Molecular Phylogenetic Taxonomy and Descriptive Analysis on Hexangium sigani Goto & Ozaki, 1929 (Digenea: Microscaphidiidae) from Three Different Siganus spp. Fishes from Red Sea, Egypt

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Abstract

Three different common species of Rabbitfishes in the Red Sea region were found to be naturally infected by Hexangium sigani Goto and Ozaki, 1929. The encountered parasites were described morphologically and morphometrically by means of light and scanning electron microscopy. Present specimens presented and exhibited a wide range of variability inside the same host and at same locality and accordingly all previous synonyms of Hexangium sigani were presented, shown here and discussed with the previously described forms. These variations included testes position relative to each other and relative to ovary, body spination and uterus extension but these differences were considered to be of minor importance. The SEM disclosed well differentiated three forms of sensory papillae; oral papillae, genital papillae and body papillae which may reflect a variation in the functions they performed. Furthermore, the true nature of male genital system in all Hexangium spp. were reviewed and elucidated the absence of cirrus sac in all known species and probably some fibrous tissues may be around the seminal vesicle. Also, key to species of Hexangium Goto and Ozaki, 1929 was added. Molecular data characterized Hexangium sigani within Microscaphidiidae and referred to an interrelationship between Microscaphidiidae & Mesometridae which in need to more future analyses to give a deeper understanding. It is worth mentioning that SEM study of this parasite was done for the first time from Egypt with an addition of many ultrastructural details; most of which are of taxonomical importance. For the first time, Siganus luridus represented a new host record of H. sigani.

Keywords: Egypt; Microscaphidiidae; Hexangium; Hexangium sigani Goto and Ozaki, 1929; Siganus; Rabbitfishes; Red Sea

Introduction

Goto and Ozaki erected Hexangium with H. sigani from Siganus fuscescens in Misaki and Takamatsu, Japan as its type species [1]. Yamaguti partly re-described this species from the same host in the Inland Sea of Japan and recorded it late from the intestine of a Siganus luridus as the second species from Siganus javus of Manila, the Philippines [4]. From the same geographical region, Anneraux described H. secundum from a single specimen obtained from Siganus guttatus of Mercedes, Samar, the Philippines [5].

Nagaty established Arthurloossia as the fourth genus in the family Mesometridae Poche, 1926 and described its type species Arthurloossia loossi which had been collected from two fish species, Hippocampus harid and Siganus canaliculatus in the Red Sea of Hurgada, Egypt [6]. Investigations clarified that Arthurloossia Nagaty, 1954 was congeneric with H. sigani with very few and insignificant variations [7]. So, Arthurloossia was reassigned as a synonym of Hexangium Goto and Ozaki, 1929. In addition, Arthurloossia loossi Nagaty, 1954 synonymized a junior synonym of Hexangium sigani due to the great identity [7]. Also, Yamaguti erected a new subfamily Hexangini in the family Angiostictidae Looss, 1902 for Hexangium [7].

Razariheliosa recorded H. sigani from non siganid fish Neoptilomycter samara (Syn. Holocentrus samara) and observed the great similarities among H. affinis, H. secundum, its specimens and Yamaguti’s description of H. sigani [8]. Accordingly, he cast doubt about the validity of H. affinis, H. secundum and H. loossi, and considered them as synonyms of H. sigani. The subsequent studies by Velasquez and Fischthal & Kuntz revealed them agreement with the previous author in considering H. affinis, H. secundum and H. loossi as synonyms of H. sigani [9,10]. Gupta and Miglani expressed a different point of view and refused these synonyms [11]. Nevertheless, subsequent reports of H. sigani have continued to accept these synonyms [12-17].

Manter described H. elongatum from Naso sp., of Fiji [18]. Since ventral body-surface of H. elongatum concaved anteriorly and modified to form accessory attachment organ, Jones and Blair eliminated H. elongatum from Microscaphidiidae Looss, 1900 and transferred it inside Mesometridae Poche, 1926 as a new genus Parawardula [19]. Jones and Blair due to the very close similarities with Wardula Poche, 1926 and Parawardula elongate [18] n. comb. as the type species [19].

Machida and Uchida described another species H. leptosomum from Naso unicornis off Okinawa [21]. This species characterized by concaved ventral body-surface anteriorly and modified to form accessory attachment organ, tandem testes, the caeca almost reach the posterior extremity and lack an oesophageal bulb so, Blair transferred this species into Mesometridae Poche, 1926 as a new genus Pseudeoxeangium with Pseudeoxeangium leptosomum n. comb. as the type species [20,21].

In 2005, Hassanine and Gibson described a new species H. brayi from Siganus luridus of Sharm El-Sheikh, South Sinai, Egypt [22]. Also, in 2013, two new species added; H. ecosmi from Siganus rivulatus of...
Red Sea, Saudi Arabia and *H. saudii* from the same fish species off Saudi coast of the Red Sea [16,23].

