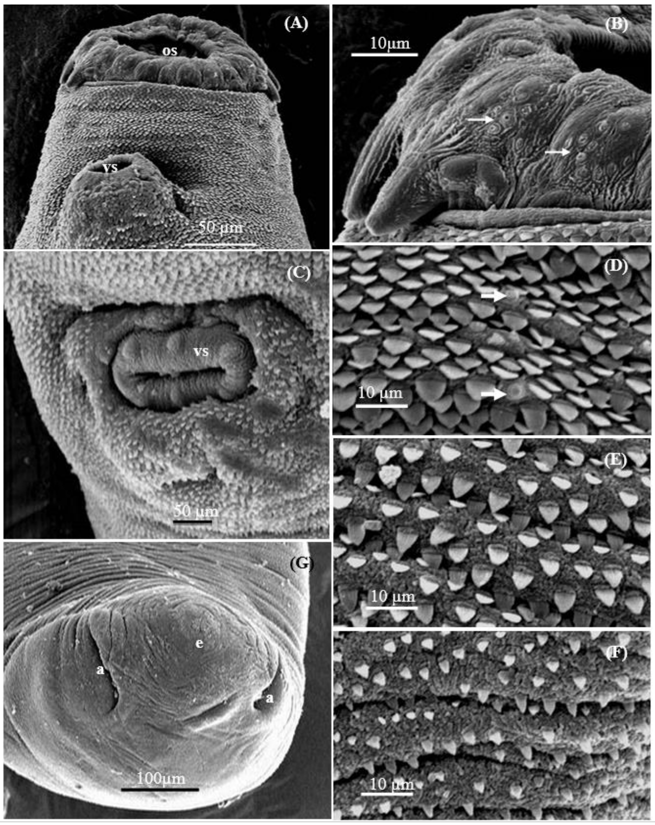

Fig. 5. Scanning electron micrographs (SEM) of *A. absconditum* juveniles. **a** Ventral view of the anterior end showing the oral sucker, ventral sucker and circumoral spines partially withdrawn into the anterior end of the body; **b** Enlarged portion of Fig. 5a showing ciliated papillae and openings of glands on the tegument above the developing spine; **c** Ventral view of the anterior end showing the ventral sucker surrounded by elevated spiny tegument; **d-f** Distribution of spines on the antero-dorsal, mid-dorsal and postero-dorsal surfaces, respectively. Note roundly swollen (type-II) papillae on the tegument; **g** Posterior end showing the two dorso-lateral slit-like anal openings and the terminal excretory pore; *a* anal opening; *e* excretory pore; *os* oral sucker; *v* ventral sucker; thin arrows ciliated papillae and openings of glands; thick arrows roundly swollen (type-II) papillae.

Discussion

The genus *Acanthostomum* [2] is characterized by the presence of a collar of tegumental spines around the oral sucker [9, 10] and one pair of anal openings near the posterior end [11]. Its morphology is nearly similar to that previously described for *Acanthostomum absconditum* [11], but with some minor morphometric measurement differences These differences are intraspecific and may probably be attributed to the over compression of the present specimens during preparation for examination.

The present ultrastructural observations showed that the opening of the oral sucker of adult stages is rounded and armed with 19 - 24 spines, which are ensheathed by a basal extension of the tegument that reaches approximately second third of each spine. This observation agrees with the results of Moravec [11]. The circum-spine tegument of the present specimens showed numerous pits from which emerge secretory granules. Such findings are not recorded in the description of Moravec [11] and Ibraheem [5]. In early juveniles, however, the tegument overlaying the oral sucker appeared completely devoid of spines and the overall body is covered by tegumental spines. As the juveniles grow up, the circum oral spines gradually protrudes from the circumoral tegument. At the same time, the tegumental spines, which cover the body, become less dense at the terminal posterior end. Ibraheem [5] considered that the ventral sucker of *A. absconditum* might be not powerful enough to make efficient adhesion in intervillous position and the attachment is primerly enhanced by the circumoral spines.

Lee et al. [12] stated that the distribution of spines helps the worm in attachment to, and abrasion of the host tissue at intervillous crypts. Tegumental spines may help the newly existed worms to establish good contact with the intestinal villi and, therefore, prevent them from being expelled with the faeces of the host [13]. The shape and distribution of the tegumental spines of digenetic trematodes depend on many factors such as the state of worm maturation, parasite habitats, and migratory behavior inside the host's intestine [14-16].

In the present study, the two types of sensory papillae, uni-ciliated (type I) and roundly swollen (type II), are characteristic only to the juvenile stages of *A. absconditum*. The type I papillae are mainly scattered on and around the oral sucker. The function of the uni-ciliated papillae has been suggested to be tango and/or chemoreceptor and that of type II has been supposed to be tango and/or pressure receptors [17-19].The papillae distributed on and around the oral sucker of the digenetic trematode *Echinoparyphium recurvatum* are probably tactile sensory receptors involved in feeding and attachment [15]. The Type I papillae which scatter on the tegument of the oral sucker have been supposed to be sensory and help in attachment to the intestinal mucosa [20, 21] Other digenetic trematodes such as those of the genera *Microphallus* and *Aspidogaster* have shown to possess fore body glands [22, 23].

In this study, we found that the entire oral sucker of *A. absconditum* along with its circumoral spines exhibit some mobility and could be able either partially or completely to retract inside the anterior end of the body. Such action has previously been recorded for *A. absconditum* and *A. spiniceps* [5], *A. macroclemidis* [9] and *A. gnerii* [24]. Other digenetic trematodes which are equipped with a similar collar of spines such as Eechinostomatids cannot retract their oral suckers and spine collars into their bodies [9].

Conclusions

In conclusion, the present study revealed the presence of two types of papillae on the body tegument of *A. absconditum*, uni-ciliated papillae (type I) on the oral sucker and roundly swollen papillae (type II) between the tegumental spines. Such findings have not previously been recorded by Ibraheem [5]. These papillae may be used by juveniles to ensure firm attachment that could be compensated by the well-developed oral sucker in the adult stage. So, as the worm grows up, these papillae gradually disappear.

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