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# Surface ultrastructure of the adult and juvenile stages of the trematode *Astiotrema impletum* (Looss, 1899) Looss 1900 (*incertae sedis*) from the Nile puffer, *Tetraodon lineatus* Linnaeus, 1758

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**Summary**

A scanning electron microscope study was performed on the surface of the trematode *Astiotrema impletum* (Looss, 1899) Looss 1900 from the Nile puffer, *Tetraodon lineatus* Linnaeus, 1758 (Syn. *Tetraodon fahaka*) for the first time. Adult *A. impletum* have a markedly large, sub-terminal oral sucker and a small ventral sucker. As with most trematodes, tegumental spines are concentrated anteriorly and are absent just anterior the ventral sucker. Spines have serrated tips on a short, tongue–shaped body. At the level of the ventral sucker the base of each spine exhibits a three bulbous-like structure that can be partially or completely withdrawn into the tegument. Sensory papillae are concentrated around the oral and ventral suckers and genital pores. Papillae are conical or knob-like, either ciliated or non-ciliated; some are protruded and others are embedded inside the tegument. In the juvenile stage, spines are smaller, less well-developed and tightly packed. The cytoplasmic processes of the posterior quarter of body show brain-like velvety appearance on the adult and small cobblestone-like on the juvenile.

**Keywords:** *Astiotrema impletum*; SEM study; sensory papillae; surface topography; *Tetraodon* sp.; Trematoda

## Introduction

The trematode, *Astiotrema impletum* (Looss, 1899) Looss 1900 was first described from the fresh and brackish water globe fish, *Tetraodon lineatus* Linnaeus, 1758. It has also been reported from the same host species in Egypt and Sudan (Odhner, 1911; Sheng-Liang & Fotedar, 1958; Fischthal & Kuntz, 1963; Khalil, 1969). Yamaguti (1958) considered *T. lineatus* as the type host of *A. impletum*. The majority of the studies provided a detailed morphological description of the adult *A. impletum* through light microscopy and have revealed a significantly larger oral sucker than ventral sucker (Bray *et al.*, 2006; Sheng-Liang & Fotedar, 1958; Siddiqui, 1958; Khalil, 1959; Fischthal & Kuntz, 1963; Siddiqi 1965). In Egypt, Hamada (2002) described the surface ultrastructure of a trematode, identified as *A. impletum* from *Oreochromis niloticus niloticus* (Linnaeus, 1758) but having the ventral sucker larger than the oral sucker. Unfortunately Hamada (2002) did not describe any other morphological features that would allow identification of her *Astiotrema* sp.. Comparing the trematode described by Hamada (2002) with specimens of the presented study however, clearly shows that two different trematode species, possibly even genera, have been involved (Table 1). Major differences in the spine and papillae distribution patterns between the two trematodes are readily evident, especially around the suckers. Given the confusion of the trematode species in the Hamada (2002) study, the aim of our study is to describes the surface ultrastructure of adult

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Table 1. Significant differences between *Astiotrema impletum* described in this study and the trematode recorded by Hamada (2002).

| Feature | Present Study | **Hamada** (2002) |
|---|---|---|
| Host | Fahaka, Nile Pufferfish<br>*Tetraodon lineatus*<br>Tetraodontiformes<br>Tetradontidae | White Tilapia<br>*Orechromis niloticus*<br>Perciformes<br>Cichlidae |
| Oral sucker much larger than ventral sucker. | yes | no |
| Ventral sucker found in the anterior quarter of the worm. | no | yes |
| Ventral sucker surrounded by numerous spines | no | yes |
| Ventral sucker with sensory papillae and tend to be in four clusters. | yes | No, papillae absent. |
| Tegumental area between the oral and ventral suckers. | Square-shaped and has relatively small spines directed postero-laterally and arranged in parallel rows. | Rectangular and naked. |

and juvenile *A. impletum* from the type host, *T. lineatus***.** Scanning electron microscopy is now commonly used in studies of digenetic trematodes as it provides valuable data for their identification and characterization of developmental stages (Scholz *et al.,* 1992; Sohn *et al.,* 2003).

