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First Record of *Lethrinitrema gibbus* Lim and Justine, 2011 (Monogenea: Ancyrocephalidae) from Gills of *Lethrinus nebulosus* (Perciformes: Lethrinidae) of the Red Sea, Egypt

Sohier A. H. Rabie*, Abdel-Nasser A. Hussein, Ali Mansour

Department of Zoology, Faculty of Science, South Valley University, 83523 Qena, Egypt

Abstract

Lethrinitrema gibbus was re-described from gills of *Lethrinus nebulosus* collected from coasts of Safaga region along the Red Sea, Egypt during the period from June 2018 to January 2019. Among 30 specimens of *Lethrinus nebulosus* examined in this study, 7 were infected (23.33%) with *Lethrinitrema gibbus*. *L. gibbus* is an ancyrocephalid monogenean parasite distinguished from other monogeneans by a male copulatory organ (MCO) which consists of a simple tapered copulatory tube with a long base distally recurved and a thin accessory piece arises from distal part of the tube. It also possesses a haptor with four well - developed anchors; ventral anchors with lateral grooves along both sides, crown-like pieces on its inner roots and a pair of pear - shaped haptoral reservoirs with tubular extensions which bifurcate distally prior to associate with the lateral grooves along both sides of the ventral anchors. In the current study, the recovered parasite was comparable to the previously described *L. gibbus*. Accordingly, it is the first record of *L. gibbus* infecting gills of *L. nebulosus* in the Red Sea, Egypt.

Keywords: *Lethrinitrema gibbus* - *Lethrinus nebulosus* - Safaga - Red Sea - Egypt.

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*Corresponding Author: Sohier A. H. Rabie E-mail: soheir_rabie@yahoo.com

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Introduction

Most studies on monogeneans in Egypt were from freshwater fishes (El-Naggar and Serag, 1986, 1987; Khidr, 1995, 1996, 1997; El-Naggar et al., 2000; El-Naggar, 2001; Morsy, 2007 and Morsy et al., 2012 a, b) and a little work was carried on marine monogenean parasites (Paperna, 1972 a, b; Bayoumy, 2003 and Morsy et al., 2011, 2014 a, b).

Lethrinids have many species but the most common genus is *Lethrinus* Cuvier, with 26- 30 species (Froese and Pauly, 2010; Randall, 2005 and Sato, 1978) especially in tropical waters of the Indo-Pacific region. There are only *Lethrinus atlanticus* Valenciennes, 1830 in the Atlantic Ocean, off West Africa (Lim and Justine, 2011). In New Caledonia, the lethrinids are one of the most important groups for fisheries (Laboute and Grandperrin, 2000); they harbour various species of monogeneans (i.e. capsalids on the pharyngeal teeth; microcotylids, diplectanids and ancyrocephalids on the gills) (Justine, 2007, 2010).

Lethrinitrema can be diagnosed by having two pyriform haptoral reservoirs, each with an elongate tubular extension bifurcates prior to contact with the superficial lateral grooves along both sides of the ventral anchors (Lim and Justine, 2011). *Lethrinitrema gibbus* was first recorded from the gills of *Lethrinus rubrioperculatus* Sato (Lethrinidae) off New Caledonia by Lim and Justine, 2011.

This study provides a full redescription of *L. gibbus* Lim and Justine, 2011 for the first time from the gills of *L. nebulosus* Forsskål, 1775 of the Red Sea, Egypt. The present study also compared this species with other previously described species from the same genus.

Materials and methods

From June 2018 to January 2019, thirty samples of *Lethrinus nebulosus* Forsskål, 1775 were collected from coasts of Safaga region along the Red Sea, Egypt (26°44' N, 33°56' E). Fish samples ranged between small and medium size. The samples were transported in a cooling box to the laboratory of Parasitology, Zoology department, Faculty of Science, South Valley University. Each collected fish was photographed, measured and weighed.

Skin surface, fins and gills were examined by naked eyes and with the help of magnifying lens for any attached parasites, lesions or external changes. Gills were exposed to hot water for relaxing monogeneans and to remove any excess gill mucus. Monogeneans were picked off the gills with a Pasteur pipette or a fine needle under a dissecting binocular microscope.

