FULL LENGTH ARTICLE

Digenean trematodes from siganid fish

*Amphacanthus sigan* at Macady Bay, Southern Hurghada, Red Sea, Egypt

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KEYWORDS

Digenetic trematodes; *Helicometra*; *Gyliauchen*; Siganid fish; Red Sea; Egypt

Abstract  

Forty fish samples of *Amphacanthus sigan* were collected from Sharm El-Naga southern Hurghada to study the parasitic infection included and present a new individual to *Helicometra* species using light microscope and re-describe another digenean trematode species using scanning electron microscope (SEM). Two trematode species were identified and described; *Helicometra siganus* n. sp. Nov belonging to *Helicometra Odhner, 1902* (family Opecoelidae Ozaki, 1925) which is different from all the listed species in many features as having an elongated body, tapering anteriorly and larger sucker ratio; the vitellaria extended from the intestinal bifurcation to the posterior extremity of the hind-body and finally the cirrus sac and cirrus longer than the other species and extended to the midpoint of esophagus level. So, these criteria are specific and sufficient to consider it as a new species. The other species, *Gyliauchen volubilis Nagaty, 1956* (Gyliauchenidae Fuki, 1929 and Ozaki, 1933) which was described before but some other constituent are not evident and appeared using the electron microscope. However, the present sample illustrated transverse striations on the body surface, the acetabulum evaginates outward, ovoid shaped and surrounded by lip-like structures and finally, the excretory pore is situated at some distance from the acetabulum at the posterior end extremity.

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Introduction

Parasitic helminths of the fishes are one of the least known parts of the Red Sea fauna (Hassanine and Gibson, 2005). However, study of fish parasitology is one of the more interesting branches for many researchers all over the world especially for the marine fishes for describing the new taxa (Al-Jahdali, 2010) either for the internal or external parasites. The species of the Opecoelid genus *Helicometra Odhner, 1902* (family Opecoelidae) are the most widespread and frequent members having many different species especially in the marine fishes of the shallow and deep-water. Sekerak and Arai (1974) reviewed the genus *Helicometra* and divided the valid species into three groups as follows: Group (a); includes species with a short cirrus sac and vitellaria follicles extending
anteriorly to the acetabular zone or the fore body; group (b); includes species with a short cirrus sac and vitellaria follicles confined to the hind body; group (c); includes species with a short fore-body and long cirrus sac extending to the hind body.

Stosscich (1903) described new species from subfamily Plagioporiinae, *Helicometra flavia* from the intestine of *Centropristis hepatus* in Italy. Linton (1910) described two new species, *H. torta* from *Epinephelus striatus* and *H. execta* from *Chloichthys bifasciatus* in Florida. Nicoll (1910) synonymized *H. fasciatus* (Rudolphi, 1819) Odhner, 1902 with *H. pulchella*. Isaichikov (1928) described another new species *H. plovorini* from intestine of *Lycoles agnostus* and *Aspidophoroides alriki* in Russia and transferred *Allocreadium labri* (Stosscich, 1886) Odhner, 1901 to genus *Helicometra* as *H. labri* (Stosscich, 1886) Isaichikov, 1928. Manter (1933) synonymized *H. labri* (Stosscich, 1886) Isaichikov, 1928 with *H. pulchella* (Rudolphi, 1819) Odhner, 1902. Ramadan (1986) described *Helicometra* Odhner, 1902 as *H. epinepheli* Yamaguti, 1934 from *Balistes aculeatus* and *Anampses caeruleopunctatus* as a new species from the Red Sea. *H. boseli* was collected from the intestine of *Holocentrus samara* from Hurghada, Red Sea, also Nagaty (1956) described *H. hypoditis* Yamaguti, 1934 from *Serranus (= Epinephelus)* sp. Aken’Ova et al. (2006) described new species named *Helicometra spreanti* in Australian waters. Many other studies on the trematodes of fishes had been carried out including the examination of fishes infested by helminth parasites in the Red Sea (started by Nagaty, 1930 till Ali, 2005) who revealed the existence of more than 89 trematode species were under more than 47 genera from which at least 14 species were new. Many other studies had been done on the Red Sea fish parasites by Hassanine (1995, 2005, 2007). On the other hand, Nagaty (1956) described *Glyphauchen volubilis* from the small intestine of *Amphacanthus sigan*, called “sigan” from Sharm El-Naga in Hurghada. Abdou and Heckmann (2001) studied the fine structure of *G. volubilis* Nagaty, 1956, which has segmented body and is not attached with the outer membrane of the tegument. They illustrated that, its body has two types of vesicles which are scattered in the tegumental matrix. Al-Jahdali (2012) studied the infra-population size or mating group size mostly affects some processes acting within the infra-population of *G. volubilis* Nagaty, 1956 from *Siganus*. Al-Jahdali and Hassanine (2012) studied the life cycle of *G. volubilis* Nagaty, 1956 and pointed out that this species was collected from *Siganus rivulatus* fishes of mangrove swamps at Aqaba Gulf.

