Ultrastructural study on the body surface of *Porrorchis indicus* (Acanthocephala: Plagiorhynchidae) from the Egyptian cuculus, *Centropus senegalensis aegyptius (*Aves : Cuculidae)

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Abstract: *Porrorchis indicus* (Das, 1957) Schmidt and Kuntz, 1967 is recovered from the small intestine of the Egyptian cuculus, *Centropus Senegalensis aegyptius* in Egypt. This acanthocephalan was identified and described by using light and scanning electron microscope for the first time. Scanning electron microscopy of the proboscis hooks, provide additional data about the surface of these taxonomic relevant structures. The proboscis in both sexes carries 20-24 vertically oriented rows each of 8 or 9 hooks which vary in length and size in relation to their position. The study revealed the striated nature of the proboscis hooks of *P. indicus*, a characteristic only reported in two acanthocephalan species. Scanning electron microscopy documented the elevated slit-like female gonopore, the ribbed surface of the eggs and the spoon-like shape of male bursa.

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1. Introduction

The acanthocephalan genus *Porrorchis* Fukui, 1929 (Eoacanthocephala: Plagiorhynchidae: Porrorchinae) was revised by Schmidt and Kuntz (1967). Most species of *Porrorchis* use birds as definitive hosts, although two species *Porrorchis hylae* (Johnston, 1914) and *P. leibyi* (Schmidt and Kuntz, 1967) have been reported to occur incidentally in mammals in southeast Asia.

P. indicus has a very limited worldwide distribution, it was described from the predatory birds from India (Das, 1957). This parasite was recorded for the first time from *Centropus senegalensis aegyptius* in Egypt by Ashmawy and El-Sokkary (1991).

Hooks are an important taxonomic feature for Acanthocephala, but there is a dearth of detailed information concerning their ultrastructure (Miller and Dunagan, 1985). The proboscis of adult acanthocephalan bears hooks of a definitive shape, size, number, and pattern of arrangement in a given species. Variations in size, shape and arrangement pattern of proboscis hooks are used in separation of species of *Echinorhynchus* (Huffman and Bullock, 1975) and *Pomphorhynchus* (Huffman and Nickol, 1979). SEM observations of proboscis hooks of several acanthocephalan species belonging to Palaeacanthocephala, Eoacanthocephala and Polyacanthocephala indicated that surface striations are rare and are so far exclusive to the hooks of two acanthocephalan species, *Dentitruncus truttae* Sinzar, 1955 (Palaeacanthocephala) reported by Dezfuli *et al*., (2008) and *Rhadinorhynchus ornatus* Van Cleave, 1918 (Eoacanthocephala) by Amin *et al*., (2009).

The present study aims to reveal for the first time, the surface ultrastructure of *P. indicus*.

2. Materials and Methods

Twenty-three males and forty-five female *Porrorchis indicus* were collected alive from the small intestine of five *Centropus senegalensis aegyptius* from Sharkia Governorate, Egypt. For light microscopy, eleven specimens (five males and seven females) were fixed in 70% ethanol, dehydrated and cleared in lactophenol. Specimens were measured with the aid of camera lucida. For scanning electron microscopy, two males and four females were fixed in cold (4°C) 2.5% glutaraldehyde (in 0.1M sodium cacodylate buffer) at pH 7.2 for 3hr, rinsed repeatedly in 0.1M sodium cacodylate buffer at pH 7.2 and 4°C, post fixed in cold (4°C) 1% osmium tetroxide in the same buffer for 1hr, dehydrated in ascending grades of ethanol and critical-point-dried. Dried specimens were coated with gold and examined with Jeol JSM-5200 scanning electron microscope. Measurements are in millimeters unless otherwise stated.

3. Results

Description: *Porrorchis indicus* (Das, 1957) Schmidt and Kuntz, 1967. (Fig. 1-18).

Adult worms have large size and pale yellow to white in colour with clear sexual dimorphism. Trunk cylindrical, elongate, thin walled, and slightly swollen near the anterior end in both sexes (Fig. 1). Mature females larger than males. Proboscis ovoid, slightly swollen (Figs. 2, 3) with 20-24 vertical rows