The collected worms were fixed in 5% formalin and before mounting, they were washed with distilled water to remove the excess of the fixative. For microscopical investigation of sclerotized parts of the haptor and a male copulatory organ, all specimens were mounted unstained by adding few drops of a mixture of alcohol 70% and glycerol (50% of each) (Mizelle 1936, 1937) under cover slips.

Illustrations from photographs, drawings (Hand drawings with micro-projector) and measurements were made using a binocular compound microscope (Model 3B 100, Germany). Measurements are in micrometers (µm) and are expressed as the mean followed by the range (minimum and maximum) and the number (n) of measurements taken in parentheses.

Results

Class: Monogenea
Subclass: Monopisthocotylea
Order: Dactylogyridea Bychowsky, 1937
Family: Ancyrocephalidae Bychowsky, 1937
Genus: *Lethrinitrema* Lim and Justine, 2011
Lethrinitrema gibbus Lim and Justine, 2011

Host: Spangled emperor, *Lethrinus nebulosus* Forsskål, 1775 (Perciformes: Lethrinidae).
Locality: Safaga, Red Sea, Egypt (26°44' N, 33°56' E).
Site of infection: Gills.
Prevalence: 7 out of 30 fish (23.33%) were infected with a total of 9 worms.
Range of intensity: 1-2 worms per infected fish.
Mean intensity: 1.28 (9/7).
Relative abundance: 0.3 (9/30).
Material examined: 4 adult specimens were temporarily mounted in a mixture of alcohol and glycerine.
Material deposited: Slides were deposited at Zoology department, Faculty of Science, South Valley University, Qena, Egypt.

Redescription (Figs. 1-12, Table 1):

[Based on 4 adult samples] Body leaf-like, 619.66 (595.03-644.3; n=2) long, with greatest width 86.21 (66.93-96.04; n=3) usually at level of testis. Cephalic region moderately broad; cephalic lobes well-developed; three pairs of head organs; four pairs of pigmented eyespots. Pharynx ovoid with width of 32.31 (26.17- 41.93; n=3). Intestine bifurcates just posterior to pharynx; caeca unite just posterior to testis, continue posteriorly as two diverticula. Body peduncle moderately narrow, long.

Haptor not well differentiated from body proper, width, 57.52 (49.44-65.6; n=2); adductor muscle well developed, connecting to tips of inner roots of ventral anchors. Two pear-shape haptoral reservoirs, each with elongate, tubular extension which bifurcates distally prior to contact with lateral groove on each side of the ventral anchor.

