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ORIGINAL PAPER

Molecular characterization and new geographical record of *Lecithochirium priacanthi* (Digenea: Hemiuridae) infecting the moontail bullseye fish *Priacanthus hamrur* (Perciformes: Priacanthidae) from the Red Sea, Egypt

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Abstract Sixty specimens of the moontail bullseye *Priacanthus hamrur* were collected at Coasts of Suez Gulf, Red Sea (Egypt) during the four different seasons of the whole year 2014 and necropsied to study the infection with metazoan parasites. Twenty-one out of 60 examined fish specimens (infection rate of 33.33 %) were found to be naturally infected by the hemiurid digenean parasite *Lecithochirium priacanthi*. The large-sized fish reaching 15–30 (23.5±4.8)cm were more intensively infected than the smaller ones. A definite seasonal effect was observed as winter was found to be the season of severe parasitic infections, while midsummer was the lowest one. The morphological and morphometric characterization of this parasite were examined by light and scanning electron microscopy. The adult worms had an elongated body measuring 1.93–2.54 (2.11±0.20)mm in length and 0.61–0.72 (0.67 ±0.02)mm in width. The body was characterized by the presence of a sub-terminal oral sucker with diameters reaching 0.12–0.16 (0.14±0.02)mm. The ventral sucker measured 0.32–0.45 (0.38±0.02)mm in diameter. The body was supplied by a short retracted portion with a blunt end that measured 0.48–0.61 (0.56±0.02)mm in length and 0.28–0.35 (0.32±0.02)mm in width. Morphological results of the present parasite were compared with other related species described previously from Perciformes. Molecular characterization based on small subunit ribosomal DNA was done to confirm the obtained morphological and morphometric results. A preliminary genetic comparison between SSU rDNA of this parasite and other species of Hemiuridae places the present specimen as a putative sister taxon to *Lecithochirium grandiporum* and *Lecithochirium caesionis*. The finding of *L. priacanthi* in Egyptian marine water fish represents a new geographical record for this parasite.

Keywords *Priacanthus hamrur* fish · Hemiuridae · *Lecithochirium* species · Morphology · Phylogenetic analysis

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Introduction

Fish are an important source of animal protein for human consumption and the impact of parasites on their populations is well documented (Williams and Jones 1994; Abdel-Ghaffar et al. 2013). Moontail bullseye, *Priacanthus hamrur* Forskål, 1775, is a marine carnivorous fish that feeds on crustacean and planctonic invertebrates, belongs to the family Priacanthidae Kamegai 1973 and is widely distributed in the Red Sea (Randall 2005). Although studies on parasitic worms from Egyptian fish are important in controlling the impact of such parasites on fish health and fish production, relatively little is known on the helminthic fauna of fish in Egypt (El-Serafy et al. 2003; Al-Bassel and Ohida 2006; Abdel-Ghaffar et al. 2013). This is probably due to the fact that helminth parasites mainly infect the internal organs, predominantly the gastrointestinal tract which, for humans, does not comprise the edible parts of fish (Barson and Avenant-Oldewage 2006). Digenetic trematodes represent the largest group of all internal metazoan parasites as they comprise about 18,000 nominal species (Cribb et al. 2001). The suborder Hemiurata is one of the most

diverse groups of digenean parasites which usually occur in the stomach and intestine of marine teleost fish (Gibson and Bray 1979; Shih et al. 2004; Al-Zubaidy 2010). These parasites have a wide geographical distribution, since then infect fish at the Great Barrier Reef of Australia and in the Indian and Atlantic Oceans (Gibson and Bray 1986). At the early time Looss (1907) studied the morphology of the family Hemiuridae and established the criteria for the identification of the genera and species as depending on the relative size relationship for the two parts of the body (soma and ecsoma) and on the relative position of organs in the post-acetabular portion of the body. The most common genus of this family is *Lecithochirium* which contains about 100 nominal species (Shih et al. 2004). Phylogenetic studies of these trematode parasites are based on characters derived from the morphology and from the life cycle are rather controversial (Blair et al. 1998). The taxonomy of this group needs actual revision by a multidisciplinary approach.

Therefore, the present study aims to confirm the systematic position and the etiology of the digenean parasite *Lecithochirium priacanthi* infecting the host fish *P. hamrur* in the Red Sea (Egypt) based on molecular analysis and offers a re-description of the morphology and morphometric analysis applying light and scanning electron microscopy.

