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Aporchis massiliensis (Digenea: Echinostomatidae) from *Larus leucophthalmus* (Aves: Laridae) from the Red Sea, Egypt, with Scanning Electron Microscopy of the Tegumental Surface

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Abstarct: *Aporchis massiliensis* Timon-David,1955 (Echinostomatidae) is recorded for the first time from the intestine of *Larus leucophthalmus* Temminck, 1825 (white-eyed gull) (new host record) in Giftun island at the Egyptian Red Sea coast (new geographical locality). The marginal collar spines are small and tapered in a single row of 46 spines, in addition to 10-12 large, blunt and irregularly distributed angle spines. The study of the surface topography using scanning electron microscopy revealed the presence of small dome-shaped papillae on both sides of the oral sucker, collar and ventral sucker. Scale-like spines are arranged on the dorsal and ventral tegumental surface in alternative rows, being densely packed anteriorly and sparsely distributed towards the posterior end. The ventro-and dorsomedian spines are deeply embedded in the tegument, with their edges exposed to the surface. The tegument at the ventral depression between the oral and ventral suckers and the posterior extremity was devoid of spines. The present study extends our knowledge of echinostomes by revealing the surface structure of *A. massiliensis*.

Key words: White-eyed gull · *Larus leucophthalmus* · *Aporchis massiliensis* · Echinostomatidae · SEM · Red Sea · Egypt

INTRODUCTION

Aporchis massiliensis Timon-David, 1955 is a large slender echinostome with a characteristic serrate lateral body margin and elongate eggs with long polar filament. This parasites may cause severe epigastric or abdominal pain accompanied by diarrhea, easy fatigue and malnutrition for its hosts [1]. It was recorded in the intestine of many species of gulls such as ; Lesser black-backed gull, *Larus fuscus* in Great Britain [2]; Herring gull in southeast France [3]; Audouin's gull, *Larus audouinii* [3]; Yellow-legged gull, *Larus cachinnans* [4] and Mediterranean gull, *Ichthyaetus melanocephalus* [5].

Larus leucophthalmus Temminck, 1825 the white-eyed gull is endemic in the Red Sea, where about 30% of the world population nests lie on the islands, at the mouth of the Gulf of Suez; preferring open ground near the shore [6,7]. This species is mostly sedentary [8,9], although it disperses from its breeding sites to occur throughout the Red Sea during the non-breeding season [10]. The species is mainly coastal. feeding aquatic organisms [11], but some Egyptian populations have adopted a scavenging role at rubbish tips and harbors [12]. The diet consists largely of fish, but also includes crustaceans, molluscs, annelids and offal [9,11].

To the best of our knowledge, no records for echinostome infection have been detected in *L. leucophthalmus*, the present study was performed to examined the trematode infection in this avian host.

Since the size, shape, number and distribution of collar and tegumental spines and sensory papillae have been used for species differentiation [13] and these structures were not described before for *A. massiliensis*, the present study used SEM to reveal the detailed structure and distribution of tegumental spines and papillae of *A. massiliensis*.

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MATERIALS AND METHODS

Three white-eyed gulls were examined in October, 2011 during a shore trip near the big Giftun island,10 miles north of Hurghada, Red Sea, Egypt (27°15´28½ N, 33°48´42½ E). The entire intestine from the esophagus till the rectum was examined for trematode infection. Worms were collected in 0.75% normal saline, vigorously shaken for relaxation then pressed between two glass slides. Specimens were fixed in 70% ethyl alcohol, stained in aceto-alum carmine, cleared in clove oil and mounted in Canada balsam. Mounted specimens were examined with Olympus CX31 trioccular microscope and imaged with an E-330 DC digital camera. The number of worms in the infection sites was recorded. Identification of the specimens followed Bray *et al.* [14].

For scanning electron microscopy, standard method was followed to prepare the specimens [15] which included critical point drying and mounting on SEM sample mounts using conductive double sided carbon tape. Samples were gold coated, examined with a JOELJXA-840A electron microscope and imaged at 30KV.