The current study is aimed to review the available species included in genus *Helicometra* and presents a description of the collected individual using light microscope and re-describe another digenean trematode species using scanning electron microscope (SEM). These species were collected from marine siganid fish *Amphacanthus sigan* at Macady Bay southern Hurghada.

**Materials and methods**

Forty fish samples were collected from the Sharm El-Naga coast southern Hurghada during summer 2011 and kept alive in aquaria. Fish samples (*Amphacanthus sigan*) were identified

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**Figure 1** *Helicometra siganus* n. sp. To the left (A) and *Glyphauchen volubilis* Nagaty, 1956 (B). O. S., Oral Sucker; Pre-ph., Pre-pharynx; Ph., Pharynx; C., Cirrus; Oes., Oesophagus; In.C., Intestinal Ceaca; V., Vitellaria; G. P., Genital Pore; Ac., Acetabulum; O., Ovary; T., Testis; U.T., Urinary tube.
according to Randall (1983) and Froese and Pauly (2004). Samples were then dissected to demonstrate the intestinal trematodes. Standard parasitological techniques were used to examine the alimentary canal of the fish. Trematodes were removed from their host fishes under a dissecting microscope and observed alive under a compound microscope. Some worms were fixed in alcohol-formalin-acetic acid (AFA) under a slight cover-slip pressure and preserved in 75% ethyl alcohol. Whole-mounts were stained in aceto-acetic alum carmine; some samples were cleared in terpineol and mounted in Canada balsam.

The collected samples of parasitic trematodes were identified according to Yamaguti (1971) and some of the available recent publications (e.g., Al-Jahdali, 2012; Al-Jahdali and Hassanine, 2012; Hassanine, 2007; Hassanine and Al-Jahdali, 2007; Mohamed and Abdel-Latif, 2007). All measurements are in millimeters (mm) except eggs in \( \mu \text{m} \). The collected trematodes were washed in isotonic saline solution for several times and relaxed. Each worm was carefully flattened between a slide and a cover slip, and then they were fixed in formal saline (25 formalin and 75 ml saline solutions) and alcohol–formalin acetic acid (AFA) and preserved in 70% ethyl alcohol (Hassanine, 2005) containing 5% glycerin for preventing dryness (Mohamed, 1998). Some other trematode samples were stained in aceto-acetic acid carmine (Mohamed and Abdel-Latif, 2007) then cleared in clove oil and mounted in DPX. Some other samples were preserved in cold glutaraldehyde to be scanned by the SEM at South Valley University. Reference sample specimens were preserved in the National Institute of Oceanography and Fisheries, Red Sea Branch, Hurghada.

**Results and discussion**

The fish *Amphacanthus sigan* was observed highly infected with several types of parasites with high number of trematodes. The present results are based on description of 17 samples of *Helicometra* (Fig. 1A) and 11 samples of *Gyliauchen volubilis* Nagaty (Figs. 1B and 2–5) as the following description.

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**Figure 2** Photography of *Helicometra siganus* n. sp. (to left) and *Gyliauchen volubilis* Nagaty, 1956 (right).

**Figure 3** Scanning electron microscope illustrated the whole length of the fluke *Gyliauchen volubilis* Nagaty, 1956 to left side and enlarged part of the oral sucker to right.
Sub-Family: Plagioporinae Manter, 1942.
Genus: Helicometra Odhner, 1902 (syn. Laqborchis Lu ¨ he Stossich, 1902)
Species: Helicometra siganus sp. Nov. (Figs. 1A and 2)