each of 8-9 hooks (Figs. 2, 3, 7, 8). Anterior hooks are posteriorly directed and well-developed with posteriorly enlarged simple roots that are much longer than the blades (Fig. 3). Apical hooks somewhat longer and thiner than middle hooks (Figs. 8, 9, 10); third and fourth hooks of each row are the largest and strongest (Figs. 8, 10); basal three or four hooks of each row comparatively smaller than the apical and middle hooks, spiniform and rootless (Figs. 3, 8, 11). Apical and middle hooks surrounded at their bases by a cub-like theca of proboscis tegument (Figs. 9, 10), while the basal hooks are embedded basally in longer cuticular cones (Fig. 11). Scanning electron microscopy of proboscis hooks revealed the presence of surface longitudinal striations that run parallel from the base to the tip (Figs. 12, 13). Neck well developed, robust and shorter than proboscis (Figs. 2, 3, 7). Proboscis receptacle cylindrical, double walled and inserted at base of proboscis (Fig. 2). Lemnisci tubular, narrow, much longer than proboscis receptacle (Fig. 1). Testes oval, contiguous, tandem within anterior third of trunk, behind the anterior swollen part (Fig.4). Cement glands four, tubular and elongate. Membranous spoon shaped bursa with dilated lacunar canals (Figs. 5, 14, 15). Female posterior end swollen with tapered end (Figs. 6, 16). Uterus long, cylindrical, widening posteriorly (Fig. 6). Gonopore subterminal, slit-like with raised orifice (Figs. 16, 17). Eggs elliptical with a corrugated surface (Fig. 18).

Male (measurements based on four specimens): Trunk 28-35 (31.5) long, 1.2-1.4 (1.3) wide. Proboscis 0.321-0.473 (0.397) long by 0.287-0.432 (0.359) wide. Anterior proboscis hooks 0.038- 0.046 (0.042) long by 0.009-0.013 (0.011) wide at base of blade; middle hooks 0.035-0.044 (0.039) long by 0.013- 0.017 (0.015) wide at base; basal hooks 0.023- 0.029 (0.026) long by 0.006-0.009 (0.007) wide at base. Neck 0.121- 0.173 (0.147) long by 0.267-0.301 (0.284) wide. Proboscis receptacle 1.041-1.153 (1.097) long by 0.286- 0.341 (0.313) wide. Lemnisci 1.935- 2.167 (2.051) long by 0.113-0.146 (0.129) wide. Anterior testis 0.707- 0.866 (0.786) long by 0.274-0.328 (0.301) wide; posterior testis 0.686- 0.746 (0.716) long by 0.269-0.314 (0.291) wide. Bursa 1.372-1.483 (1.427) long by 0.493-0.613 (0.553) wide.

Female (measurements based on seven gravid specimens): Trunk 42-48 (45) long by 1.4-1.8 (1.6) wide. Proboscis 0.354-0.491 (0.422) long by 0.324-0.458 (0.391) wide. Anterior proboscis hooks 0.042-0.051 (0.046) long by 0.008-0.013 (0.011) wide at base of blade; middle hooks 0.037-0.045 (0.041) long by 0.014-0.017 (0.015) wide at base; basal hooks 0.025- 0.028 (0.026) long by 0.006-0.008 (0.007) wide at base. Neck 0.132-0.174 (0.153) long by 0.298- 0.326 (0.312) wide. Proboscis receptacle 1.086-1.195 (1.141) long by 0.256-0.321 (0.289) wide. Lemnisci 2.145-2.382 (2.263) long by 0.076-0.096 (0.086) wide. Eggs 0.031-0.053 (0.042) long by 0.014-0.022 (0.018) wide.