Materials and methods

A total of 60 specimens of the moontail bullseye fish *P. hamrur* were trapped alive during the four different main seasons of the year 2014 at the coasts of Suez Gulf, Red Sea at Hurghada City in Egypt (geographical coordinates of 27° 14′ 20″ N and 33° 50′ 9″ E). Fish were identified according to Randall (1995) and showed 15–30 (23.5±4.8)cm for total length with a difference between males (25±3.2 cm) and females (22±4.6 cm). Collected fish were transported alive to the Laboratory of Parasitology Research, Zoology Department, Faculty of Science, Cairo University, where they were dissected and examined for the presence of digenean parasites. The viscera and the gills were removed and examined together with body surface and cavities. The recovered parasites were treated in different ways. For light microscopic examination and for whole mount examination, digenean parasites were flattened by repression, stained with Semichon's aceto-carmine and examined, and then identified according to morphological criteria proposed by Yamaguti (1971). The descriptive terminology of the organs followed the guidelines of Gibson and Bray (1979, 1986), Gibson (2002), and Bartoli and Gibson (2007). For scanning electron microscopy, adult flukes were fixed in 2.5 % cold-buffered glutaraldehyde for at least 4 h, washed in the same buffer, post-fixed in osmium tetroxide, and then immersed into 2 % tannic acid for 8 h. Specimens were then dehydrated in a graded series of ethanol, infiltrated with amyl acetate. After critical drying, specimens were mounted on aluminum stubs, coated with gold, and then examined and photographed with high-resolution at a SEM JOEL 6100 Electron Microscope Unit at the Micro-analytical center of the Faculty of Science, Beni Suef University, Egypt. All drawings were made with the aid of camera Lucida. Measurements are given in millimeters as the range followed by the mean±SD in parentheses.

For molecular analysis, some of the collected parasites were immediately fixed alive in 96 % ethanol which was replaced later with 1 M Tris-EDTA buffer via repeated washings. gDNA was extracted using QIAamp1 DNA Mini Kit (Quiagen, GmbH, Germany) following the manufacturer-recommended protocols. The SSU rDNA gene was amplified by PCR using forward primer (SB8: GGGTGGATTTATTAGAACA G) and reverse one (PB: CCGTCAATTCMTTTRAG TTT). PCR amplicons were either gel excised or purified directly using Qiagen Qiaquicke columns, then cycle-sequenced from both strands using ABI BigDye chemistry, alcohol-precipitated, and run on an ABI Prism 310 automated sequencer with using forward primer (SB3: GGAGGGCAAGTCTGGTGC) and reverse ones (SB9: TTTCACC TCTAACACCGC and A27: CCATACAAATGCCCCCGTCTG). Contiguous sequences were assembled, edited using Sequencher™ (Gene Codes Corp., Ver.3.11), and then submitted to the GenBank. Newly generated sequence was aligned with 22 gene sequences previously published using CLUSTAL-X (Kumar et al. 2004), then combined using DNASTAR (Window Ver.3.12e) in order to estimate the phylogeny of the hemiuroids. Maximum parsimony tree was constructed with the software PAUP (Sworfford 1993).

Results

The stomach of adult specimens of *L. priacanthi* (Digenea: Hemiuridae) were found to be parasitized by moontail bullseye *P. hamrur* (F: Priacanthidae) reaching a prevalence rate of 33.33 % in mean. The highest percentage of infection was recorded in winter reaching 73.33 % (11 specimens out of 15). The rate decreased gradually to 40.0 % (6 out of 15) and 20.0 % (3 out of 15) in spring and autumn, respectively. The lowest value of infection was detected in summer when only 6.66 % (1 out of 15) fish were infected. Prevalence and intensity of infection were positively correlated with the host size. Host sex showed no effect in this respect.