RESULTS

Examination of the entire intestine of two male and one female white-eyed gull revealed the presence of one *echinostome species*; *A. massiliensis.* Worms were distributed throughout the entire ileum at a mean intensity of 24.5±8.5. The present study is the first record of this echinostome from the white-eyed gull, *L. leucophthalmus* and a new geographic locality of this *echinostome species.*

Description (based on 15 carmine stained mature specimens) (Fig. 1)

Body is very long, slender and filamentous, measuring 22 ± 4.5 X 0.3±0.1 mm at the level of anterior body. The maximum width of the pseudosegmented body is attended above the level of the ovary (0.8 ± 0.3 mm). Head coller notches are at the ventral side. Collar spines are arranged in a single marginal row of 46 small tapered spines (0.014±0.002 X 0.006mm) and 10-12 large, blunt irregularly distributed angle spines (0.029±0.004 X 0.015±0.001 mm). Pharynx is globular (0.24±0.03X0.19 mm). Intestinal furcation is preacetabular, the ceca extend to the posterior extremity. Testes are subequal, tandem, ovoid and close together near the posterior extremity. Anterior testis measures 0.63±0.02X0.25mm, while the posterior measures 0.72±0.02 X 0.41mm. Cirrus is slender, sinuous and enclosed within a cirrus sac that extends posteriorly behind the ventral sucker and encloses cylindrical kidney-shaped seminal vesicle (0.41±0.01 X 0.15mm). Ovary is spherical, submedian, close to the anterior testis and measures 0.18±0.003 mm in diameter. Mehlis' gland is avoid and larger than the ovary. Vetellaria are follicular extending in two vertical rows on both sides of uterus, overlapping ceaca, restricted middle and ending at the level of ovary. Vetellarian field is 28-32% of total body length. Uterus is interceacal, long, spirally coiled and extends anteriorly without descending arms. Vagina opens in the genital opening. Genital opening is preacetabular, immediately behind the intestinal fucation. Excretory pore is dorsal and subterminal at the posterior extremity, excretory vesicle is Y- shaped and immediately bifurcates at the end of posterior testis. Eggs are operculated (0.16 X0.05mm) and provided with a filament at one pole. Bundles of longitudinal muscle fibers are easily distinguished in the body wall extending from the level of the cirrus till the end of the body.

Scanning electron micrographs show that the tegumental surface is transversely ridged with serrate appearance medially and posteriorly (Figs.10 and 14). The oral and ventral suckers are close to each other. The oral sucker is subterminally situated at the anteroventral side of the body (Fig.4). The surface of the ventral sucker carries numerous, randomly distributed, small, dome-shaped papillae (Fig. 3). Collar spines are partially embedded in the tegument, with their distal end free above the tegument (Figs. 2, 7). Tegument of the oral sucker and the longitudinal ventral depression between oral and ventral sucker is spineless (Fig. 4). Several small dome-shaped papillae are observed on both side of oral sucker and collar surface (Fig. 2). The tegumental spines at the anterolateral (Figs. 4, 5) and anterodorsal (Figs.7, 8) surfaces are scale-like with curved edges, being slightly tapered at the dorsal surface. The spines on both surfaces are arranged in alternative rows and densely packed at the level of the ventral sucker (Fig.5). Immediately posterior to the ventral sucker, the spines become sparsely distributed (Fig. 6). Tegumental spines behind the fore body are more wide than the anterior ones and partially embedded in the tegument (Fig. 9). The tegumental surface of the ventro- and dorsomedian regions forms transverse serrate ridges that carry alternative rows of spade-shaped spines (Figs. 10, 11, 13). The spines are completely covered by the tegument (Fig.12). The tegumental ridges diverge towards the posterior end (Fig.14) with only traces of plate-like spines (Fig.15). The tegument at the posterior extremity is devoid of spines (Figs.14, 16).