The most structuring taxonomy of *Hexangium* Goto and Ozaki, 1929 recognized only four accepted species; *H. brayi*, *H. sigani*, *H. ecosami* and *H. saudi* [24]. All these species are commonly known in the Red Sea region [17,23].

As part of an on-going study of the digenean trematodes parasitizing some Red Sea fishes, the main purpose of this study was to increase our knowledge by the endohelminths of fish from the Red Sea through clarifying the morphological variations in internal organs' shape and distribution and ultrastructural description of a questionable Microscaphidiid species *Hexangium sigani* collected from some Rabbitfishes, of Sharm El-Naga. Also, molecular characterization of *Hexangium sigani* within Microscaphidiidae. Lastly, providing a key to the species inside this genus.

**Materials and Methods**

**Morphological data**

A total of ninety four Rabbitfishes: seventy *Siganus rivulatus* Forsskal and Niebuhr, sixteen *Siganus sutor* Valenciennes and eight *Siganus luridus* Ruppell (Perciformes: Siganidae), were caught by small trawl in the Red Sea off Sharm El-Naga, Egypt, during the period from July 2011 to August 2012. Fish were transported as alive as possible with good aeration and cooling immediately to the laboratory; the alimentary tract from the esophagus to the anus removed and examined for endohelminths under a dissecting microscope and the surrounding peritoneal cavity examined by the aid of a magnifying hand lens. Digeneans were relaxed in 1 part unfiltered sea water to 3 parts tap water peritoneal cavity examined by the aid of a magnifying hand lens. Digeneans were observed, fixed in 10% formalin solution [27], preserved in 70% ethanol, stained with alum carmine and then under very slight cover slip pressure in a 5% buffered formal saline solution [27], preserved in 70% ethanol, stained with alum carmine and mounted in a mixture of distyrene and a plasticizer dissolved in toluene-xylene (DPX). Drawings were prepared with the aid of a Zeiss Universal compound microscope using micro-projector or camera Lucida (PZO 01852 10x). Measurements for the species description are expressed in micrometers (µm) with ranges and means indicated; the number [n] of measurements is also noted where needed (Table 1). Comparative measurements were taken from the original species descriptions unless otherwise stated. If needed, some critical measurements that were not available from the original descriptions were calculated from original illustrations and are identified herein. The fish host was identified according to criteria established by [28-31]. The identification more confirmed through the fishbase website (http://www.fishbase.org). Digenea identification based on Bray [32]. Ecological terms follow Bush et al. [33].

Specimens were deposited in the Zoology Department Museum, Faculty of Sciences, South Valley University (SUV), Qena, Egypt.

**Ultra-structural data**

For scanning electron microscopy; the relaxed specimens were fixed for 6 h at 4°C in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer then washed several times in the same buffer. The post fixation carried out in 1% osmium tetroxide for 2 h, specimens washed two times cacodylate buffer then dehydrated in ascending grades of ethanol series, transferred into pure acetone. Samples were then processed in a critical point drier "Bomler-900" with Freon 13 then sputter coated with gold in a Technics Hummer V and viewed with a JEOL JSM-5400LV SEM operated at 15 kV in electron microscopy unit, Assiut University [34].

**Molecular data**

Total genomic DNA was extracted using DNA Extraction Kit (QIAamp DNA Blood Mini Kit (Cat. No. 51140). A region from bp 92 to bp 112 off the 18S nuclear ribosomal DNA was chosen for the forward primer (5′-GGT TCC TTA GAT CGT ACA TGC-3′), bp 498 to 519 bp for the reverse primer (5′-GTA CTC ATT CGA ATT CAG GAG C-3′).

PCR amplifications were carried out using Taq PCR Master Mix Kit (Qiagen, Cat.201443) in a total volume of 25 µl consisting of 12.5 µl of Taq PCR Master Mix, 0.5 µl of each primer, and 1 µl of DNA template, made up to 25 µl with Invitrogen™ ultraPURE™ distilled water.

The PCR Amplification was performed in a thermal cycler (Eppendorf) with the GastB programme after an initial denaturation step to hot start the polymerase at 95°C for 15 min, 30 cycles of 1 min at 95°C, 2 min at 56°C and 3 min at 72°C and an elongation step at 72°C [34]. Amplified DNA was purified using a QIAquick Gel Extraction Kit (Qiagen, Cat. No. 28704) according to the manufacturer’s protocol. Amplified DNA fragments were sequenced directly using the ABI Prism Big Dye Terminator V3.1 Cycle sequencing Kit on an ABI 310 DNA automated sequencer (Applied Biosystems) reactions were done in 20 µl mixture reaction, according to the instructions of the manufacturer, using the same primers used for PCR amplification. Sequencing had been carried out at Genetic Engineering Research Department (VACSERA), Cairo, Egypt.

Newly generated 18S sequences were aligned with sequences of Superfamily Paramphistomoida taxaa available on GenBank (Table 2). Alignments were performed using Clustal W tool in MEGA v7.0.26 software. The resultant alignments were refined by eye using MEGA v7.0.26 and the ends of each fragment were trimmed to match the shortest sequence in each alignment.