The body is elongated, tapering anteriorly, aspinose and rounded posteriorly, its length is ranging between 1.59–1.65 mm by 0.44–0.61 mm width (Fig. 1A). The oral sucker is small and rounded in shape and located at the anterior terminal, with ventral aperture. The oral sucker diameter is varied between 0.11 mm and 0.13 mm at maximum. The acetabulum is rounded to oval in shape, located at the end of anterior half of the body, measuring 0.25 mm length by 0.31 mm width. The pre-pharynx is not distinguished and a muscular well developed pharynx was observed and measuring 0.05 × 0.08 mm. The esophagus is cylindrical not so long, measuring 0.11 mm length. It bifurcates in the middle of the anterior half of the body into two long and cylindrical intestinal caeca, running blindly to the posterior extremity of the body. The testes are paired, diagonal, with irregular shape and located in the posterior half near the end of the body. The anterior testis measures 0.27 mm length by 0.11 mm width, while the posterior testis measures 0.165 mm length by 0.32 mm width. The ovary is irregular in shape, located pre-testicular mass and measures 0.25 mm length by 0.18 mm width. Cirrus pouch is less developed, while the cirrus is well developed, located between the ventral sucker and intestinal bifurcation, opening before the intestinal bifurcation and measures 0.27 mm length. The vitellaria are small follicules and numerous in numbers, running in two parallel rows along the sides of the body, from the level before the intestinal bifurcation to the posterior extremity of the body. The uterus is convoluted and extending from pre-ovarian to the level of the acetabulum, containing eggs. Eggs are rounded, embryonated, yellow in color, very large in size with a polar filament and measure 0.065 mm length by 0.041 mm width. The excretory vesicles are short tubular with I-shaped and open at the extremity posterior end of the body. There are many different species described and included within the genus Helicometra (Odhner, 1902), where most of the collected and described species are listed in Tables 1 and 2 and collected from marine fishes. Reversat et al. (1989) showed that the genus Helicometra consists neither a complex of strictly specific species nor a single very polymorphous species with broad specificity. Indeed, the existence of at least three species is shown with different degrees of specificity for their final hosts of fishes (teleosts).

The present species is closely related to Helicometra (Odhner, 1902) due to the general characters and morphology which related to most of the illustrated species.
To the level of acetabulum and ovary. Ovary shape: Lobed, Lobed, Lobed, Lobed, Unlobed, Lobed, Lobed, Unlobed.

Digenetic trematodes from siganid fish: From anterior to the third of acetabulum. Overlapping the anterior border of the acetabulum. To posterior border of the acetabulum. Overlapping the anterior third of the acetabulum. Lobed. From anterior to the anterior third of the acetabulum. To the anterior border of the acetabulum. To the posterior end of the acetabulum. To the intestinal bifurcation to the posterior end.

**Table 1** Helicometra species with a short cirrus sac and vitellaria folicles extending anteriorly to the acetabular zone or the fore body.

<table>
<thead>
<tr>
<th>Characters</th>
<th><strong>H. pulchella (Radcliffe, 1819)</strong></th>
<th><strong>H. pulchella (Radcliffe, 1819)</strong></th>
<th><strong>H. pulchella (Radcliffe, 1819)</strong></th>
<th><strong>H. pulchella (Radcliffe, 1819)</strong></th>
<th><strong>H. pulchella (Radcliffe, 1819)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Body length</td>
<td>0.21–0.7</td>
<td>0.21–0.7</td>
<td>0.21–0.7</td>
<td>0.21–0.7</td>
<td>0.21–0.7</td>
</tr>
<tr>
<td>Fore body</td>
<td>0.21–0.7</td>
<td>0.21–0.7</td>
<td>0.21–0.7</td>
<td>0.21–0.7</td>
<td>0.21–0.7</td>
</tr>
<tr>
<td>Total body length</td>
<td>0.21–0.7</td>
<td>0.21–0.7</td>
<td>0.21–0.7</td>
<td>0.21–0.7</td>
<td>0.21–0.7</td>
</tr>
<tr>
<td>Oral sucker</td>
<td>0.18–0.23</td>
<td>0.18–0.23</td>
<td>0.18–0.23</td>
<td>0.18–0.23</td>
<td>0.18–0.23</td>
</tr>
<tr>
<td>Aacetabulum</td>
<td>0.18–0.23</td>
<td>0.18–0.23</td>
<td>0.18–0.23</td>
<td>0.18–0.23</td>
<td>0.18–0.23</td>
</tr>
<tr>
<td>Sucker ratio</td>
<td>0.18–0.23</td>
<td>0.18–0.23</td>
<td>0.18–0.23</td>
<td>0.18–0.23</td>
<td>0.18–0.23</td>
</tr>
<tr>
<td>Testes shape</td>
<td>0.21–0.23</td>
<td>0.21–0.23</td>
<td>0.21–0.23</td>
<td>0.21–0.23</td>
<td>0.21–0.23</td>
</tr>
<tr>
<td>Extension of cirrus sac</td>
<td>From anterior to the level of the pharynx</td>
<td>From anterior to the level of the pharynx</td>
<td>From anterior to the level of the pharynx</td>
<td>From anterior to the level of the pharynx</td>
<td>From anterior to the level of the pharynx</td>
</tr>
<tr>
<td>Ovary shape</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Extension of vitellaria follicles</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Egg length (mm)</td>
<td>30–100</td>
<td>30–100</td>
<td>30–100</td>
<td>30–100</td>
<td>30–100</td>
</tr>
<tr>
<td>Egg width (μm)</td>
<td>11–42</td>
<td>11–42</td>
<td>11–42</td>
<td>11–42</td>
<td>11–42</td>
</tr>
</tbody>
</table>

(All measurements are expressed in mm except eggs in μm).