Microscopic examination

The adult worms examined were flattened, elongated with tapered conical anterior end and cylindrical posterior one (Figs. 1, 2, 3, 4, 5, 6, 7, 8, 9). They measured 1.93–2.54 (2.11±0.20)mm in length and 0.61–0.72 (0.67±0.02)mm in

width (at its widest point). The oral sucker was found subterminally and had a diameter of 0.12–0.16 (0.14±0.02)mm thus reaching approximately of 1/3 the diameter of the ventral sucker which measured 0.32–0.45 (0.38±0.02)mm in diameter and was located at the posterior part of the anterior third of the body. The pharynx measured 0.07–0.10 (0.08±0.01)mm in length and was followed by a short esophagus. The caecum appeared with a bifurcating tube, which terminated blindly before the front of the ecsoma. The sinus-sac was ovoid, well-developed, and opened by the genital pore. Two large ovoid testes were located laterally at the left median third part of the body and measured 0.17–0.21 (0.19±0.02)mm in diameter. The seminal vesicle was constricted into two portions: a large proximal part and a short distal one, which was connected to the pars prostatica by a short glandular duct. The ovary was oval, measured 0.19–0.26 (0.22±0.03)mm in diameter and was located at the left of the median line at the posterior end of the middle third of the body. The uterus was long, coiled, filled with eggs, and did not extend into ecsoma. It was located with its main bulk between the ovary and the testes and stretched from the ventral sucker to the end of the intestinal caeca. The uterus narrowed at the level of ventral sucker to form a metraterm. The vitellaria were reduced to two compact, lobated masses just posterior to the ovary. A retracted ecsoma with a blunt end was found at the posterior end of the body and measuring 0.48–0.61 (0.56±0.02)mm in length and 0.28–0.35 (0.32±0.02)mm in width.

Taxonomic summary

Parasite: *Lecithochirium priacanthi* Yamaguti (1970) family Hemiuridae Looss (1899).

Type host: Moontail bullseye *Priacanthus hamrur* Forskål (1775) belonging to Perciformes, family Priacanthidae Kamegai (1973).

Site of infection: Stomach of the infected fish host.

Host locality: Coasts of Suez Gulf at the Red Sea at Hurghada City, Egypt.

Prevalence of infection: 21 out of 60 specimens of the examined fish were found to be naturally infected (33.33 %).

Etymology: The specific name of the parasite derives from *Priacanthus* which is the generic name of the host fish from which the parasite was isolated for the first time.

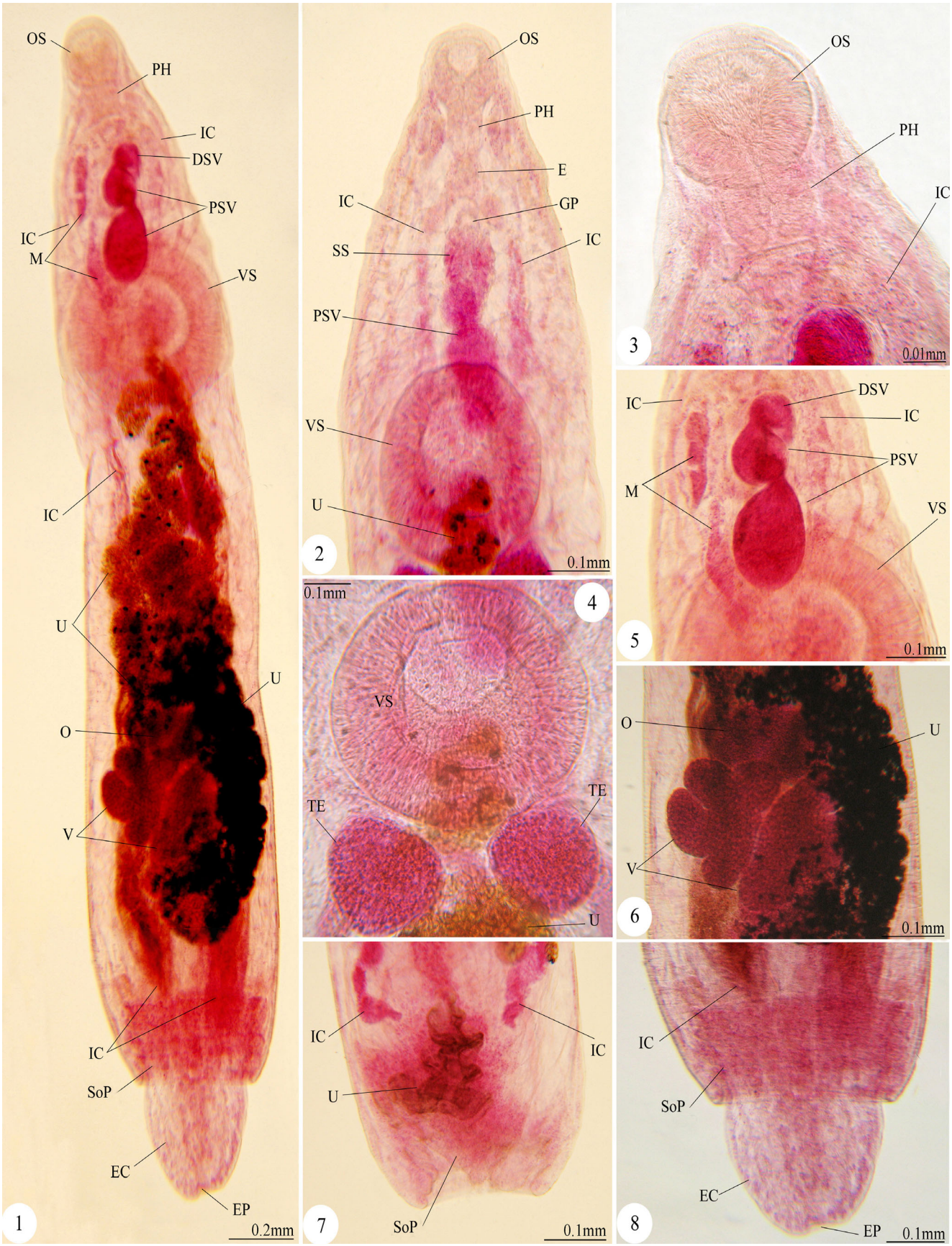
Material deposition: Voucher specimens were deposited at the Zoological Museum, Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt.