**Results**

**Morphology**

*Family Microscaphidiidae Looss, 1900*

(Syn. Angiodictyida Looss, 1902)

*Genus Hexangium* Goto and Ozaki, 1929

Syn. *Arthurloosia* [6]
**Hexangium sigani Goto and Ozaki, 1929** (Figures 1-4)

(Syn. Arthuroloossia loossi [6]; Hexangium affine [4]; Hexangium loosi [6,7]; Hexangium secundum [5].

**Hosts:** Siganus rivulatus Forsskal and Niebuhr; Siganus sutor Valenciennes; Siganus luridus Rüppell (Perciformes: Siganidae).

**Locality:** Northern Red Sea, off Sharm El-Naga, Makadi Bay, Southern Hurghada, Egypt (26°55.16’N, 33°56.05’E-26°53.59’N, 33°59.49’E, depth=0.5-2.5 m; July/2011-August/2012).

**Site of infection:** Intestine.

**Deposit material:** Deposited in Zoology Department, Faculty of Science, South Valley University (SVU), Qena, Egypt.

**Prevalence:** 44/70 S. rivulatus (62.9% infected); 2/16 S. sutor (12.5% infected); 2/8 S. luridus (25% infected).

**Intensity:** 1-8 worms/host specimen.

**Mean intensity:** 2.64 (116/44) in S. rivulatus; 2 (4/2) in S. sutor; 1 (2/2) in S. luridus.

**Relative density/abundance:** 1.66 (116/70) in S. rivulatus; 0.25 (4/16) in S. sutor; 0.25 (2/8) in S. luridus.


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**Figure 1.** Photomicrographs of the adult digenean parasite of *Hexangium sigani* showing: A & B. Ventral view of whole mount preparation of the mature worm infecting Siganus rivulatus. C & D. Ventral view of whole mount preparation of the immature worm infecting Siganus rivulatus and revealing X-shaped ceca. E. Ventral view of whole mount preparation of the immature worm infecting Siganus sutor. Scale bar=500μm.

**Figure 2.** Line diagram of *Hexangium sigani* showing: A. Ventral view of whole mount preparation of the adult worm infecting Siganus rivulatus. B. Ventral view of whole mount preparation of the adult worm infecting Siganus sutor. C. Ventral view of whole mount preparation of the immature worm infecting Siganus sutor and revealing X-shaped ceca. D. The oval operculated eggs. Scale bar (A-C)=500 μm & Scale bar (D)=100 μm.

Abbreviations: AT: Anterior testis; Es: Esophagus; EB: Esophageal bulb; EV: Excretory vesicle; Ce: Cecum; MG: Mehlis gland; Ov: Ovary; PT: Posterior testis; Ph: Pharynx; SV: Seminal vesicle; Ut: Uterus; VF: Vitelline follicle; VS: Ventral sucker.

Re-description: (Based on 18 mature and 7 immature specimens. Morphological features illustrated in Figures 1-3. Measurements, morphometric percentages and morphometric ratios are given in Table 1). Living specimens fleshy of white color and with sluggish movement body dorsoventrally flattened, elongate, stout, with almost straight and smooth margins (in some specimens, body provided with minute spines, especially anteriorly), maximum width at level of mid-body or slightly anterior it. Anterior end tapering (in immature specimens) to rounded (in mature specimens); posterior endless round to tapering possessing a median knob-like protuberance. Tegument smooth in almost specimens but some specimens provided with minute spines, especially anteriorly. Oral sucker absent and replaced by well-developed pharynx. Pharynx spherical, without sacs, ventro-subterminal slightly terminal with conspicuous, oval aperture directed anteroventrally. Ventral sucker absent. Oesophagus moderately long, moderately wide, about 1/10 of body length, very slightly convoluted to straight, without esophageal glands. Esophageal bulb present, very weakly developed, smaller than pharynx and very difficult to be observed in mature specimens. Intestinal bifurcation at the end of anterior forth of body. Ceca 2, simple, straight to very slightly undulating, equal in length (In

**Table 1.** Parasite Name and Host Details

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<th>Locality</th>
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<td>Siganus javus</td>
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<tr>
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<td>Siganus guttatus</td>
<td>Mercedes, Samar, Philippine Islands</td>
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<td>Celebes Sea</td>
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<td>Ghardaga, Egypt in Red Sea</td>
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<td></td>
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<td>Malabon, Rizal, Luzon Island, Philippines</td>
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<td></td>
<td>Siganus canaliculatus &amp; Stolephorus commersoni</td>
<td>Puerto Philippines, Princesa, Palawan Island,</td>
</tr>
<tr>
<td></td>
<td>Johnius borneensis</td>
<td>Bay of Bengal, at Puri, Orissa</td>
</tr>
<tr>
<td></td>
<td>Siganus rivulatus &amp; Siganus luridus</td>
<td>Sharm El-Naga, safaga Egypt in Red Sea</td>
</tr>
</tbody>
</table>