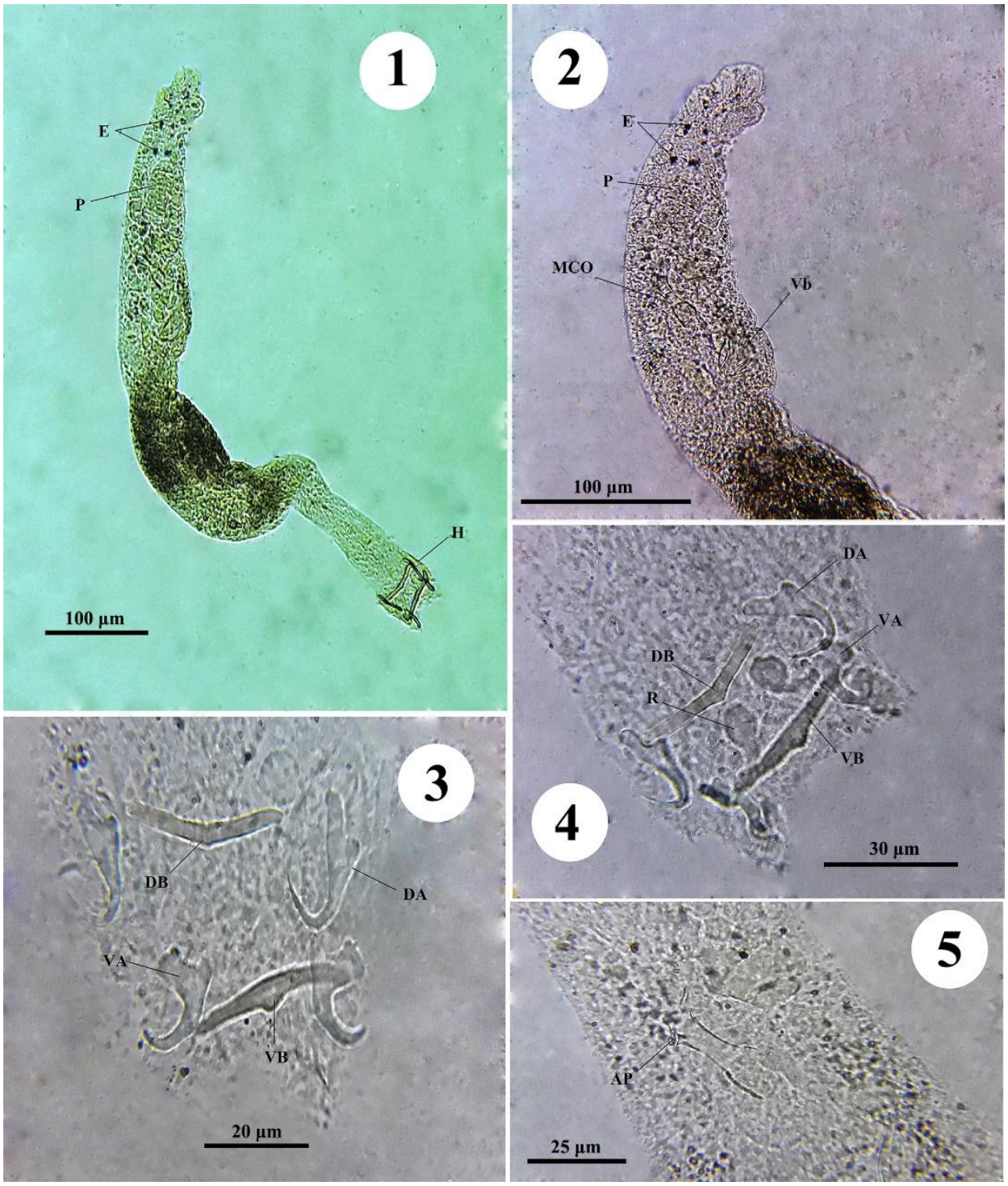
Four well-developed anchors; Dorsal anchors, inner length 22.52 (21.52-23.53; n=2), outer length 17.93 (17-18.87; n=2), well-developed inner roots 8.92 (8.54-9.31; n=2), outer root 3.24 (2.82-3.66; n=2), point 9.76 (9.46-10.06; n=2); ventral anchors, inner length 17.9 (16.88-18.92; n=2), inner root 3.47 (3.21-3.73; n=2) with a crown-like piece on its tip, outer length 16.46 (15.27-17.65; n=2), outer root 4.11 (3.91-4.31; n=2), point 7.87 (7.46-8.29; n=2).Two well-developed bars; Dorsal bar V-shaped, 44.41 (42.12-46.17; n=2) long, 4.68 (4.37-5; n=2) wide; ventral bar with a median prominence, 42.83 (42.74- 42.92; n=2) long, 5.87 (5.86- 5.89; =2) wide. 14 similar larval hooks, length 8.73 (6.9-10.56; n=2).

Testis ovoid, post ovarian 70.48 (60.78-80.19; n=2) in length, 51.09 (49.47-52.72; n=2) in width. Male copulatory organ (MCO) consists of a simple tapered copulatory tube, length 36.03 (31.31- 39.15; n=4), with a long base, recurved distally; thin accessory piece arises from distal end of the tube. Ovary in mid-body, pre-testicular. Vaginal bulb dextro-lateral, muscular. Vitelline system extensive from level of intestinal bifurcation to post intestinal region; transverse vitelline ducts conspicuous in the proximal part of uterus.