**Materials and Methods**

A total of 40 Nile puffer *Tetraodon lineatus* were collected by local fisherman between January 2017 and September 2018 from Nile basin and El-Ibrahimia canal next to El-Minia city (28° 04' − 28° 06' N and 30° 45' − 30° 46' E), Upper Egypt. Fish were taken to the laboratory in a portable aquarium fitted with an air pump. Fish were examined and the recovered adult and juvenile trematodes were placed in 0.75 % physiological saline. After washing in saline, worms were immediately fixed for 2 hours in hot 4% neutral buffered formalin (see de Chambrier *et al.* (2009)) and then washed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) at 4° C for 2 h. and post fixed for 3 h using 1% osmium tetroxide in the same buffer at 4° C. After washing in buffer, worms were

Table 2. Statistical parameters of dimensions and indices of the adult and juvenile *Astiotrema impletum* (Looss, 1899) Looss 1900 from the Nile puffer fish *Tetraodon lineatus* Linnaeus, 1758 in Egypt. (Metric characters are in mm.)

| **Morphological parameters** | **Adult** | | | | **Juvenile** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **n** | **SE** | **Mean** | **SD** | **n** | **SE** |
| Total body length | 2 | 0.3860 | 8 | 0.1367 | 1.19 | 0.1400 | 8 | 0.0574 |
| Maximum width | 0.5 | 0.1455 | 8 | 0.0515 | 0.46 | 0.0770 | 8 | 0.0315 |
| Oral sucker length | 0.27 | 0.0489 | 8 | 0.0122 | 0.24 | 0.0200 | 8 | 0.0083 |
| Oral sucker width | 0.34 | 0.1132 | 8 | 0.0128 | 0.27 | 0.0340 | 8 | 0.0173 |
| Ventral sucker length | 0.16 | 0.0351 | 8 | 0.0132 | 0.15 | 0.0270 | 7 | 0.0097 |
| Ventral sucker width | 0.18 | 0.0348 | 8 | 0.0131 | 0.15 | 0.0320 | 7 | 0.0114 |
| Ant. testis length | 0.27 | 0.0443 | 8 | 0.0156 | 0.13 | 0.0450 | 6 | 0.0183 |
| Ant. testis width | 0.23 | 0.0512 | 8 | 0.0181 | 0.12 | 0.0400 | 6 | 0.0166 |
| Post. testis length | 0.34 | 0.0504 | 8 | 0.0178 | 0.15 | 0.0640 | 6 | 0.0264 |
| Post. testis width | 0.27 | 0.0296 | 8 | 0.0104 | 0.13 | 0.0650 | 6 | 0.0265 |
| Cirrus sac length | 0.32 | 0.0977 | 7 | 0.0369 | 0.12 | 0.0400 | 3 | 0.0233 |
| Ovary length | 0.16 | 0.0349 | 8 | 0.0123 | - | - | - | - |
| Ovary width | 0.18 | 0.0370 | 8 | 0.0130 | - | - | - | - |
| Egg length | 0.040 | 0.0055 | 8 | 0.0021 | - | - | - | - |
| Egg width | 0.0124 | 0.0028 | 8 | 0.0012 | - | - | - | - |