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**Figure 3.** Line diagram of some posterior ends of Hexangium sigani showing: A. Overlapping obliquely tandem testes. B. Contiguous opposite testes. C. Tandem testes. D. Symmetrical testes. Scale bar=500 μm. Abbreviations: AT: Anterior testis; EV: Excretory vesicle; LT: Left testes; Ov: Ovary; MG: Mehlis gland; RT: Right testes; PT: Posterior testis; UT: Uterus

**Figure 4.** Scanning electron micrographs of Hexangium sigani infecting Siganus rivulatus showing: A. Ventral view of the mature worm. B. High magnifications of the forebody. C. High magnifications of pharynx disclosing strong musculature and oral papillae. D. High magnifications of body surface at posterior end revealing circular furrows.
### Oes. length

| Oes. length | 1,450–1,650 | 400–450 | 560–850 | 1/8 - 1/6 length of worm | 300–690 | 248–380 | 600–890 | 412–432 (420) |

### Oes. length %


### Oes. bulb L × W


### Oes. bulb L %

| Oes. bulb L % | 2–3 | 4–5 | 3 | --- | 5 | --- | 3–4 | --- | 3 | (3) |

### Ph. length: Oes.

| Ph. length | Oes. bulb L | 1:0.56–0.66 |
| --- | --- |

### Ph. Width: Oes.

| Ph. Width | Oes. bulb W | 1:0.56–0.66 |
| --- | --- |

### Cirrus sac length

| Cirrus sac length | 330–500 | 100–120 | Absent | Absent | Absent | Absent | 86–91 × 63–103 | Absent | Absent |

### Ovary length × width


### Ovary length %

| Ovary length | 6 | 4 | 6 | 5–6 | 7–8 | 5–6 | 6–7 | 5–7 | 5–6 | 5–6 |

### Anterior Testes L × width


### AT length %

| AT length | 11 | 8–9 | 7 | 10–11 | 17–19 | --- | 5–8 | 10–14 | 12–14 |

### Posterior Testes L × width


### PT length %

| PT length | 11 | 8–9 | 7 | 10–11 | 17–19 | --- | 7–13 | 12–13 | 12–14 |

### Mean testes L: Ovary L

| Mean testes L: Ovary L | 1:0.50 | 1:0.42–0.46 | 1:0.80 | 1:0.50–0.55 | 1:0.38–0.40 | 1:0.45–0.56 | 1:0.72–0.95 | 1:0.40–0.54 | 1:0.40–0.49 |

### Mean testes W: Ovary W

| Mean testes W: Ovary W | 1:0.50 | 1:0.42–0.53 | 1:0.80 | 1:0.67 | 1:0.52–0.67 | 1:0.63–0.80 | 1:0.65–0.90 | 1:0.50–0.56 | 1:0.44–0.52 |

### OS to genital pore%

| OS to genital pore% | 5 | --- | --- | --- | 5–7 | --- | 9–10 | --- | 7–8 |

### Oes. bulb to ovary%

| Oes. bulb to ovary% | 65 | --- | 63 | --- | 55–70 | --- | --- | --- | 63–70 (67) |

### Prebilabral distance %

| Prebilabral distance % | 17 | --- | 22 | --- | 16–18 | --- | 21 | 21–23 (22) |

### Post-caecal distance %

| Post-caecal distance % | 25 | --- | 22 | --- | 18–26 | --- | 26 | 26–28 (27) |

### Pre-gential pore distance %

| Pre-gential pore distance % | 11 | --- | --- | 10–11 | 10–18 | --- | 12–13 | 13–14 (13) |

### Prevelite distance %

| Prevelite distance % | 24 | --- | 34 | --- | 17–33 | --- | 30 | 25–29 (28) |

### Post-vitelite distance %

| Post-vitelite distance % | 32 | --- | 20 | --- | 21–33 | --- | 35 | 28–34 (30) |

### Preevian distance %

| Preevian distance % | 86 | --- | 85 | --- | 83–86 | --- | 89 | 88–89 (89) |

### Post-ovarian distance %

| Post-ovarian distance % | 9 | --- | 9 | --- | 6–11 | --- | 6 | 8–11 (9) |

### Pre-testicular distance %

| Pre-testicular distance % | 69 | --- | 70 | --- | 71–76 | --- | 75 | 70–72 (71) |

### Post-testicular distance %

| Post-testicular distance % | 13 | --- | 15 | --- | 8–11 | --- | 4–8 | 12 | 7–13 (11) |

### Post-uterine distance %

| Post-uterine distance % | 8 | --- | 8 | --- | 4–8 | --- | 4 | 6–9 (8) |

### egg length × width


Footnote:

All dimensions, measurements and percentages are calculated to [0] decimal places; all ratios are calculated to 2 decimal places.