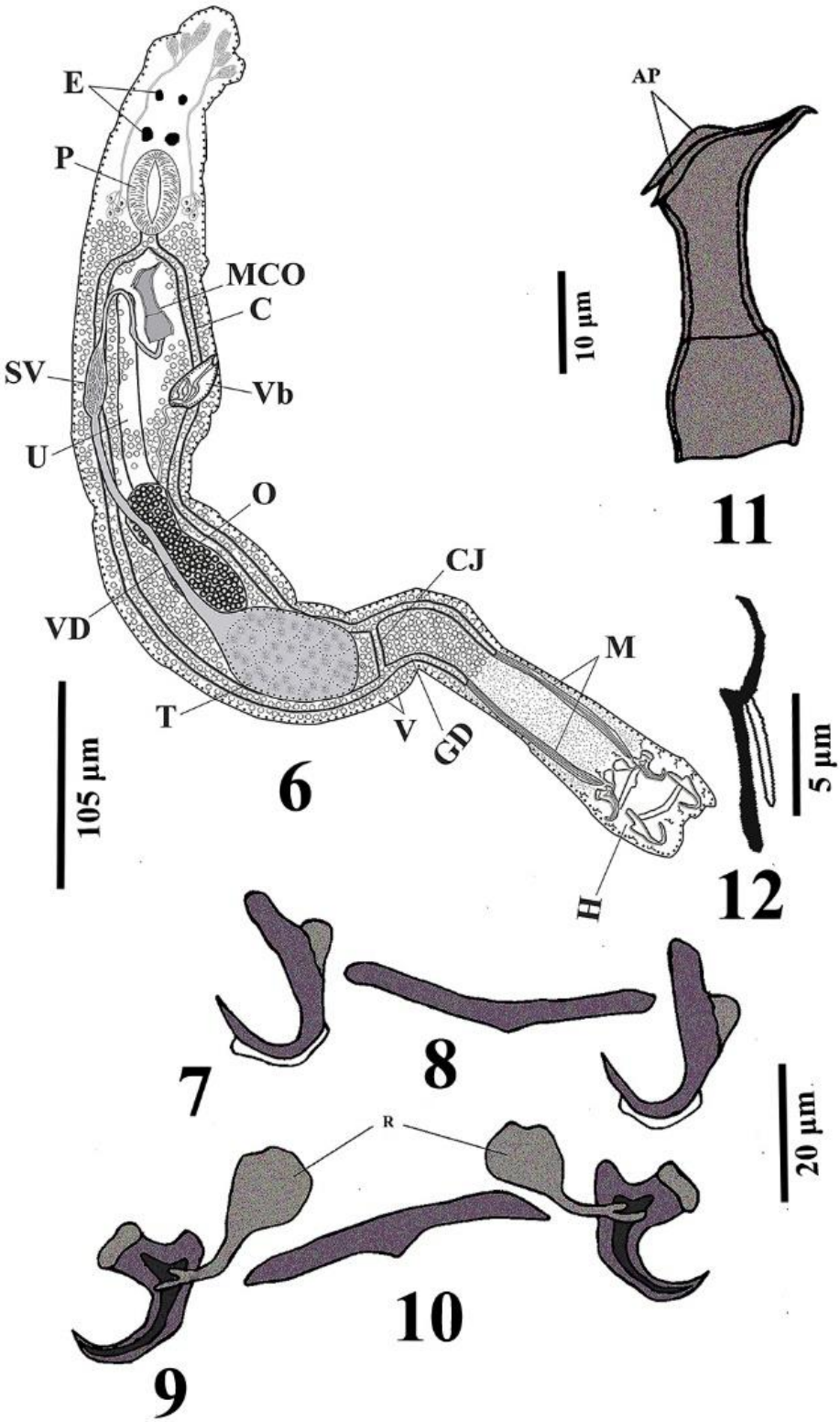
Remarks

Lethrinitrema gibbus Lim and Justine, 2011 belonging to members of *Lethrinitrema* due to some features characterizing it from other previously described monogenean species. This species having four well-developed anchors; ventral anchors, each with a crown-like piece on the edge of its inner root and a lateral groove on each side; a pair of pear-shaped haptoral reservoirs in which its tubular distal ends bifurcate prior to contact with lateral grooves on both sides of the ventral anchors; a prominent dextrolateral, muscular vaginal bulb and a simple tapered copulatory tube with a thin accessory piece arises from its distal end.