Molecular analysis

A sequence of 630 base pairs (bp) of SSU rDNA was recovered from the present hemiurid species and deposited in the GenBank under the accession number KJ872582. Pairwise comparison of the isolated gDNA sequence of *L. priacanthi* with a range of other Hemiuroidea species and genotypes revealed a unique sequence. Comparison of the nucleotide sequences showed that SSU rDNA of this species revealed 94 % sequence identity with KC166146 *Lecithochirium grandiporum*, followed by 93 % with AY222200 *Lecithochirium caesionis*, and 90 % with KC985235 *Lecithochirium microstomum* which represented the highest three BLAST scores. Phylogenetic analysis produced a neighbor-joining tree constructed with partial sequences and showed that Digenea consistently formed two major clades (Fig. 10). One represents the order Diplostomida which contains members of family Clinostomatida and the other belonged to Plagiorchiida and contains members of the Hemiurata, which were represented by the families Hemiuridae, Didymozoidae, Derogenidae, and Lectithasteridae. The sequence divergences detected between closely related Hemiuridae species varied from low values such as 2.4 % in *L. grandiporum* to high values as 8.7 % in *L. microstomum*. Phylogenetic relationships were studied using maximum likelihood, maximum parsimony, and neighbor-joining methods. It was shown that the present hemiurid species is deeply embedded in the genus *Lecithochirium* with close relationships to other *Lecithochirium* species especially to *L. grandiporum* and *L. caesionis* as putative sister taxons.