2. Calculated from figures of the original description; Annereaux (Figure 1) [5], Nagaty (Figure 6 & 7) [6], Velasquez (Figure 10-13) [9], Gupta & Tandon (Plate 3, Figure 1) [37].

3. Calculated from measurements given in the original description.

4. Neither given in the original description nor available from the published illustrations.

**Table 1.** Comparison of the measurements, morphometric percentages and morphometric ratios of *Hexangium sigani* between the present specimens against the previously described questionable synonyms and forms.
some specimens, left cecum slightly longer than right one), with the same width along the entire length, terminate anterior to testicular level directly or reach to anterior margins of anterior testis. Ani absent (Figures 1A-1C; Figures 2A-2C).

Testes 2, variable in shape (rounded, elliptical, oval, pyriform), entire, smooth, subequal, with varied position (symmetrical, side-by-side, oblique, obliquely tandem), occupy the anterior 2/3 area of last body fourth, well-separared from the posterior end, separated from each other by small distance or contiguous and sometimes the inner lateral margins overlap each other. Cirrus sac absent. Seminal vesicle sinuous, tubular, often concealed by uterus, winding from some distance posterior to intestinal bifurcation. Distal extremity of the seminal vesicle narrowing anteriorly to insignificant genital atrium, opening into short hermaphroditic duct below halfway between pharynx and intestinal bifurcation. Genital pore pre-equatorial, median (sinistral), at mid-oesophageal level or slightly posterior directly (Figures 1A-1F; Figures 3A-3D).

Ovary entire, spherical to oval, smooth, median or sinistro-submedian, immediately post testicular or separated by short distance, smaller than both testes. Seminal receptacle absent. Mehlis gland median, well-developed, large, pyriform, postero-lateral to sinistral rim of ovary, just anterior to excretory vesicle. Uterus comes out laterally from Mehlis gland forming a small uterine seminal receptacle then passes anteriorly between testes and extends in intercecal space forming many convolutions. The distal portion of uterus narrowing at intestinal bifurcation level and unites below mid-oesophageal level with distal portion of seminal vesicle forming hermaphroditic duct. Hermaphroditic duct moderately short, cylindrical and protractible outside body surface (Figures 1A-1F; Figures 3A-3D). Vitellarium follicular; field extends lateral, partly medial, to ceca, distributed in second and third quarters of body, (extends beneath the intestinal bifurcation level by small distance and terminate at cecal ends.), confluent medially, overlaps over intestinal ceca. Follicles numerous, few in number, large, irregularly shaped and arranging themselves roughly in 4 longitudinal rows (Figures 1A-1F; Figures 2A-2C). Eggs numerous, highly dense, oval, moderate in size, non-operculate, thin-shelled, without filaments or knob (Figure 2D).

Excretory vesicle V-shaped with variable sizes; excretory arms short, divided at level of ovary gives off single duct on each side which subsequently divides into three long stems reaching level of oesophagus, excretory pore subterminal (Figures 1B-1D and 1F; Figures 2A-2D).

Ultra-structure description (Figures 4 and 5)

SEM examination illustrates that the body dorsoventrally flattened, elongate, stout, with almost straight and smooth margins, maximum width slightly posterior to mid-body level on (Figure 4A). Anterior endless round (Figure 4B), posterior endless round possessing a median, transverse, narrow, slit-like sub-terminal excretory pore (Figure 4D). The enlarged hind part of the body illustrated absence of spines and papillae at this region. Also, it is divided by thin circular grooves. These grooves become crowded towards the posterior extremity (Figure 4D).

Pharynx sub-elliptical, ventro-subterminal or slightly terminal (situated on top of a rather globular cecal end) with conspicuous, oval, wide, aperture (mouth opening) directed anteroventrally. Cephalic extremity slightly swollen, probably by contraction of muscular pharynx. Body with a slight constriction just posterior to pharynx, and another slight constriction at level of genital pore (Figure 4A and 4B). Inside pharynx, three large domed structures occupy most of internal space of mouth opening; these structures may represent extensions from the muscular layer of pharynx. Also, several, small, sessile, rounded, well-developed, randomly distributed and different sized spherical papillae aggregated on inner tegmental surface of pharynx on (Figure 4C).

At genital pore level, cylindrical moderately long hermaphroditic duct comes out from the genital pore. Several, small, sessile, rounded, well-developed, randomly distributed and different sized spherical papillae aggregated on outer tegmental surface of hermaphroditic duct on (Figure 5A and 5B). Also, hermaphroditic duct’s surrounding region is represented as a depression in body surface and very crowded by randomly distributed and different sized spherical papillae on (Figure 5C).

The worm was incised at middle of the body to examine eggs from its uterus. The eggs are smooth, non-operculate. Another different sized spherical papillae of few numbers and randomly distributed on body surface observed on (Figure 5D and 5E).