Based on morphological and morphometrical characters, the present specimens were identified as *L. gibbus* Lim and Justine, 2011. There also were some differences in measurements between the present specimens and the original specimens of *L. gibbus* Lim and Justine, 2011 in which most of the measured parameters as the total length, the greatest width, haptor width, dorsal and ventral anchors, ventral bar width, hook length and



Figs. (1-5): Light Photomicrographs of *Lethrinitrema gibbus* Lim and Justine, 2011 infecting *Lethrinus neblosus* Forsskål, 1775. **Fig. (1):** The whole mount of *L. gibbus* showing cephalic lobes; four eyespots (E); pharynx (P) and the haptor (H) with anchors and connecting bars, scale bar 100 µm. **Fig. (2):** The anterior portion of the worm focusing on cephalic lobes; four eyespots (E); ovoid pharynx (P); male copulatory organ (MCO) and vaginal bulb (Vb), scale bar 100 µm. **Figs. (3&4):** High magnification of the haptor consisting of two pairs of anchors, two dorsal anchors (DA), two ventral anchors (VA); two connecting bars, dorsal bar (DB), ventral bar (VB) and two pear-shaped haptoral reservoirs (R), scale bars 20 µm, 30 µm. **Fig. (5):** The male copulatory organ (MCO) consisting of a simple tapered copulatory tube with a long base and a thin accessory piece (AP) arises from distal end of the copulatory tube, scale bar 25 µm.



Figs. (6-12): Line drawings of *L. gibbus* Lim and Justine, 2011 infecting *L. neblosus* Forsskål, 1775. **Fig. (6):** Dorsal view of whole mount preparation of the mature worm. **Fig. (7):** Dorsal anchors. **Fig. (8):** Dorsal bar. **Fig. (9):** Ventral anchors with two pear-shaped reservoirs. **Fig. (10):** Ventral bar. **Fig. (11):** Male copulatory organ with a thin accessory piece. **Fig. (12):** Hook. Scale bars: **Fig. 6**, 105 µm; **Figs. 7-10**, 20 µm; **Fig. 11**, 10 µm; **Fig. 12**, 5 µm.

Abbreviations; E, eyespots; P, pharynx; MCO, male copulatory organ; C, caecum; Vb, vaginal bulb; SV, seminal vesicle; U, uterus; O, ovary; VD, vas deferens; T, testis; V, vitellaria; GD, gut diverticulum; CJ, caecal junction; M, muscles; H, haptor; AP, accessory piece; R, reservoirs.

Table (1): Measurements of *Lethrinitrema gibbus* Lim and Justine, 2011 in the present study compared to some of the previously described species from the same genus *Lethrinitrema*.