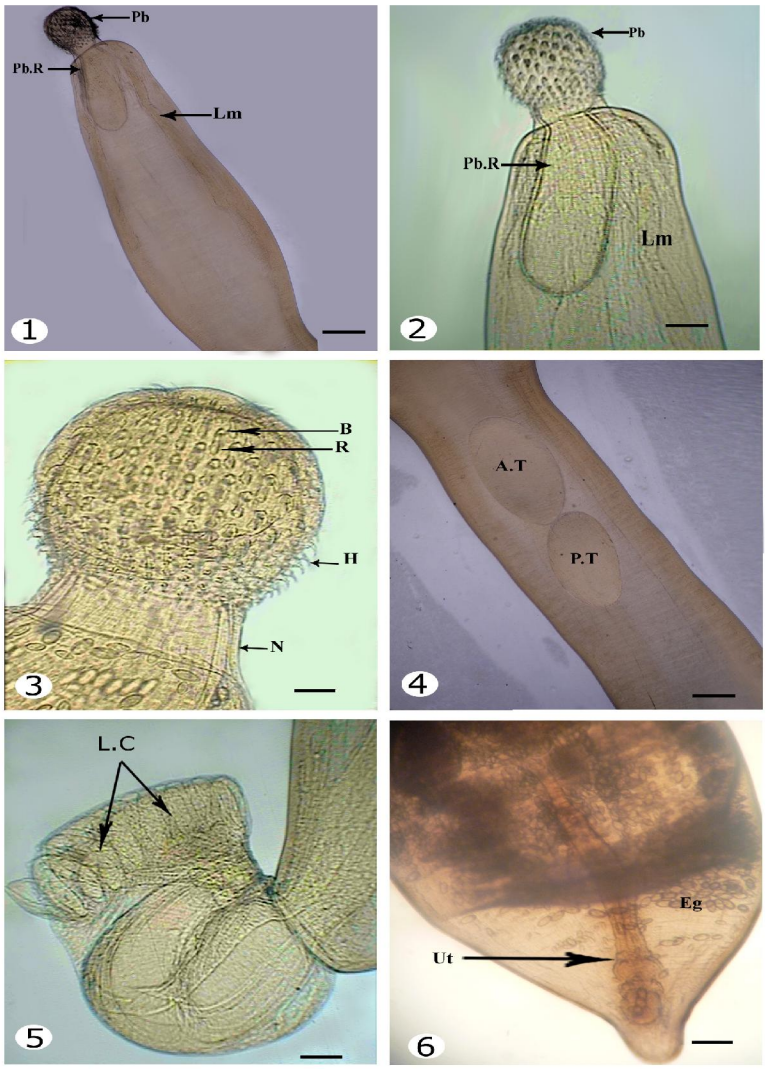
4. Discussion

The genus *Porrorchis* currently includes 17 species (Amin, 1985; Golvan, 1994; Salgado-Maldonado and Cruz- Reyes, 2002; Amin *et al*., 2008) distributed throughout southeast Asia, India, the Philippines, Australia, Madagascar, Africa and southeastern Mexico. Most species are parasites in birds, although four species, *P.chauhani, P.crocidurai* (Gupta and Fatma, 1985), *P.hydromuris* (Edmonds, 1957) and *P.nickoli* (Salgado-Maldonado and Cruz-Reyes, 2002) have been recorded in mammals. Two additional species, *P.hylae* (Johnston, 1914) and *P.leibyi* (Schmidt and Kuntz, 1967) have been reported as incidental in mammals.

Porrorchis indicus was reported for the first time in the predatory birds from India by Das (1957), who listed it under the synonym *Pseudoporrorchis indicus*. Later, this parasite was reidentified to *Porrorchis indicus* by Schmidt and Kunts (1967). It was recorded in Egypt by Ashmawy and El- Sokkary (1991) from the Egyptian cuculus *Centropus senegalensis aegyptius*, they studied the histopathological effects of this parasite on the gut wall of the infected birds.

In the present study, *P. indicus* is described by using scanning electron microscope for the first time. The description of this species agrees with the characteristics of *P. nickoli* given by Salgado-Maldonado and Cruz-Reyes (2002), and *P. tyto* reported by Amin *et al*. (2008) in (1) the presence of anterior swelling of the cylindrical trunk in both sexes, and ovoid proboscis provided with vertical rows of hooks, (2) the first four hooks in each row are strong, with well-developed simple roots and the remaining hooks are spiniform and rootless, (3) the double-walled proboscis receptacle inserted at the base of the proboscis. (4) Posterior swelling expanded to Fan-shaped in female worms of *P. tyto*. Other characteristics include tandem, oval testes and long, tubular cement glands. However, *P. indicus* can be distinguished from *P. nickoli* and *P. tyto* by the number of rows on the proboscis where *P. indicus* has 20-24 rows of 8 or 9 hooks per each, while *P. nickoli* has 22-24 rows of 7or 8 hooks per row and *P. tyto* has 25-29 rows each having 10-11 hooks, and the lemnisci are flat, tongue-shaped in *P.nickoli*, while in *P. tyto* the lemnisci equal, stout, may extend to level of anterior testis, but in *P. indicus* the lemnisci are tubular and narrow. *P. indicus* can also

be differentiated from *P. nickoli* by the position of the male reproductive system, which occupies the anterior third of the trunk, behind the anterior



Figs. 1- 6. Light photomicrographs of *Porrorchis indicus*.

Fig. 1. Anterior swollen part of the trunk showing the proboscis (Pb), lemnisci (Lm) and proboscis receptacle (PbR). Scale bar = 400 µm.