Discussion

Hemiuridae are a family which comprises the most common parasitic digenean flukes inhabiting the digestive tract of marine fish (Bullard et al. 2011; Abdel-Ghaffar et al. 2013). *Lecithochirium* is the most common genus which includes at least more than 100 described species (Shih et al. 2004; Surekha and Lakshmi 2005; Morsy et al. 2012). In the present study, the moontail bullseye fish *P. hamrur* was found to be naturally infected by the digenean parasite *L. priacanthi* in a rate of 33.33 % as an average percentage of infection which increased during winter and decreased during summer. These results are consistent with findings of Abdel-Ghaffar et al. (2013) who reported that the infection of *Anguilla anguilla* with *L. grandiporum* was 41.66 % and also increased during winter reaching 31.66 % but decreased to 10 % during summer. In addition, Bauer (1957) found that fish suffered from high degrees of parasitic infections during hibernation in winter when they are in a state of exhaustion. Generally, there are so many variations and combination of characters described for the genus *Lecithochirium* that make the selection of distinguishing characteristics difficult and thus is mostly arbitrary (Bartoli et al. 2005). Therefore, one or more of the following three characteristics such as the presence or absence of a pre-acetabular pit, presence or absence of elevations in the wall of the oral cavity, and the characters of the male vesicle within the sinus sac seem to be the most appropriate criteria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◀ **Figs. 1–8** Photomicrographs of *Lecithochirium priacanthi* parasite infecting the fish *Priacanthus hamrur*. *1* Whole mount preparation of the adult worm showing oral sucker (*OS*), ventral sucker (*VS*), pharynx (*PH*), intestinal caeca (*IC*), distal seminal vesicle (*DSV*), proximal seminal vesicle (*PSV*), ovary (*O*), metraterm (*M*), large coiled uterus (*U*), multilobed vitelleria (*V*), and evaginated ecsoma (*EC*) projected from somatic pore (*SoP*) and ended by excretory pore (*EP*). *2–8* High magnifications of (*2*, *3*) the anterior part of the worm body. Note the presence of esophagus (*E*), sinus sac (*SS*) opened by genital pore (*GP*). *4* Ventral sucker (*VS*) and the two spherical testes (*TE*). *5* Distal seminal vesicle (*DSV*), proximal seminal vesicle (*PSV*), and metraterm (*M*). *6* The lobed vitellaria (*V*), ovary (*O*), and coiled uterus (*U*). *7*, *8* High magnifications of posterior part of the worm showing the invaginated (*7*) and the evaginated (*8*) ecsoma from the somatic pore (*SoP*) and ended by excretory pore (*EP*)

(Manter 1954). According to Yamaguti (1971), the outstanding diagnostic generic features of *Lecithochirium* are the male gonads, the type of the fish host, and the harboring site inside the host. Surekha and Lakshmi (2005) stated that the genus *Lecithochirium* have exclusive taxonomic characters such as a well or poorly developed ecsoma, a presomatic pit, a ventrocervical groove, a bipartite, or tripartite occasionally coiled seminal vesicle. The vitellarium is condensed and usually divided into 6–7 in oval to digitiform lobes and the uterus has massive coils. The morphology of the parasite specimens recovered from *P. hamrur* presents the diagnostic generic features of the genus *Lecithochirium* by showing an elongated body provided with two symmetrical testes, multilobed vitellaria, a large coiled uterus, and a retracted ecosoma. The present species is compared morphologically and morphometrically with other *Lecithochirium* species as is shown in Table 1. The appearance is closely related to that of *L. priacanthi* recorded previously by Yamaguti (1953) from *P. hamrur* (Bleeker) off New Caledonia with a little difference in body measurements as stated in Table 1. The present species resembles *Lecithochirium magnaporum* Manter (1940) and differs from *L. microstomum* Chandler 1935 by the possession of a large genital pore. In *Lecithochirium synodi* Manter, 1931, the sinus sac is complete and encloses the anterior prostatic vesicle, a fact which agrees with our results.

In general, morphological data alone provide less resolution than molecular data do. The morphology in addition to phylogenetic analysis using SSU rDNA genes enhances significantly the chance for an accurate differentiation between the different species (Vilas et al. 2005). This method offers essential criteria for the identification of new species and for re-description of an inadequately described species (Testini et al. 2011). The general structure of the dendrogram obtained in the present study is consistent with previous analyses by Olson et al. (2003), which were constructed using maximum likelihood and maximum parsimony revealing the same gross topology and showing that it splits into two major clades. One of these clades is leading to members of Diplostomida and the other clade to the members of Plagiorchiida represented by a large number of independent lineages of families Hemiuridae, Didymozoidae, Derogenidae, and Lectithasteridae. From the