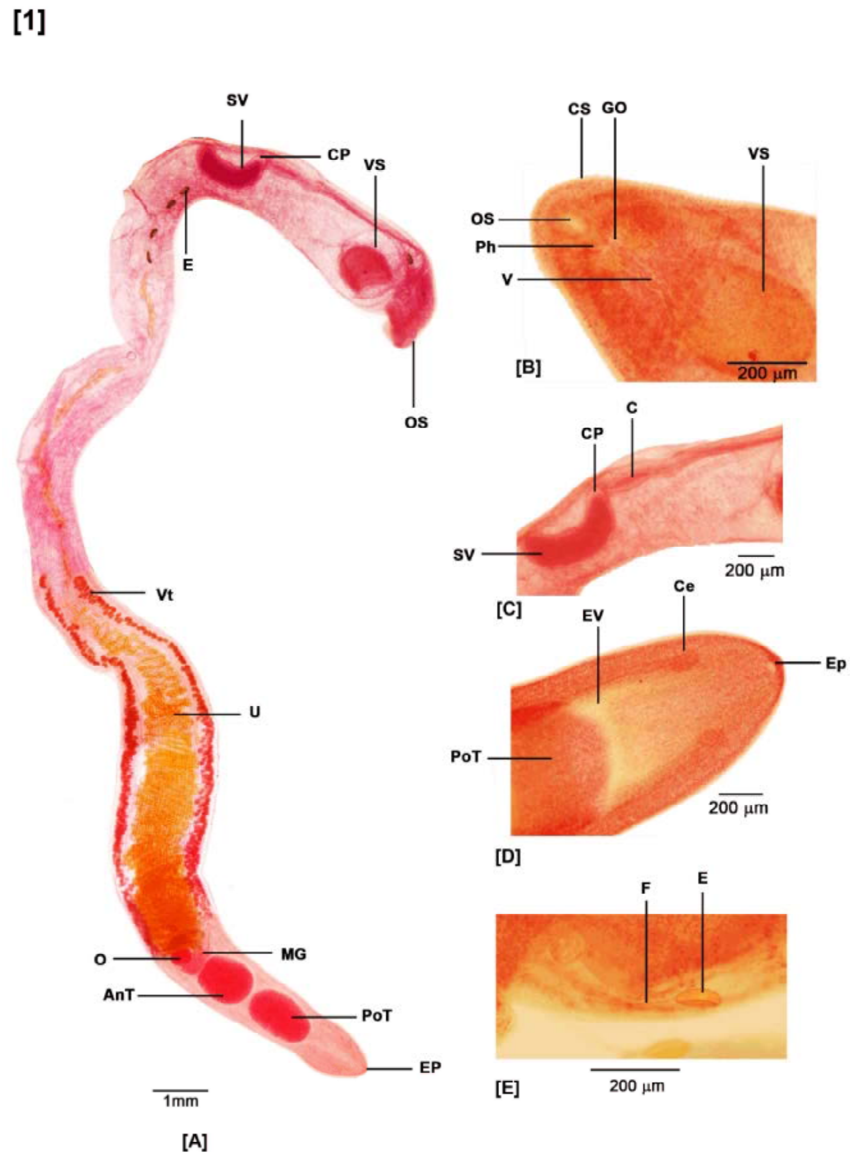


Fig. 1: Carmine stained preparation of *Aporchis massiliensis*

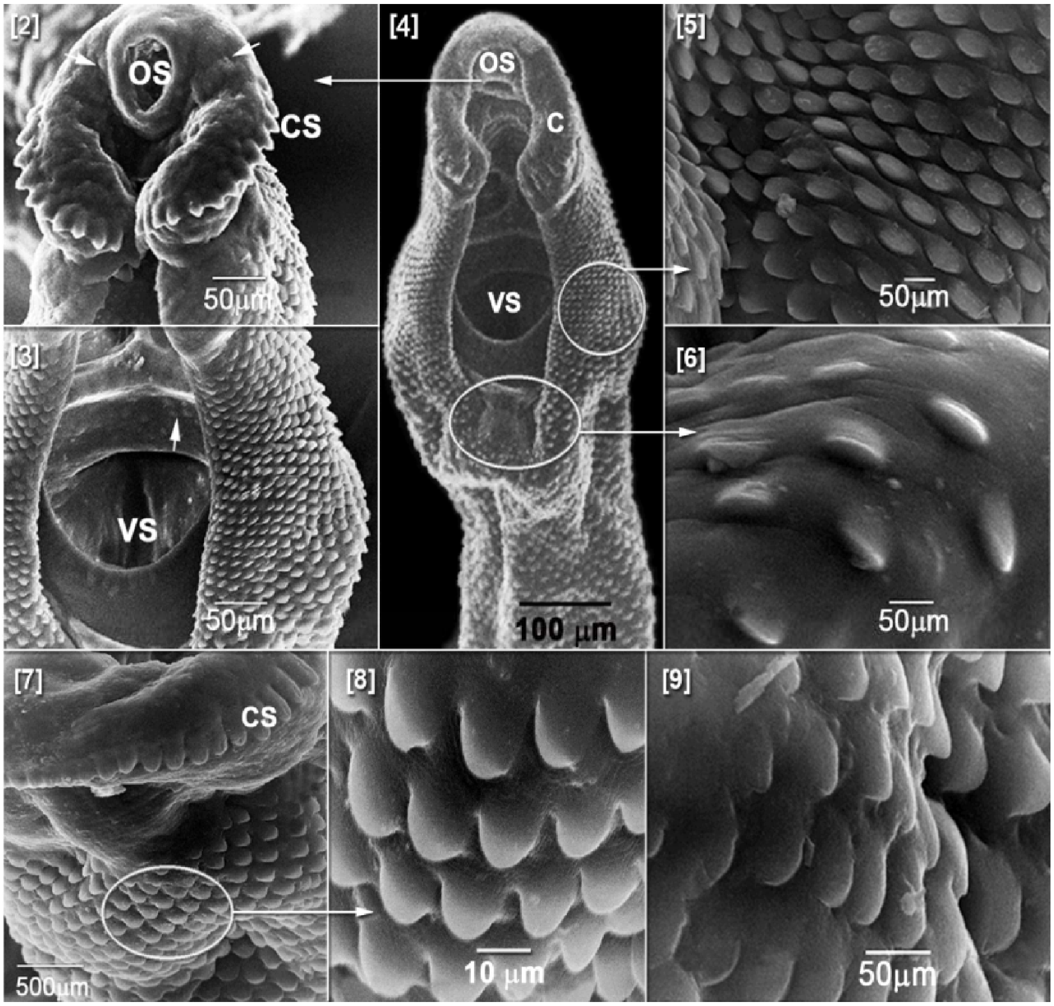
A. Whole mount of the fluke shows the long filamentous body with short forebody. Subterminal oral sucker (OS) close to the ventral sucker (VS). Testes avoid, tandem and near the posterior end, posterior testis (PoT) larger than anterior testis (AnT). Cirrus (C) slender and sinuous. Cirrus pouch (CP) encloses cylindrical, kidney - shaped seminal vesicle (SV), Ovary (O) avoid and close to anterior testis. Vetellaria (Vt) overlap ceaca, restricted middle and end at the level of the ovary. Mehlis' gland (MG) avoid and larger than the ovary. Uterus (U) very long, spirally coiled and encloses large number of mature eggs (E).

B. High magnification of the forebody shows oral sucker (OS) surrounded by collar spines (CS). Globular pharynx (Ph). Vagina (V) opens by genital opening (GO) postfucal and anterior to the ventral sucker (VS).

C. High magnification shows the cylinder and sinuous cirrus (C). Cirrus pouch (PC) encloses cylindrical and kidney-shaped seminal vesicle (SV).

D. High magnification of the posterior extremity shows Y-shape excretory vesicle (EV), just behind the posterior testis (PoT) and opens at excretory pore (EP). Note the extending of the intestinal ceaca (Ce) almost until the end of the body.

E. High magnification shows the operculated eggs (E) with long filament (F).



Figs. 2-16: Scanning electron micrographs of *Aporchis massiliensis*

Fig. 2: Oral sucker (OS) surrounded with collar spines(CS) that are partially covered by the tegument. Note the presence of small dome-shaped papillae on both sides of the oral sucker (arrows).

Fig. 3: Ventral sucker showing the surface with numerous, randomly distributed, small dome-shaped papillae (arrows).