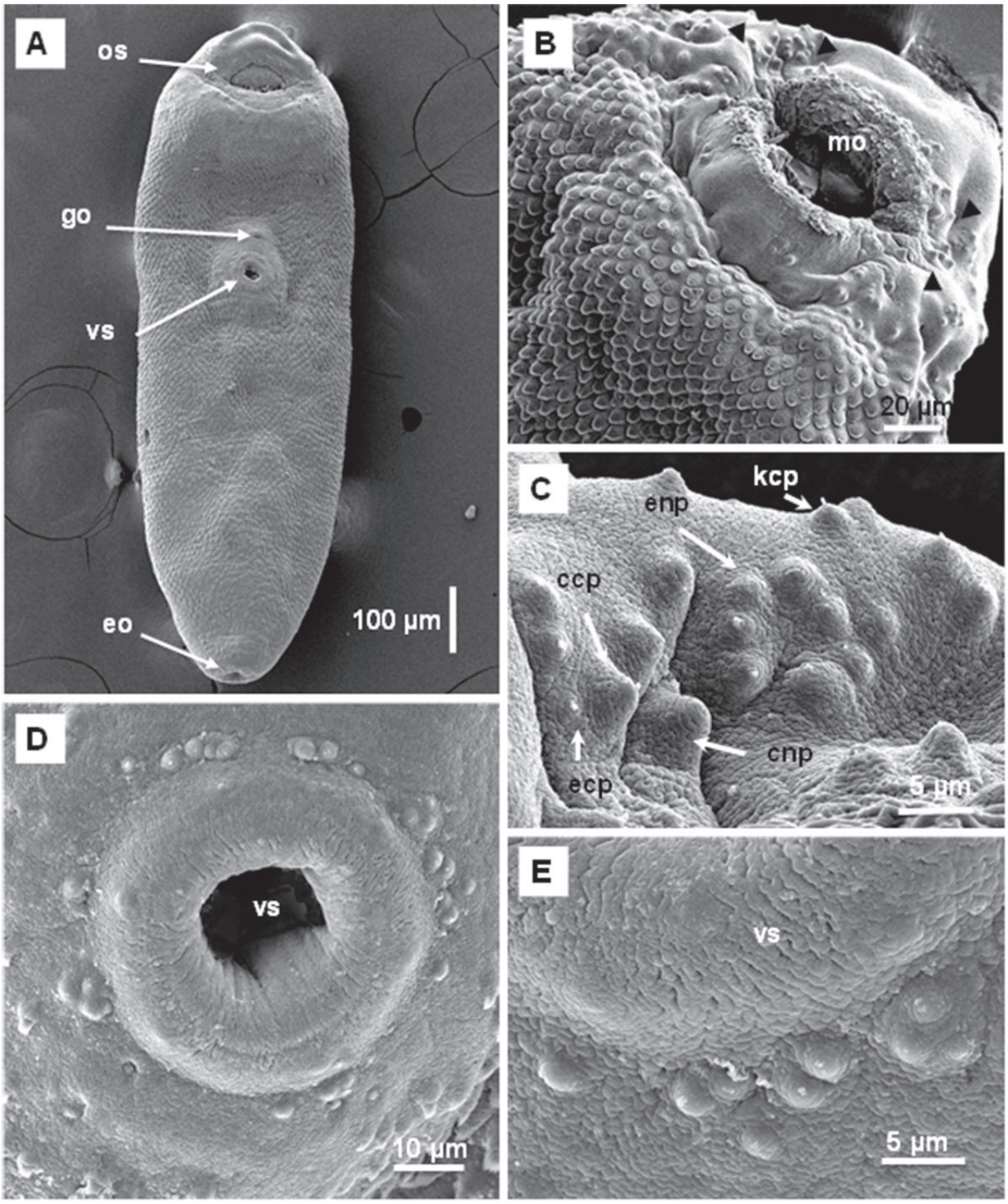


Fig. 1. Scanning electron micrographs (SEM) of adult *Astiotrema impletum*.

A – Ventral view of whole body. B – Distribution of sensory papillae around oral sucker and mouth. C – Different shapes of sensory papillae on oral sucker. D – Randomly distributed papillae on naked tegument surrounding ventral sucker. E– Solitary knob-like ciliated papillae and grouped papillae in vicinity of ventral sucker. *Abbreviations:* ccp – conical ciliated papillae; cnp – conical non-ciliated papillae; ecp – embedded ciliated papillae; enp – embedded non-ciliated papillae; eo – excretory opening; go – genital opening; kcp – knob-like ciliated papillae; mo – mouth opening; os – oral sucker; vs – ventral sucker; arrowheads indicate sensory papillae.