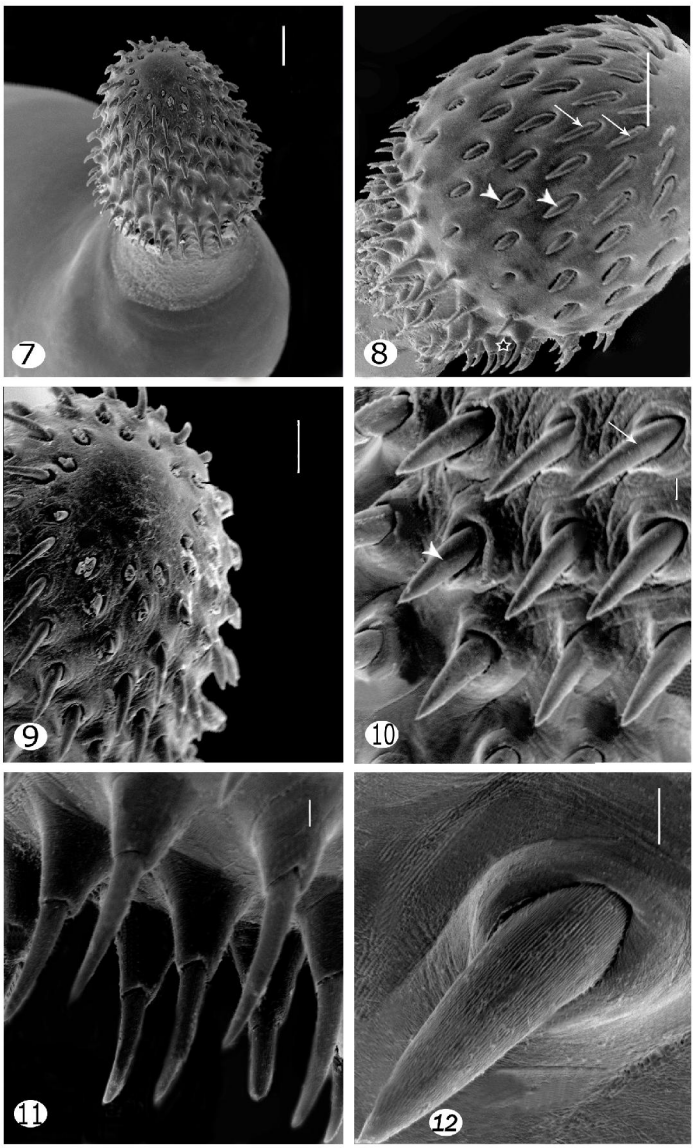
Fig. 2. Anterior part of the body. Pb the ovoid proboscis, Lm lemnisci; PbR proboscis receptacle. Scale bar = 165 µm.

Fig. 3. Anterior part of the body showing the proboscis hooks and the neck (N). B blade of the hook; R root of the hook. Scale bar= 40 µm.

Fig. 4. Anterior third of the trunk showing the anterior testis (AT) and the posterior testis (PT). Scale bar = 400 µm.

Fig. 5. Posterior part of the male showing the bursa. LC lacunar canals. Scale bar = 40 µm.

Fig. 6. Posterior part of the female showing the uterus (Ut), eggs (Eg) and the tapered end. Scale bar = 40 µm.



Figs. 7-12. SEM micrographs of *Porrorchis indicus.*

Fig. 7. Anterior part of the body showing the ovoid proboscis and the neck. Scale bar = 100 µm.

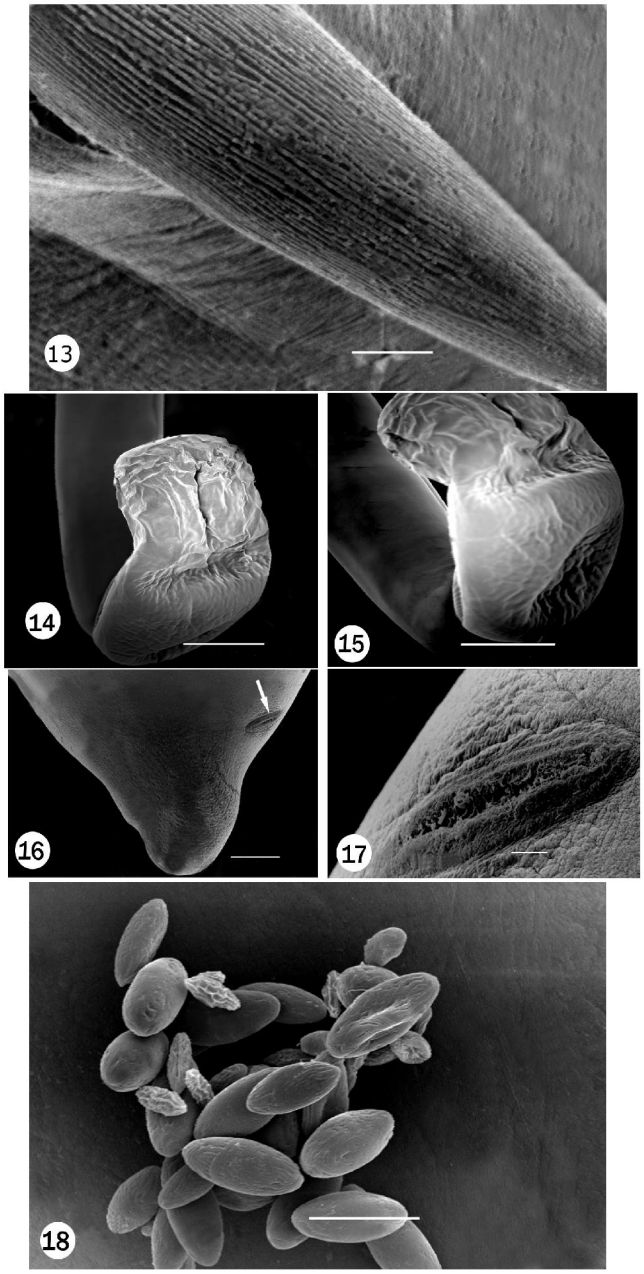
Fig. 8. Proboscis showing the apical hooks (arrow), middle hooks (arrowhead) and basal hooks (star) of the proboscis. Scale bar = 100 µm.