Aspect	*L. dossenus* (Lim and Justine, 2011)	*L. gibbus* (Lim and Justine, 2011)	*L. chrysostomi* (Young, 1968)	*L. fleti* (Young, 1968)	*L. lethrini* (Yamaguti, 1937)	*L. gibbus* (present study)
Total body length	1,148 (970–1,546)	1,288 (1191-1458)	(506–693)[a]	(748-1210)[a]	(1400-1700)	619.66 (595.03-644.3)
Maximum body width	152 (121–201)	204 (158-236)	(51-122)[a]	(137-175)[a]	---------	80.75 (66.93-96.04)
Pharnyx width	---------	---------	---------	---------	---------	32.31 (26.17-41.93)
Testis length	---------	---------	---------	---------	---------	70.48 (60.78-80.19)
Testis width	---------	---------	---------	---------	---------	51.09 (49.47-52.72)
Haptor width	92 (75–123)	127 (96–181)	---------	---------	---------	57.52 (49.44- 65.6)
		Dorsal anchor				
Inner length	32 (30–33)	31 (29–31)	---------	---------	---------	22.52 (21.52-23.53)
Outer length	24 (23–25)	22 (21–23)	---------	---------	---------	17.93 (17 - 18.87)
Inner root	2 (11–14)	12 (11–14)	---------	---------	---------	8.92 (8.54 - 9.31)
Outer root	3 (2–3)	3 (2–4)	----------	---------	---------	3.24 (2.82- 3.66)
Point length	13 (11–15)	15 (12–16)	---------	---------	---------	9.76 (9.46- 10.06)
		Dorsal bar				
Length	32 (32–33)	45 (41–48)	(91-92)[a] 41[b];34[c]	(54-60)[a] 27[b];32[c]	(54-80)	44.41 (42.12-46.17)
Width	5 (3–5)	4 (3–5)	---------	---------	---------	4.68 (4.37- 5)
		Ventral anchor				
Inner length	20 (20–21)	21 (19–22)	---------	---------	---------	17.9 (16.88- 18.92)
Outer length	21 (20–24)	23 (22–24)	---------	---------	---------	16.46 (15.27-17.65)
Inner root	12 (11–13)	12 (9–13)	----------	---------	---------	3.47 (3.21– 3.73)
Outer root	3 (2–3)	4 (3–6)	---------	---------	---------	4.11 (3.91- 4.31)
Point length	10 (8–13)	8 (7–9)	---------	---------	---------	7.87 (7.46-8.29)
		Ventral bar				
Length	37 (35–39)	41 (37–46)	(81-100)[a] 43[b];37[c]	(44-50)[a] 31[b];37[c]	(45-57)	42.83 (42.74-42.92)
Width	7 (6–8)	9 (7–11)	---------	---------	---------	5.87 (5.86- 5.89)
Hook length	12 (11–13)	12 (11–13)	---------	---------	---------	8.73 (6.9- 10.56)
Copulatory organ length	25 (24–30)	40 (40–45)	(65-76)[a] 32[b];27[c]	(34-36)[a] 23[b];27[c]	150	36.03 (31.31 - 39.15)
Host	*Lethrinus rubrioperculatus*	*Lethrinus rubrioperculatus*	*Lethrinus Chrysostomus*	*Lethrinis fletus*	*Lethrinus haemopterus*	*Lethrinus nebulosus*
Locality	Off New Caledonia,	Off New Caledonia,	Off Heron Island, Australia	Moreton Bay, Australia	Off Japan	Red Sea, Egypt
Site of location	Gills	Gills	Gills	Gills	Gills	Gills

- All measurements in the present study are in micrometers (**µm**) and are expressed as the mean followed by the range (minimum and maximum) in parentheses.
- [a] From measurements given in the original text.
- [b] From figures, based on a scale in legend.
- [c] From drawings in the original text of Young (1968).
- --------- Neither given in the original description nor available from published illustrations (figures or drawings).

the male copulatory organ of the present specimens were small compared with those described by Lim and Justine, 2011. There are some parameters which hadn't been measured in the original specimens of Lim and Justine, 2011 as pharynx width, testis length and testis width. Some measured parameters in the present specimens were relative to the measurements of *L. gibbus* examined by Lim and Justine, 2011 as outer roots of the dorsal and ventral anchors, dorsal bar length, dorsal bar width, point length of ventral anchor and length of the ventral bar.

The differences in measurements of *L. gibbus* in the present study and in the original study performed by Lim and Justine, 2011 are due to a little number of the present measured specimens (n=4) compared with the number of measured specimens by Lim and Justine, 2011 (n=14). The rest of recovered worms (5 worms) were destroyed during the method of collection and most of its details were not clear. All of measured parameters between the present specimens and previously recorded specimens from the same genus *Lethrinitrema* are shown in the table (1).