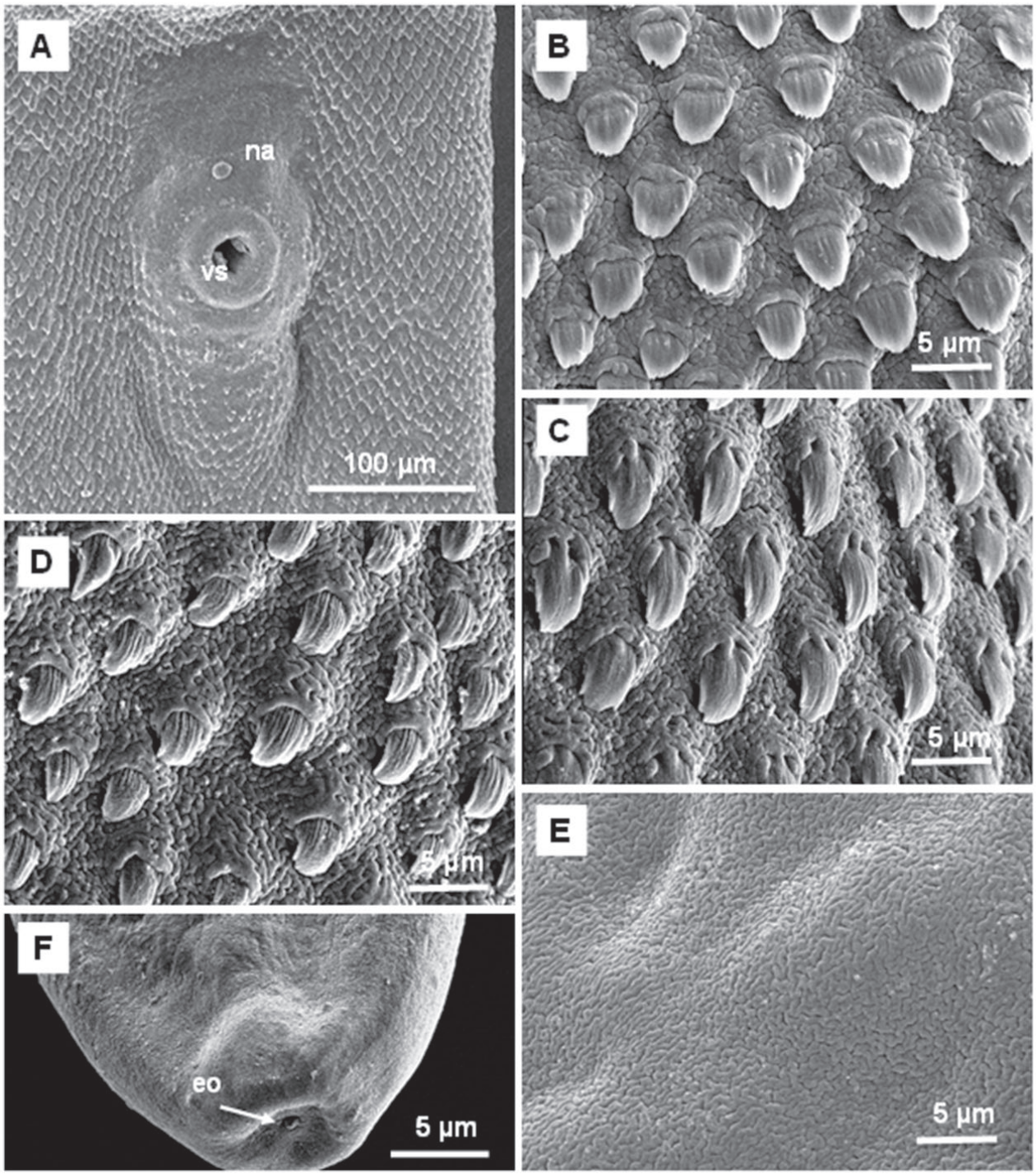


Fig. 2. SEM of ventral surface of adult *A. impletum*.

A – Naked area around and anterior ventral sucker. B – Tegumental spines of anterior ventro -lateral surface of body, just posterior to oral sucker. C – Tegumental spines on the ventro- lateral part, at level of ventral sucker, are long and pointed. D – Tegumental spines on the ventro -lateral part, at level of third quarter of body are smaller, fewer and more corrugated or with parallel surface ridges. E – Cytoplasmic processes on tegument of posterior end have a vermiculate, brain-like velvety appearance. F – Excretory opening is slightly shifted towards ventral surface. *Abbreviations*: eo – excretory opening; na – naked area in front of ventral sucker; vs – ventral sucker.

dehydrated in ascending ethanol series and acetone, and dried in a critical-point drying machine using liquid carbon dioxide as a transitional medium. Dehydrated worms were mounted on metallic stubs and coated with gold under vacuum for 10 min. at a setting of 10 – 15 mA using a JEOL JFC-1100E ion sputtering device. Examination was with a JEOL JSM-5400 LV stereo scan device at an acceleration of 15 kV.

For light microscopy, the isolated worms were washed in 0.75% physiological saline solution, fixed in 10% neutral buffered formalin, stained in acetic alum carmine, dehydrated in an ascending series of ethanol, cleared in clove oil, and then mounted in DPX (Morsy *et al.,* 2018). Drawing was made by using a camera Lucida. All measurements were presented in millimeters.

**Ethical Approval and/or Informed Consent**

Animal care and handling were carried out in accordance with all the relevant national regulations and institutional policies.