Molecular phylogeny (Figure 6)

The genotypes of *Hexangium sigani* Goto and Ozaki, 1929 (401 nucleotides) recorded in GenBank with accession number KT070706.
This sequences aligned with 10 reference sequences representing all the available and appropriate species of the Paramphistomoid (Table 2); 1 species from the Cladorchiidae Fischoeder, 1901, one species from the Mesometridae Poche, 1926, two species from the Microscaphidiidae Looss, 1900 and three species from the Paramphistomidae Fischoeder, 1901, together with four species belongs into the Pronocephaloidea; AY222115 [39] [Labicola], AY222114 [40] [Notocotylus], AY222116 [39] [Opisthotrematidae] and AY222113 [39] [Rhabdiopeoeidae], for out-group comparisons.

All 12 sequences (including out-groups), are aligned over 365 positions (trimmed to parallel the shortest sequence length) and the genetic distance among them. Phylogenetic analysis of this dataset resulted in the Paramphistomidae forming a monophyletic clade (Figure 6) with strong support (BI=100, ML=100, MP=100, NJ=100, ME=100) to the exclusion of out-group taxa.

The Paramphistomidae divided into two distinct sister clades; Cladorchiidae/Paramphistomidae clade of a differentiated support value (BI=65, ML=51, MP=72, NJ=90, ME=90), and Mesometridae/Microscaphidiidae clade with of weak support (BI=54, ML=51, MP=53, NJ=53, ME=55).

Cladorchiidae clade which represented by only one species Solenorchis travassosi Hilmy, 1949 (Syn. Indosolenorchis hirudinaceus

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<td>Australia</td>
<td>AY222116</td>
<td>Olson et al. [39]</td>
</tr>
<tr>
<td>Rhabdiopeoeus taylori</td>
<td>Dugong dugon</td>
<td>Australia</td>
<td>AY222113</td>
<td>Olson et al. [39]</td>
</tr>
</tbody>
</table>

Table 2. Sequence data representing species of the Paramphistomoidea and outgroups determined in the present study, together with key 18S reference sequences (see GenBank accession nos.) and epidemiological information.

Figure 6. The genetic relationships among members of the Paramphistomoidea inferred from partial sequence of the 18S rDNA locus following analysis using Bayesian inference (BI) method based on the following parameters; the best-fit model (SYM+G), nst=6, rates=gamma & statefreqpr=fixed (equal). Average standard deviation of split frequencies=0.008912 & number of the generations applied=900,000. The nodal values arranged in the following order (BI/ML/MP/NJ/ME).
Crusz, 1951), resolved in all resulting trees as basal clade to all species of Paramphistomidae. Paramphistomidae species resolved as a distinct monophyletic clade with strong support (BI=97, ML=86, MP=76, NJ=97, ME=97).

**Neohexangium zebrazomatissi** Machida, 1984 resolved as basal to the well-supported Mesometra/Hexangium clade (BI=94, ML=76, MP=76, NJ=85, ME=85). The genus Hexangium resolved as strongly supported monophyletic clade (BI=99, ML=92, MP=95, NJ=99, ME=99); Hexangium sigani and Hexangium sp. resolved as two sister clades. In spite of the somewhat strong support and the strong relationship between Hexangium sigani and Hexangium sp., the species H. sigani has a longer branch length than Hexangium sp., which means that the former has number of nucleotides substitutions more than in the later indicating that both species are different.

**Discussion**

**Morphology**

Present specimens consistent with Jones criteria which indicate that the newly collected specimens belong to the superfamily Microscaphidiidae Looss, 1900 [32]. These criteria are; excretory pore at posterior extremity, absence of both sucker and cirrus sac, excretory system partly reticulate, metraterm absent, pharynx present and functionally replaces oral sucker. Furthermore, since ventral body-surface not modified as attachment organ, this places present specimens into the family Microscaphidiidae Looss, 1900 [20].

The combined features; vitelline follicles entirely anterior to testes and caeca terminate anteriorly to testes, places present specimens in the genus Hexangium Goto and Ozaki, 1929. Comparison among newly collected specimens and the valid species in the genus Hexangium indicated that present specimens identical to Hexangium sigani Goto and Ozaki, 1929 since sharing in; overall appearance, egg size range, possessing median and genital pore at mid-oesophageal level, alike in vitellarium distribution, ovary position and shape and parasitizing in same host group. In addition, almost of allometric measurements extremely similar and clearly have converging range such as; body width, pharynx length, oesophagus length, oesophageal bulb length, ovary length, testes length and post-testicular distance as a percentage of body length. Also, mean testes width/ovary ratios and pharynx/oesophageal bulb ratios very identical.

Goto and Ozaki (1929) indicated in the description of H. sigani to the large body size, oblique testes and figured structure resembling a thin-walled cirrus-sac. Both Tubangui & Masilungan and Annereaux stated another two species H. affine and H. secundum, reported from a single specimen and differed from the type species H. sigani in size and arrangement of the testes where; both H. affine and H. secundum and have a smaller body size and symmetrical testes [4,5]. H. secundum characterized from H. affine by larger ovary, larger in dimensions and very inconspicuous cirrus-sac. Present specimens exhibited a wide variability in testes positions [symmetrical, side-by-side, oblique, obliquely tandem] and ovary size, this is consistent with description of H. sigani, H. affine and H. secundum.