Discussion

There are nine species belonging to *Lethrinitrema*, *L. gibbus* Lim and Justine, 2011, *L. dossenus* Lim and Justine, 2011, *L. chrysostomi* (Young, 1968) Lim and Justine, 2011, *L. fleti* (Young, 1968) Lim and Justine, 2011, *L. lethrini* (Yamaguti, 1937) Lim and Justine, 2011, *L. nebulosum* Sun, Li and Yang, 2014, *L. zhanjiangense* Sun, Li and Yang, 2014, *L. grossecurvitubum* (Li and Chen, 2005) Sun, Li and Yang, 2014 and *L. austrosinense* (Li and Chen, 2005) Sun, Li and Yang, 2014, all parasitized on gills of *Lethrinus* species (Lethrinidae). The presence of *Lethrinitrema* species only on gills of *Lethrinus* spp. suggests a history of host-parasite co-evolution (Lim and Justine, 2011). *L*ethrinitrema *gibbus* differs from the rest of *Lethrinitrema* species in the detailed morphology and measurements of its male copulatory organ, anchors, bars and haptoral reservoirs.

The presence of haptoral reservoirs can be found in many monogenean species as *Allomurraytrema spari* (Yamaguti, 1958) Byrnes, 1986; *Calceostomella inermis* (Parona and Perugia, 1889) Palombi, 1943; *Cichlidogyrus halli typicus* El-Naggar and Kearn, 1989; *Chauhanellus australis* Kearn and Whittington, 1994, *C. youngi* Kearn and Whittington, 1994; *Hamatopeduncularia arii* Yamaguti 1953, *H. major* Kearn and Whittington, 1994 and *H. pearsoni* Kearn and Whittington, 1994. All of these species distinguished by having two haptoral reservoirs. There are another monogenean species having four haptoral reservoirs as members of *Sundatrema* Lim and Gibson, 2009; *Parancylodiscoides* Caballero and Bravo-Hollis, 1961; *Bravohollisia* Bychowsky and Nagibina, 1970; *Caballeria* Bychowsky and Nagibina, 1970 and *Ancyrocephaloides* Yamaguti, 1938 (Lim and Justine, 2011).

There are many differences between all of the mentioned species and *Lethrinitrema* spp. in the following features; the presence or absence of lateral grooves in the anchors; the shape and size of anchors, bars and male copulatory organ and they also differ in the type of hosts (Lim, 1994, 1995a, b, 1996; Lim and Gibson, 2008; Lim and Justine, 20011 and Wong et al., 2008). This genus also differs from members of *Haliotrema* and *Ancyrocephalus* in the intestinal patterns, i.e. the intestinal crura apparently separate, but actually united separately in *Haliotrema* spp. vs the intestinal crura not united posteriorly in *Ancyrocephalus* spp. vs the intestinal caeca form a cyclocoel just posterior to testis and continue as two posterior gut diverticula in *Lethrinitrema* species (Lim and Justine, 2011 and Yamaguti, 1963).

Lim and Justine (2011) recorded *Lethrinitrema gibbus* from the gills of *Lethrinus rubrioperculatus* off New Caledonia, but the present specimens were recorded from the gills of *L. nebulosus* Forsskål, 1775 of the Red Sea, Egypt. Haiyan (2013) recorded this species for the first time from the gills of *L. xanthochilus* from China.

Lethrinitrema spp. recorded from *L. nebulosus* Forsskål, 1775 were *Lethrinitrema fleti* (Young, 1968) Lim and Justine, 2011, *L. nebulosum* Sun, Li and Yang, 2014, *L. grossecurvitubum* (Li and Chen, 2005) Sun, Li and Yang, 2014, *L. zhanjiangense* Sun, Li and Yang, 2014, *L. austrosinense* (Li and Chen, 2005) Sun, Li and Yang, 2014 and the two unidentified species examined by Sun et al., 2014; *Lethrinitrema* sp.1 and *Lethrinitrema* sp.2. To our knowledge, it is the first record of *L. gibbus* Lim and Justine, 2011 from gills of *Lethrinus nebulosus* Forsskål, 1775 in the Red Sea, Egypt.

Conclusion

From the present study, we conclude that *Lethrinitrema gibbus* Lim and Justine, 2011 is recorded for the first time from gills of *Lethrinus nebulosus* Forsskål, 1775 (new host) of the Red Sea, Egypt (new locality). More scanning, transmission electron microscope and molecular studies are recommended to justify the exact taxonomic position of this species. Due to insufficient numbers of examined specimens in this study, we also recommend with more collection of these samples from their lethrinid hosts and from different marine localities to estimate their biodiversity in these regions.

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