**Results**

Adult worms were bearing egg and varied from 1.7 to 2.63 mm long with mean of 2 mm. Juvenile worms lacked eggs, varied from 1 to 1.5 mm with a mean of 1.19. Other worms in both categories were selected for study with light microscope (Table 2).

*Adult Worm*

SEM characterization was based on 10 specimens. The oral and ventral suckers are well-developed; the former is markedly stronger and their relative size ratio is 1.8 – 1, respectively (Fig. 1A). The sub-terminal oral sucker is sub-globular and has no distinct lips. The smaller ventral sucker, however, is nearly rounded and in a median position at the beginning of the second third of the body. The genital opening is slightly sinistral to the midline of the body, just anterior ventral sucker (Fig. 1A).

Sensory papillae randomly surround only both suckers and genital opening. Sensory papillae of the oral sucker are numerous and either solitary or in groups of two to eight; they scatter on the lateral sides of the mouth opening close to the corners (Fig. 1B). They show some variation in size and shape, ranging from knob-like to conical; the majority appears to have a single cilium. Some of these papillae are obviously protruded with a cilium, others are embedded inside the tegument (Fig. 1C). Papillae around ventral sucker (Fig. 1D) are smaller and fewer than those of the oral sucker. Most papillae vary in size and are knob-like with short cilia (Fig. 1E). Papillae are slightly smooth and arranged either solitary or clusters of two to five. There are fewer papillae anterior the ventral sucker and around the genital opening.

The dorsal and ventral surface of the anterior part of *A. impletum* is densely covered with backwardly directed spines. Spines gradually disappear on the posterior quarter of the body. Spines are absent from just anterior the ventral sucker and near the genital opening (Fig. 2A). Ventral spines are closely spaced short, blunt and tongue-shaped with serrated tips (Fig. 2B). Posterior to the ventral sucker, spines increase in length. The base of each spine exhibits three bulbous-like structures (Fig. 2C). The latter are observed sometimes hidden, either partially or completely, inside the tegument. Near the posterior end spines decrease in size and density and have a more corrugated surface (Fig. 2D) before disappearing. The naked posterior quarter and of the body surface has a vermicular or brain-like appearance (Fig. 2E). The shape and distribution of dorsal and ventral spines are similar. The excretory pore is at the posterior extremity, in an indentation and slightly shifted ventrally (Fig. 2F).

*Juvenile Stage*

SEM characterization was based on 35 specimens. The immature worm generally appears like a smaller adult: dorso-ventrally flattened and blunt at both ends (Fig. 3A). The oral sucker is therefore large, wide, sub-terminal and lacking distinct lips. The ventral sucker, slightly anterior of mid-body, is somewhat rounded and slightly elevated above the body tegument (Fig. 3A). Relative size ratio of oral sucker to ventral one is about 1.7 – 1, respectively. Sensory papillae are few, with same distribution as that of the adult. On the oral sucker, they occur either solitary or in clusters of two to six (Fig. 3B). Few sensory papillae are scattered away from the genital opening. The latter has a position seen in adult worms (Fig. 3C). About 14 variable knob-like sensory papillae in four loose clusters surround the ventral sucker. Some of them have a short cilium (Fig. 3D). The body is covered by smaller backwardly directed spines, with the same distribution seen on adult. Posterior oral sucker, spines are tongue-shaped with broad bases and corrugated surfaces. The tegument at the base of each individual spine protrudes upwards, forming a pocket-like structure that surrounds the root of the spine (Fig. 3E). On both sides of the ventral sucker, the spines are much corrugated and are smaller (Fig. 3F). As in the adult, spines gradually disappear at the posterior extremity. The cytoplasmic processes on the naked posterior part of the body have a fine granulate or small cobblestone-like appearance (Fig. 3G).