**Table 2** A comparison between the present species and other species of Helicometra.

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Body length</td>
<td>0.27–0.49</td>
<td>0.35–0.63</td>
<td>3.56–4.53</td>
<td>1.42</td>
<td>1.52–2.61</td>
<td>2.16–2.3</td>
<td>0.97–1.99</td>
<td>1.48–1.58 (1.59–1.69 ± 0.025)</td>
</tr>
<tr>
<td>Body width</td>
<td>0.39–0.69</td>
<td>0.48–0.5</td>
<td>0.44–0.66</td>
<td>0.49</td>
<td>0.46–0.52</td>
<td>1.21–1.01</td>
<td>0.35–0.64</td>
<td>0.43–0.48 (0.44–0.61 ± 0.079)</td>
</tr>
<tr>
<td>Oral sucker</td>
<td>0.18–0.24</td>
<td>0.18–0.24</td>
<td>0.18–0.24</td>
<td>0.11–0.13</td>
<td>0.11–0.13</td>
<td>0.11–0.13</td>
<td>0.11–0.13</td>
<td>0.12 ± 0.13</td>
</tr>
<tr>
<td>Aacetabulum</td>
<td>0.23–0.36</td>
<td>0.21–0.35</td>
<td>0.23–0.33</td>
<td>0.22–0.23</td>
<td>0.21–0.23</td>
<td>0.14–0.20</td>
<td>0.14–0.20</td>
<td>0.21 ± 0.17</td>
</tr>
<tr>
<td>Sucker ratio</td>
<td>0.101.4</td>
<td>0.101.4</td>
<td>0.101.4</td>
<td>0.051</td>
<td>0.0103</td>
<td>0.0104</td>
<td>0.0104</td>
<td>0.0106 (0.0106 ± 0.0005)</td>
</tr>
<tr>
<td>Anterior testes</td>
<td>0.29</td>
<td>0.29</td>
<td>0.29</td>
<td>0.195–0.214</td>
<td>0.195–0.214</td>
<td>0.195–0.214</td>
<td>0.195–0.214</td>
<td>0.195–0.214</td>
</tr>
<tr>
<td>Posterior testes</td>
<td>0.32–0.47</td>
<td>0.32–0.47</td>
<td>0.32–0.47</td>
<td>0.14–0.24</td>
<td>0.14–0.24</td>
<td>0.14–0.24</td>
<td>0.14–0.24</td>
<td>0.14–0.24</td>
</tr>
<tr>
<td>Ovary</td>
<td>0.14–0.25</td>
<td>0.14–0.25</td>
<td>0.14–0.25</td>
<td>0.14–0.25</td>
<td>0.14–0.25</td>
<td>0.14–0.25</td>
<td>0.14–0.25</td>
<td>0.14–0.25</td>
</tr>
<tr>
<td>Vitellaria</td>
<td>From acetabulum to posterior end</td>
<td>From acetabulum to posterior end</td>
<td>From cecum to posterior end</td>
<td>From cecum to posterior end</td>
<td>From cecum to posterior end</td>
<td>From cecum to posterior end</td>
<td>From cecum to posterior end</td>
<td>From cecum to posterior end</td>
</tr>
<tr>
<td>Eggs</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.052</td>
<td>0.052</td>
<td>0.052</td>
<td>0.052</td>
<td>0.052</td>
</tr>
<tr>
<td>Hosts</td>
<td>Holocentrus samara</td>
<td>Holocentrus samara</td>
<td>Holocentrus samara</td>
<td>Holocentrus samara</td>
<td>Holocentrus samara</td>
<td>Holocentrus samara</td>
<td>Holocentrus samara</td>
<td>Holocentrus samara</td>
</tr>
</tbody>
</table>