Fig. 9. Top view of the proboscis showing apical hooks. Scale bar = 50 µm.

Fig. 10. Lateral view of proboscis shows apical hooks (arrow) and the middle hooks (arrowhead). Scale bar = 10 µm.

Fig. 11. Basal hooks of the proboscis. Scale bar = 10 µm.

Fig. 12. An enlargement of one middle proboscis hook showing that the surface striations, run parallel toward the tip of the hook. Scale bar = 10 µm .



Figs. 13-18. SEM micrographs of *Porrorchis indicus*.
Fig. 13. High magnification of proboscis hook showing parallel striations. Scale bar = 5 µm .
Figs. 14, 15. Posterior part of male showing the spoon-like bursa. Scale bare = 500 µm.
Fig. 16. Posterior part of female showing the subterminal gonopore and the tapered end. Scale bar = 100 µm.
Fig. 17. The elevated orifice and slit-like female gonopore. Scale bar = 10 µm.
Fig. 18. Eggs released from the body cavity of a female showing elliptical shape and corrugated surface. Scale bar = 50 µm.

swollen part in *P. indicus*, while in case of *P.nickoli* it occupies the posterior half of the trunk, and in *P. tyto* the testis pre-quatorial, close together and to proboscis receptacle.

The acanthocephalan proboscis hooks are taxonomic relevant structures and the information pertaining to their development and morphology is rather limited. The anatomical and cellular origin of the hooks has been a matter of controversy; Van Cleave and Bullock (1950) stated that hooks are wholly noncellular in origin. Later, the dermal origin or secretion of these structures was reinforced by Hyman (1951). However, the basement membrane was suggested as origin of hooks for *Polymorphus minutus* (Crompton and Lee, 1965). With regard to *Acanthocephalus ranae*, Hammond (1967) reported that the connective tissue layer is the site of origin for hooks. Morphogenesis of the proboscis hooks of *Moniliformis moniliformis* by light and electron microscopy established that hooks have their origin from muscle tissue and that they are of cellular nature (Hutton and oetinger, 1980), with muscular origin supporting the function of retraction or extrusion or both (Taraschewski, 2000).

In a functional context, many aspects of Acanthocephala morphology are interpreted as structural adaptations that favor effective attachment to the host digestive tract wall (Van Cleave, 1952). The proboscis hooks are thought to be primary attachment structures (Aznar, *et al*., 1999). Taraschewski (2000) divided the acanthocephalan species into three categories based on proboscis activity and depth of penetration within the host tissue.

In the present work, SEM of *P. indicus* revealed the presence of surface striations on the proboscis hooks, as a characteristic feature of this species, which contrast with the smooth hooks of most acanthocephalan species. This finding represents the third report of striations in acanthocephalan proboscis hooks. Surface striations of proboscis hooks have been previously reported in two acanthocephalan species, *Dentitruncus truttae* (Dezfuli *et al*., 2008) and *Rhadinorhynchus ornatus* (Amin *et al*., 2009). Dezfuli *et al*., (2008) speculated that such striations may provide more effective attachment to the host intestinal wall.

The well-defined, slit-like female gonopore, the raised female genital orifice, the corrugated surface of eggs, and spoon-like male gonopore are reported in *P. indicus* for the first time by SEM. No reference to any of those features was found in other acanthocephalans except in *Rhadinorhynchus ornatus* (Amin *et al*., 2009).

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