**Discussion**

The genus *Astiotrema* Looss, 1899 includes about 28 species described from tortoises, snakes and fish in Asia and Africa (Besprozvannykh *et al.*, 2015; Zhokhov *et al.*, 2017). *Astiotrema impletum* has been previously studied from the fresh water globe fish *T. lineatus* in Egypt and Sudan using light microscopy (Fischthal & Kuntz, 1963; Khalil, 1969; Mehra, 1931; Odhner, 1911; Sheng-Liang & Fotedar, 1958). Hamada (2002) has done the only surface topography. Lack of data on SEM morphology of most species prevents analysis of systematic relationships within the genus. There have been successive revisions that have evaluated the validity of species resulting in many synonyms (Bhalerao, 1936; Gupta,

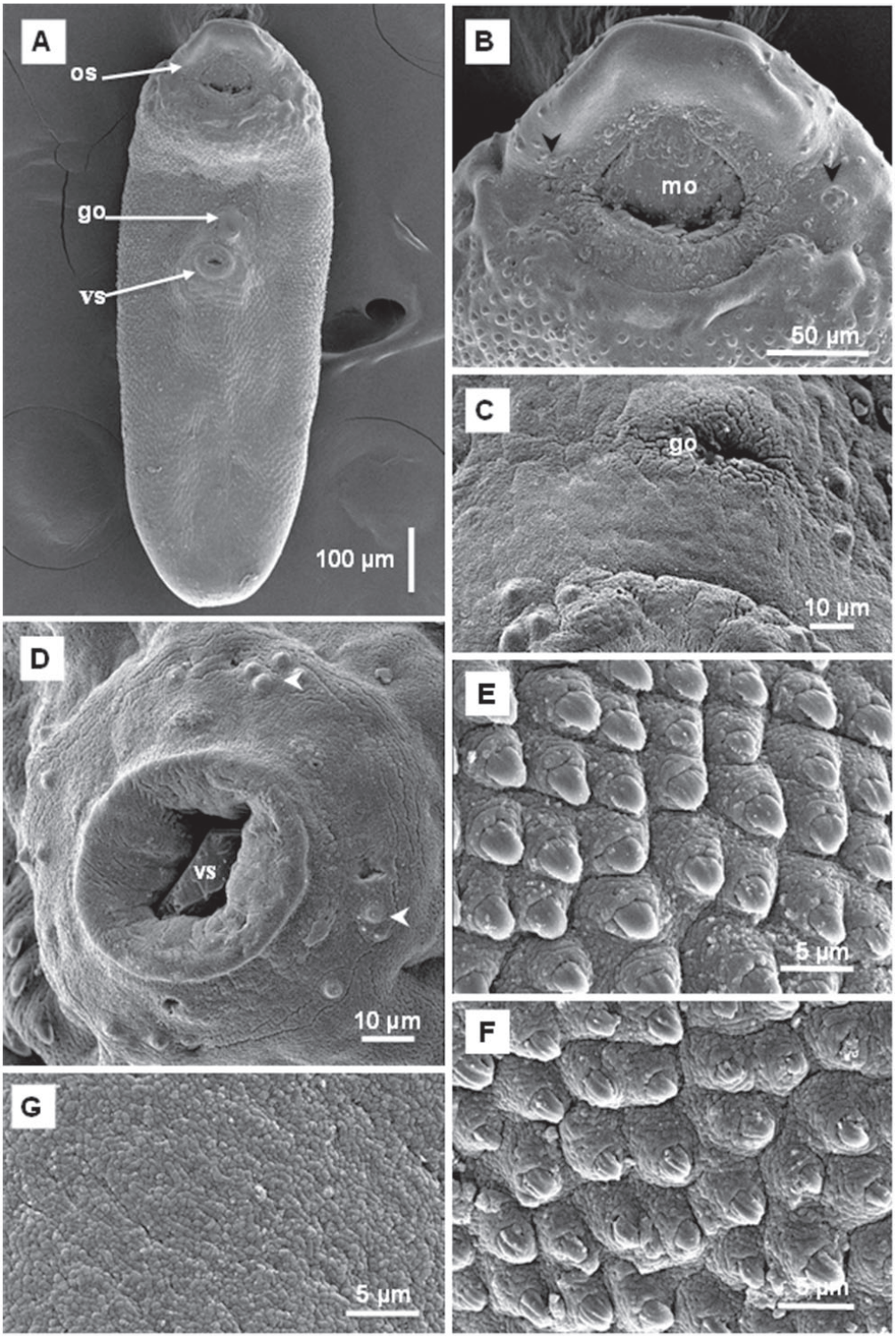


Fig. 3. SEM of juvenile *Astiotrema impletum*.