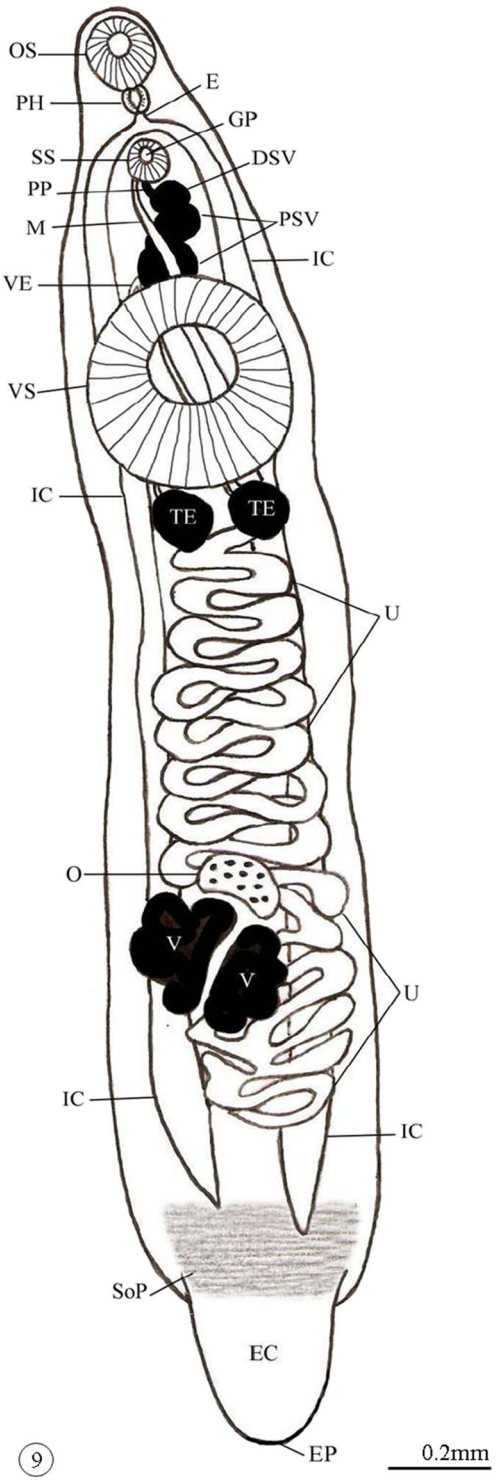

Fig. 9 Line diagram of the adult *L. priacanthi* showing oral sucker (*OS*), ventral sucker (*VS*), pharynx (*PH*), intestinal caeca (*IC*), sinus sac (*SS*), genital pore (*GP*), distal seminal vesicle (*DSV*), proximal seminal vesicle (*PSV*), testes (*TE*), vasa efferentia (*VE*), ovary (*O*), metraterm (*M*), large coiled uterus (*U*), multilobed vitellaria (*V*), evaginated ecsoma (*EC*), somatic pore (*SoP*), and excretory pore (*EP*)

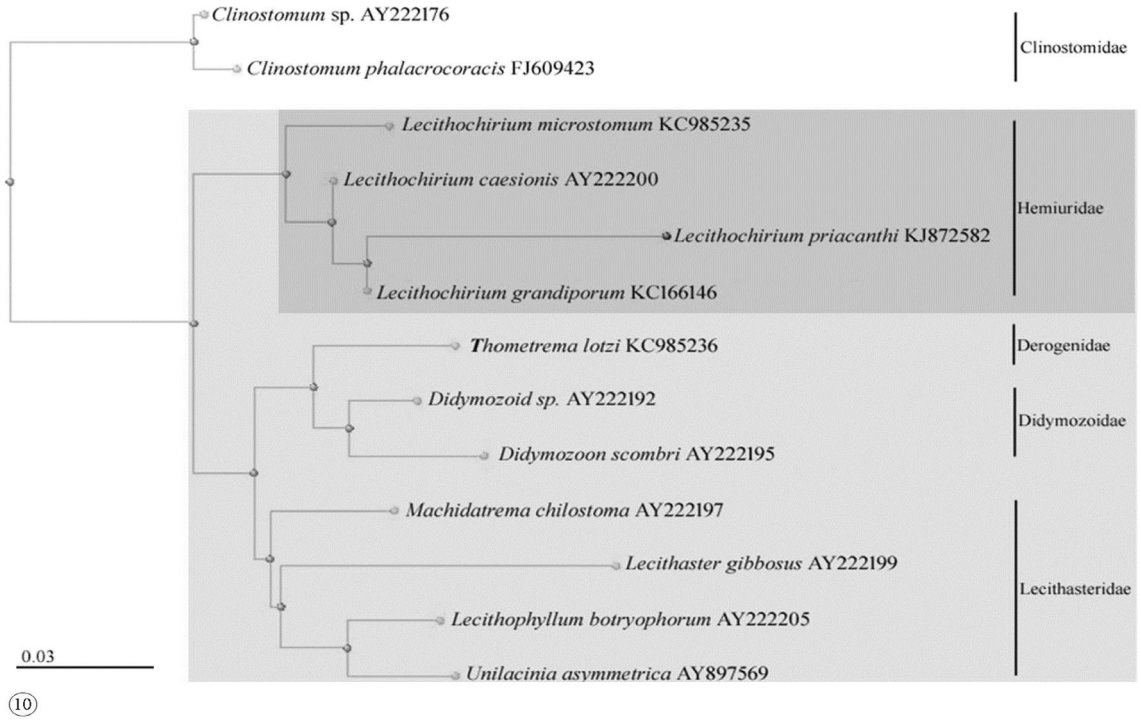

Fig. 10 Dendrogram showing the phylogenetic relationship between the present *L. priacanthi* with other Hemiuroidea species recovered from the GenBank