(All measurements are expressed in mm).
in Tables 1 and 2. On the other hand, Yamaguti (1971) separated the genus Helicometra into subgenera as follows: Subgenus Helicometra (Helicometra) Odhner, 1902 in which, the vitellaria are extended to fore- and hind-body and subgenus Helicometra (Metahelicometra) Yamaguti, 1971, in which vitellaria are commencing at pre-ovarian level. Sekerak and Arai (1974) reviewed the genus Helicometra and divided the valid species into three groups as follows: Group (a) which includes species with a short cirrus sac and vitellaria follicles extending anteriorly to the acetabular zone or the fore body; group (b) that includes species with a short cirrus sac and vitellaria follicles confined to the hind body; group (c) that includes species with a short fore-body and long cirrus sac extending to the hind body. On the other hand, Hassanine (2007) revised the proposed groups into four groups including all species of Helicometra possessing a terminal, infundibuliform oral sucker. These groups are re-described as follows:


The present sample *Helicometra siganus* sp. nov. agrees typically and related with the concepts of group IV of genus *Helicometra*, and similar to its mentioned species in containing short fore-body and a long cirrus sac which extent posteriorly to the ventral sucker, but differs significantly from them in having a distinctly obvious pharynx, a distinct esophagus, a very large sucker ratio where the oral sucker reached 1/5 of the ventral sucker, and small vitelline follicles less clustered and arranged in two lateral rows on each side of the body. The vitelline follicles grouped into small clusters are only known in *H. borneoensis* (group I). However, the new species differs from each of the known species of different groups in several other characteristics such as having a larger and elongated fore-body nearly reached about 1/4 of the total length, a cirrus sac extending posterior to about 1/2 the body at distance of the ventral sucker and not reach to the ovary. The vitelline follicles are terminating anteriorly at level of a middle part of the esophagus before the intestinal bifurcation (vs. at the same level of the cirrus opening). It could resemble *H. interrupta* but differentiated from it in having a longer fore-body representing 1/4 of the body length, vitelline follicles terminating anteriorly at the level of the midpoint of the esophagus and before the intestinal bifurcation. Moreover, the present species is characterized by having big sucker ratio, having larger egg sizes (65 μ vs. sizes of 40–28.33 μ). From the point of view of these differences, the present sample is considered as a new species. Generally, five species of Helicometra are so far known from the Red Sea fishes: *H. fasciata* [described as *H. hypodytis* Yamaguti, 1934 by Nagaty (1956), as *H. epinepheli* Yamaguti, 1934 by Ramadan (1986) and as *H. marmoratae* Nagaty and Abdel-Aal, 1962 by Nagaty and Abdel-Aal, 1962], *H. equilata* [described as *H. bosei* Nagaty, 1956 by Nagaty (1956)], *H. nasae* Nagaty and Abdel-Aal (1962), *H. interrupta* Hassanine 2005 and *H. aegyptense* [described by Hassanine (2007)].

So, and from the listed Tables 1 and 2 of different species of Helicometra we can conclude that, the present sample differs from all the listed species in many features, where the species is unique in having the following characters: 1- The body was elongated and tapering anteriorly; 2- The sucker ratio (anterior: posterior) was larger (1/5 of the body) than all listed species in Tables 1 and 2; 3- The vitellaria extended from the beginning of intestinal bifurcation to the posterior extremity of the hind-body; 4- The cirrus sac and cirrus were longer than the other species and extended to the midpoint of esophagus level. So, these characters are specific and sufficient to consider it as a new species, and proposed the name *H. siganus*, where the specific name *siganus* refers to the scientific name of Siganus fish from which it was collected.
Digenetic trematodes from siganid fish *Amphacanthus sigan* at Macady Bay

Scanning electron microscope study shows that the body of the parasite is c-like shaped with strong cuticle and divided into two parts; the anterior part is broad in which the oral sucker is situated (Fig. 3), while at the posterior part the acetabulum is located (Fig. 4). Moreover, the genital pore is situated at the beginning of the second part of the fluke (Fig. 5). The tegument of the body is bending on it self ventrally and appeared as if it is segmented. The oral sucker is ovoid shaped and protrudes dorsally. The opening of oral sucker is oval in shape and surrounded by a transverse striated tegument. The genital pore opening evaginates outwards like a papilla, oval in shape and situates behind the oral sucker directly. There are transverse striations on the body surface. The acetabulum evaginates outward, ovoid shaped and surrounded by lip-like structures. The excretory pore is situated at a distance from the acetabulum at the posterior end extremity (Fig. 5). Finally, from the above description and the illustrated figures and plates, it is pointed out that the collected sample is the same *Gyliauchen volubilis* Nagaty, 1956 which described by several authors such as; Nagaty (1956), Abdou and Heckmann (2001), Al-Jahdali (2012, 2013) and Al-Jahdali and Hassanine (2012).

Finally, the presence of these two parasites with a large number in the intestinal canal affect the health of mentioned fish economically and may transferred to human by hazardous and consequently affect the human health.

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