Fig. 4: Ventral view of the forebody showing distribution of the tegumental spines, an oral sucker (OS), collar (c) and ventral sucker (VS). Note that the tegument of the oral sucker and the longitudinal ventral depression between oral and ventral sucker lacks spines.

Fig. 5: Tegument at the anterolateral regions showing scale-like spines with curved edges.

Fig. 6: Tegument immediately posterior to the ventral sucker showing few and sparsely distributed scale-like spines.

Fig.7: Dorsal view of the anterior extremity showing alternative rows of scale-like slightly tapered tegumental spines. Note that the collar spines (CS) are partially covered by the tegument, with the distal end of the spines free from the tegument.

Fig. 8:: Tegument at the anterodorsal regions showing scale-like spines with curved and slightly tapered edges.

Fig. 9: Tegument behind the fore body showing wide and partially embedded spines.

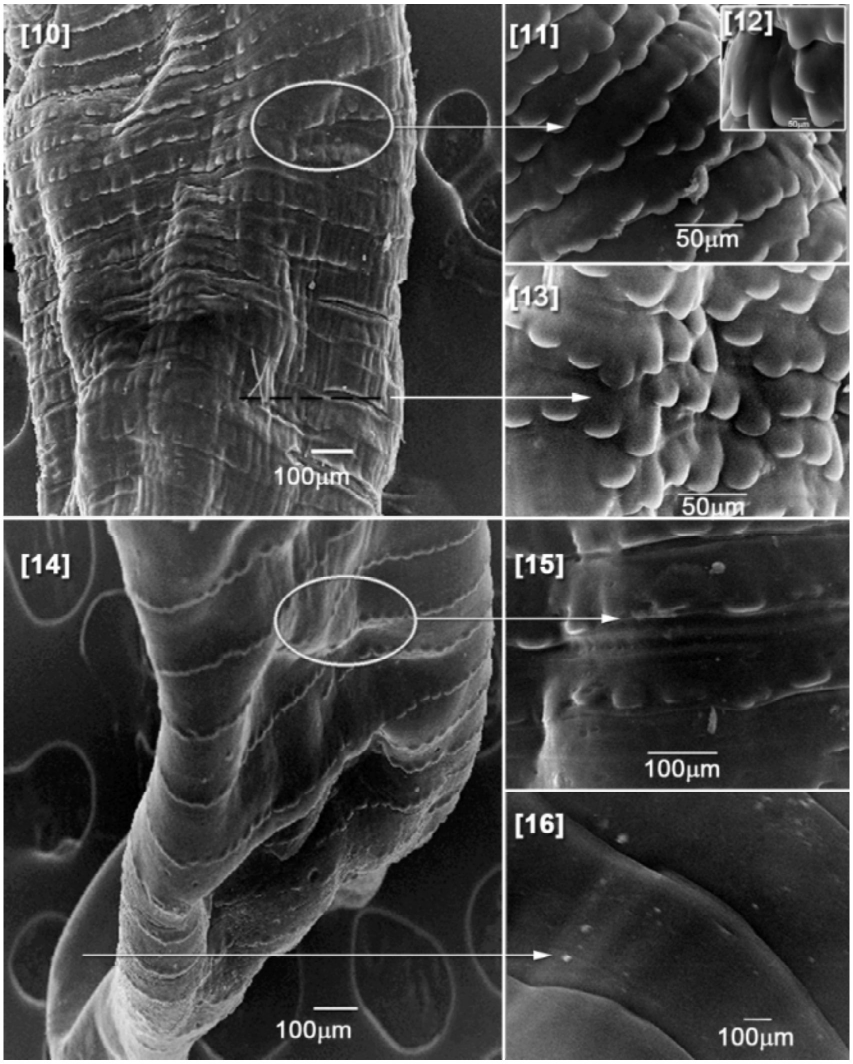


Fig. 10: Tegument in median region with transverse pseudoserrate ridges that carries alternative rows of spade-shaped spines.
Fig. 11: Tegument in ventro-median region with alternative rows of spade-shaped spines.
Fig. 12: Tegumental spines in ventro- and dorso median regions showing that the spines are deeply embedded in the tegument and completely covered with the tegument.
Fig. 13: Tegument in dorso-median region showing alternative rows of spade-shaped spines.
Fig. 14: Tegument in the posterior region with diverged transverse pseudoserrate ridges.
Fig. 15: Tegument of both dorsal and ventral posterior regions showing traces of plate-like spines.
Fig. 16: Posterior extremity showing that the tegument lacks spines.