data obtained herein, Hemiuridae species revealed a separate line in the Plagiorchiida as is strongly supported by the obtained molecular data. Some clades, which are strongly supported by molecular data, lack, however, corresponding morphological synapomorphies. This leads to the conclusion that both kinds of data are valuable to describe the relationships among the Digenea (Testini et al. 2011). Phylogenetic relationships within the Hemiuroidea, which are a group with a complex taxonomic history and controversial views regarding its structure and contents, were studied by Blair et al. (1998). No significant difference was found between the phylogenies obtained based on morphology and on the analysis of sequences of V4 domain of 18S, showing that the Hemiuroidea are a monophyletic group and is consistent with others (Neefs et al. 1990). Previous molecular phylogenetic studies have demonstrated a high degree of sequence similarity between the subset of *Lecithochirium* species (Bullard 2010; Abdel-Ghaffar et al. 2013). The present investigation showed at least 90 % sequence similarity to all *Lecithochirium* species described previously. Moreover, the present analysis revealed that *L. priacanthi* is deeply embedded

Table 1 Comparative measurements (in millimeters) of the present *L. priacanthi* with other described previously

Related species	Host	Dimensions of							
		Body length	Body width	Oral sucker	Ventral sucker	Pharynx	Testes	Ecsoma length	Ecsoma width
L. magnaporum Manter (1940)	*Paralabrax humeralis*	1.404.728	0.337–0.450	0.135–0.150	0.262–0.292	0.054–0.075	0.060–0.102	–	–
L. priacanthi Yamaguti (1953)	*Priacanthus hamrur*	2.30	0.68	0.132	0.40	0.075	0.18	0.525	0.35
L. microstomum Timi et al. (1999)	*Engraulis anchoita*	0.74–1.592 (1.034)	0.224–0.496 (0.323)	0.088–0.144 (0.119)	0.155–0.308 (0.207)	0.052–0.077 (0.062)	0.023–0.117 (0.055)	–	–
L. microstomum Florencia et al. (2011)	*Porichthys porosissimus*	0.72–2.15 (1.279)	0.20–0.40 (0.281)	0.075–0.122 (0.095)	0.145–0.310 (0.200)	0.037–0.057 (0.045)	0.037–0.065 (0.051)	0.10–0.20 (0.175)	–
L. grandiporum Morsy et al. (2012)	*Saurida tumbil*	1.63±0.20 (1.20–1.93)	0.4±0.02 (0.31–0.52)	0.15±0.02 (0.12–0.18)	0.17±0.02 (0.15–0.28)	0.06±0.02 (0.03–0.08)	0.11±0.02 (0.08–0.15)	0.40±0.02 (0.35–0.52)	–
L. grandiporum Abdel-Ghaffar et al. (2013)	*Anguilla anguilla*	1.3–1.85 (1.59±0.20)	0.29–0.48 (0.3±0.02)	0.13–0.18 (0.15±0.02)	0.14–0.25 (0.16±0.02)	0.04–0.08 (0.07±0.02)	–	0.35–0.56 (0.49±0.03)	–
L. priacanthi (present study)	*Priacanthus hamrur*	1.93–2.54 (2.11±0.20)	0.61–0.72 (0.67±0.02)	0.12–0.16 (0.14±0.02)	0.32–0.45 (0.38±0.02)	0.07–0.10 (0.08±0.01)	0.17–0.21 (0.19±0.02)	0.48–0.61 (0.56±0.02)	0.28–0.35 (0.32±0.02)

in the genus *Lecithochirium* with a close phylogenetic relationship to *L. grandiporum* and *L. caesioni* as close-related sister taxons. Therefore, this study is considered as a full redescription of *L. priacanthi* infecting the marine fish *P. hamrur*.

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