A – Ventral view of whole body. B –Distribution of sensory papillae on and around oral sucker. C – Genital opening. D - Ventral sucker surrounded by a number of randomly distributed sensory papillae. E – Tegumental spines on anterior ventro -lateral side of body, just posterior to oral sucker. Note pocket-like tegument covering bases of spines. F – Tegumental spines on ventro -lateral surface of body at level of ventral sucker. The spines are small in size and with parallel surface ridges or corrugated. G – Cytoplasmic processes of tegument display a fine granulated or small cobblestone-like appearance on the posterior end of body. *Abbreviations*: go – genital opening; mo – mouth opening; os – oral sucker; vs – ventral sucker; arrowheads indicate sensory papillae.

1954; Khalil, 1959; Sheng-Liang & Fotedar, 1958; Siddiqi, 1965). Besprozvannykh *et al.* (2015) discussed the contradictory status of species within the genus *Astiotrema*. Only *A. reniferum* (looss, 1898) and *A. impletum* have been reported from freshwater fish (Agrawal, 1966; Sheng-Liang & Fotedar, 1958; El-Naggar, 1991).

There is a serious question of the correct identity of the trematode studied by Hamada (2002), identified as *A. impletum* **,** from *Oreochromis n. niloticus*. As already noted, Hamada (2002) indicated that her specimens had a ventral sucker significantly larger than the oral sucker. According to Siddiqui (1958), Khalil (1959) and Siddiqi (1965) however, the oral sucker of *A. impletum* is significantly larger than the ventral one. In addition, her specimens came from tilapia while *A. impletum* had never been reported from any other fish host except *T. lineatus*. That fact led Mehra (1931) to concluded that *A. impletum* is a unique species known only from *T. fahaka*. (see Scholz *et al*. (2018)) for history of trematode infections in fish from Egypt). Because of the difference in sucker morphology and the question of host specificity, we consider our study as the first description of the surface topography of *A. impletum* and its juvenile stage for reasons of morphology and on specimens from the type-host, *T. lineatus*.

In the present study, SEM analysis showed that the tegument of adult and juvenile *A. impletum* is covered by spines. This pattern is similar to that of diverse trematodes (Bennett, 1975; El-Naggar *et al*.,1993b; Ibraheem, 2006; Dumbo *et al*., 2019): dense anteriorly with gradually decrease in density and size towards the posterior end. Sheng-Liang and Fotedar (1958) had earlier detected dense distribution of spines or cuticular scales on the anterior third of adult *A. impletum*, and a sparse distribution on the posterior part. Similar observations have been recorded in trematodes such as *Fasciola hepatica* by Bennett (1975), *A. reniferum* by El-Naggar *et al*. (1993b), *Orientocreadium batrachoides* by El-Naggar *et al.* (1993a), *Acanthostomum* spp. by Ibraheem (2006) and *Masenia nkomatiensis* by Dumbo *et al*. (2019). Hong *et al*. (2004) considered reduction of spines on the posterior half of body of *Macroorchis spinulosus* as an adaptation for increasing absorptive surface area. In the present study, each tegumental spine exhibits a three bulbous-like base, originating from the underlying tegument. In many cases, these structures were partially or completely withdrawn inside the tegument. This suggests to us that individual spines may have, to some extent, ability for functional mobility that may enhance abrasion of intestinal mucosa to produce tissue debris for feeding. On the other hand, they may act as a locomotive apparatus.

The tegument of the present trematode showed a fine granulated or small cobblestone-like appearance on the body surface of the juvenile stage that is gradually differentiated into a velvety, vermiculated appearance on the adult. Similar developmental differentiation has been reported in some other trematodes like *Heterophyopsis continua* (Hong *et al*., 1991), *Metagonimus miyatai* (Chai *et al*., 1998) and *Macroorchis spinulosus* (Hong *et al*., 2004). Gupta *et al.* (2017) considered the rough, granulated or cobblestone-like processes on the body of *Allogenarchopsis bareilliensis* as an efficient adaptive feature for increased absorption.

Sensory papillae were detected above the oral sucker, around the ventral sucker and the genital opening of both adults and juveniles. They were more numerous on the adults. Similar observations were detected on *Clonorchis sinensi* by Fujino *et al*. (1979) and *Himasthla alincia* by Han *et al.* (2003).

Bakke (1976) stated that absence of spines on rim of oral and ventral suckers of *Leucochlorodium* sp. is necessary for a smooth seal to host's mucosa.

**Conflict of Interest**

The Authors state that they have no conflict of interest.

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