Nagaty added a fourth genus in the family Mesometridae Poche, 1926 called Arthurloosia contains one species Arthurloosia loossii [6]. This species appeared to be congeneric with H. sigani with few variations; spiny tegument, especially anteriorly and absence of cirrus-sac. The subsequent study by [7] considered Arthurloosia Nagaty, 1954 a synonym of Hexangium Goto and Ozaki, 1929 because Mesometridae characterized by ventral body-surface entirely concave or concave only anteriory, modified to form accessory attachment organ [19]. The genus Arthurloosia has no such this structure so it reassigned into Microscaphidiidae and subsequently Arthurloosia loossii Nagaty, 1954 transferred as Hexangium loossii [6,7].

Within more detailed study on H. sigani, against the other species H. affine, H. secundum and H. loossi, Razarheliosia presented an overview about the validity of these species and suggested that they might be conspecific [8]. Velasquez specimen of H. sigani indicated to the discernibility of the cirrus sac and variability in testes position and arrangement [9]. Except for the arrangement of the testes H. affine, H. secundum and Yamaguti specimens fall within the range of measurements i.e. Velasquez concluded that H. affine and H. secundum as objective synonyms of H. sigani [3,9]. Fischthal and Kuntz results consist with the previous conclusion by Velasquez with consideration of H. loossi as another objective synonyms of H. sigani with a sign to a presence of cirrus sac with protractible cirrus [9,10]. Only in re-description of H. sigani by Gupta and Miglani, they concluded disagreement with the views of previous authors [11]. Nevertheless, subsequent reports of H. sigani by researchers and present study have continued to accept these synonyms [11-17,39].

The present observations illustrated absence of cirrus sac and this consistent with almost previous observations except [1], [4] and [10]. Al-Jahdali revealed in his description of Hexangium saudi to absence of cirrus sac [23]. On the other hand, Hassannine and Gibson indicated in the description of Hexangium brayi that "cirrus-sac weakly developed, but large, elongate-claviform, extending anteriorly from near middle of body to genital pore containing elongate, saccular seminal vesicle and inconspicuous prostatic complex" [22]. The present worker thinks that presence of weakly developed cirrus-sac but large with absence of illustrations that differentiate between seminal vesicle and cirrus-sac is not convincing and not very logic (Figure 1) [22]. So, we support absence of cirrus sac in all species of the genus Hexangium. Also, male genital system represented only by large elongate seminal vesicle and its distal end joins that of the uterus to form hermaphroditic duct.

The previous comparison among all described forms of H. sigani revealed some morphological variations confined between; absence or presence of tegumental spines, testes arrangement, larger or smaller of body dimensions and ovary size. These variations fall within slight range of variability and not enough on its own here, to indicate lack of conspecificity so, these differences are considered to be of minor importance.

Host-parasite data illustrates that the genus Hexangium parasites intestine of marine teleosts (many families) and distributed in tropical and subtropical Indo-Pacific [20]. Present specimens have the same host-parasite data as they reported from marine fish (siganus) and geographically collected from off northern Red Sea region, Egypt. Moreover, present specimens and almost previously described forms of Hexangium sigani reported from several species of siganid fishes; Siganus fuscescens, Siganus sp., Siganus javus, Siganus guttatus, Siganus canaliculatus, Siganus argenteus, Siganus spinus, Siganus vermivulatus, Siganus sutor and Siganus rivulatus, Siganus lirdius (present study) [1-6,9,11,13,15-17,39]. Furthermore, Hexangium sigani reported from other fishes belong to other families; Hippocampus harid [Labridae], Neoponiphon sarama [Holocentridae], Stolephorus commersonnii [Engraulidae] and Johnius borneensis [Sciadidae] [6,8,10,11].

As a result of the similarities in host-parasite data of all described forms of H. sigani and present study especially geographic locality, the slight morphological changes and differences in allometric...
measurements between present study and the previously described forms of *H. sigani* and between all synonyms can be attributed significantly to host-induced variability where; present specimens reported from three different siganid fishes *Siganus rivulatus*, *Siganus luridus* and *Siganus sutor*. This change of host affect directly on three main measurements which are body length, body width, and suckers width and therefore, any measurement related to the previous measurements may be labile.

Only three digenean trematodes were reported from *Siganus luridus*; *Gyliauchen volubilis* Nagaty, 1956 from Red Sea, Egypt [41, 42], *Hexangium brayi* Hassanine & Gibson, 2005 from Sharm El-Sheikh, South Sinai, Egypt [22] and *Proglyiauchen magnacetaalbum* Al-Jahdali, 2013 from the coast of Rabigh, Saudi Arabia [23]. No any record of *Hexangium sigani* reported from *Siganus luridus* i.e. *Siganus luridus* represents a new host record of *Hexangium sigani* Goto and Ozaki, 1929 for the first time.