DISCUSSION

Gulls were recorded as hosts of different species of echinostomes [3-5,16-19]. *A. massiliensis* has been previously recorded in five gull species; Herring gull *"Larus argentatus"* from southeast France [20], Lesser black-backed gull "*larus fuscus*" [2], Audouinii's gull *"Larus audouinii"* from Chafarinas islands [3], yellow-legged gul *"Larus cachinnans"* from Medes islands [4] and Mediterranean gull "*Ichthyaetus melanocephalus*" from southern Italy [5]. The geographical distribution of all previous records were restricted to the western Mediterranean area.

In the Egyptian Red Sea coastline, three species of gulls have been recorded; Pallas's gull "*larus ichthyaetus*" [9]; Sooty Gull *"Larus hemprichii"* [21]; and white-eyed gull *"Larus leucophthalmus*" [12,21]. The white-eyed gull is one of the global conservation concern and the most important in the Red Sea Egyptian islands as this area holds the largest breeding population known in the world [22]. Jennings *et al*. [6] estimated that 30% of the world's population of this species breeds on these islands. The population of this bird feeds on mollusks (Gastropoda and Bivalvia), crabs and fishes that the coast offers throughout the year [8], in addition to colonies of the algae *Cystoseria* [23] which has been reported to host encysted cercaria of *A. massiliensis* [24].

The present study records the white-eyed gull, *L. leucophthalmus* as a new host record for *A. massiliensis* and Giftun islands in the Red Sea, Egypt as new geographical locality for this echinostome. High intensity of infection with the reported echinostome in both male and female examined hosts may be explained on the bases that these birds have high prey diversity.

Many significant characteristics of echinostomes have been considered in identification of the species understudy. The most considered were the number, shape, arrangement and relative size of the collar spines; position of testes and ovary; position and structure of the cirrus; structure of internal seminal vesicle; size and shape of body; length of fore body; uterine and postesticular fields and nature of tegumental armature.

Among the six species of the genus *Aporchis* Stossich, 1905; *A. massiliensis* is closely similar to *A. continuus* Mc Cauley and Pratt,1960. The two species differ in that the angle spines of *A. massiliensis* are shorter and irregularly arranged and the eggs are bigger and has longer filament. These structures were also considered in identification.

To the best of our knowledge, no studies dealt with the surface topography of *A. massiliensis*. SEM confirmed that the tegumental surface structure of *A. massiliensis* is generally similar to those previously reported in different species of the family Echinostomatidae [13, 15 -29] in which the tegument of the anterior region of the body was densely covered with scale-like spines and the spines become sparse toward the posterior part of the body.

The shape and distribution of tegumental spines of echinostomes are associated with the fluke maturation, parasitic niche and whether they migrate or not [30]. Han *et al.* [29] noted that the distribution pattern of tegumental spines may aid in the locomotion of the worms in the intervillous space of the definitive host.

SEM observations revealed the partially embedding of the colar and ventro and dorsomedian body spines, where the sclerotized spines could be easily differentiated from the surrounding tegumental tissue by their markedly different refractivity. This finding agrees with the report of Zamparo *et al.* [31] on the echinostome *Petasiger combesi*. Additionally, the absence of tegumental spines in the ventral depression between the oral and ventral suckers in the species understudy is supported by the previous reports of Lee *et al*. [15], Køie [32], Fried *et al*. [33] and Han *et al*. [29].

SEM revealed the presence of small dome-shaped papillae on both sides of the oral sucker and the rim of the ventral sucker, such structures were not described before in this species. The functions of these papillae was suggested to vary according their shape and position on the body. Oral papillae might be tango- and chemoreceptors [34] or tactile sensory receptors [30].

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