**Ultra-structure description**

Present study revealed presence of one main tegumental structure, sensory papillae. These papillae differentiated into three forms; oral papillae, genital papillae and body papillae. Each of these forms exhibited a moderately wide range of variations both in size and in distribution. Hayunga indicates that changes in the microenvironment of helmintes are usually reflected in variations in the structure of the tegument [43].

Presence of different types of sensory papillae on different locations over body tegument of *H. sigani* may reflect a variation in the functions they performed as following; 1) oral papillae could be involved in contact reception during food detection or feeding as mentioned by [44]. 2) Body papillae might record pressure changes as the tegument stretches as reported by [45]. 3) Genital papillae could be involved in contact reception during fertilization process or could be involved in cross-fertilization between two flukes or in selecting the site of attachment as mentioned by Ashour in his explaining the reason for the abundance of the sensory papillae on lateral sides of ventral surface of body and on the two suckers [46].

**Molecular phylogeny**

According to the resultant Phylogenetic trees, it was observed that both families Cladorchiidae and Paramphistomidae very close to each other. Also, both families Mesometridae and Microscaphidiidae much related to each other despite of somewhat weak support values. This weak support value can be explained as result of flow of information used in study and more new sequences are needed.

On other hand, Paramphistomidae/Cladorchiidae clade appeared distant from Mesometridae/Microscaphidiidae clade and the well-supported values for each clade sustained this assumption. Another evidence is the host-parasite listed in the previous table (Table 2) where; all species used in the phylogenetic analysis of Cladorchiidae and Microscaphidiidae species are from fishes. This is consistent exactly with the host-parasite data reported by Jones who indicated that Paramphistomidae are only obtained from the alimentary tract of mammals [47]. Also, mammals represent one of the main groups from which Cladorchiidae are collected [48]. On the other side, host-parasite data of Microscaphidiidae referred that marine and freshwater fishes and turtles are representative hosts to this family [20]. In addition, Mesometridae parasitizes inside intestine of mainly herbivorous marine fishes [19].

Microscaphidiidae clade is paraphyletic, based on the inclusion of a single sequence representing *Mesometra* sp. from the Mesometridae with strong support values. The previous result matched exactly [37]. Insertion of *Mesometra* sp. within Microscaphidiids very interesting and further more studies are needed. Mesometridae has similar biological features with the family Microscaphidiidae where all Mesometrids reported from "herbivorous marine fishes (Sparidae, Acanthuridae); off Mediterranean Sea and, rarely, Pacific and Atlantic Oceans" [19]. Furthermore, marine fishes represent one of the major hosts of Microscaphidiids especially (Acanthuridae, Siganidae....etc.) and Microscaphidiids are "cosmopolitan but probably absent from cold-temperate and polar regions" [20]. Morphologically, the Mesometrids are characterized from Microscaphidiids only by modification of ventral body-surface to form accessory attachment organ [19,49].

The interrelationship among the genera *Neohexangitrema*, *Mesometra* and *Hexangium* can be attributed to several reasons; 1) the great similarities in body structures 'shape, distribution and position with slight changes represented by; body elongation, absence or presence of spines and their distribution, Pharynx position, Caeca extension in the hind body, variability of genital pore position at oesophageal level, entire testes positioned in the posterior third of body and their position against each other, entire to slightly lobe ovary positioned closely posterior to testes, interradial uterus passes between testes, presence or absence the metraterm and distribution of vitellarium with regard to ceca, arose in association, with an ecological shift. 2) The host parasite data from the previous table indicated that the genera *Mesometra* Lühe, 1901, *Neohexangitrema* Machida, 1984 and *Hexangium* Goto and Ozaki, 1929 parasitized on different families; Sparidae, Acanthuridae and Siganidae (respectively) from the same Order Perciformes. This consistent with data reported by Jones and Blair in which *Mesometra* obtained from the intestine of Sparidae, and the results of Blair in which *Neohexangitrema* reported from Acanthuridae and *Hexangium* obtained from many families especially Siganidae [19,20].

Finally, we concluded that reliance on only a single taxon of the most speciose Mesometrids genus are not satisfying enough to clarify this interrelationship between to the two families wherefore incorporation of other sequences of type-ta in future analyses will give a deeper understanding.

**Key to species of Hexangium Goto & Ozaki, 1929**

1a. Ecsoma present.... *Hexangium ecsomi* [16].

1b. Ecsoma absent [2].

2a. Vitelline follicles are arranged in rosette-like groups, caeca distinctly short and more distant from the testes.... *Hexangium saudii* [23].

2b. Vitelline follicles not collected in groups, caeca long and very close to testes [3].

3a. Body shape distinctly pyriform, caeca terminations dilate and saccular, vitelline follicles confined to the intercaecal field..... *Hexangium brayi* [22].

3b. Body shape distinctly elongate, caeca terminations undifferentiated, vitelline follicles overlapping or lateral to the caeca..... *Hexangium sigani* [1].

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References
