On the occurrence of adult females of *lernaea* species (Crustacea: Copepoda) parasitic on goldfish *Carassius auratus* (Linnaeus) in some commercial aquaria in Egypt

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ABSTRACT

The parasitic copepod *Lernaea* species infects the goldfish *Carassius auratus* and induces lernaeosis in commercial aquaria in Egypt. The early stages of *Lernaea* parasites appeared macroscopically as whitish spots. At about 15-24°C, the infected fish with primitive stages were reared for reaching adult females. The former stages gradually change into brownish color and well adapted to attach to body surface of the host fish, causing at least 5 mm inflamed bloody ulcers in the skin, fins, and muscles. Various postmetamorphic adult females of *Lernaea* parasites were differentiated, sketched, measured, and compared with previous related species. The majority of the present females were synchronized on one fish specimen. The adult females were different according to the shape and size of holdfast organ. For the present females, the distinguishable taxonomical character was the wide variability in shape of anchors and their processes (sub-horns). In addition, the variation in general morphology for each form was discernible. Moreover, *Lernaea* was found as economically important parasites. The massive infection reached about 29 parasites/ infected fish during winter. The infected fish became nervous, loosing scales with severe damage particularly at the bases of fins. They also appeared stressed, exhausted and stopped feeding. *Lernaea* parasites also induced disruption, haemorrhage, proliferation or necrosis and edema along the path of penetration in the host tissue. Approximately two months later, white spots appeared on the naturally infected fish and after nearly two weeks, most of them extruded without further modifications.

Key words: Parasitic copepods, Lernaeosis, *Lernaea*, Gold fish, *Carassius auratus*, Egypt.

INTRODUCTION

Isopoda, Branchiura and Copepoda are major groups of Crustacea that contain fish parasites (Özel *et al*., 2004) while the latter group includes learnaeid parasites. The majority of the cyclopoid family Lernaeidae have undergone extensive morphological adaptations hiding their close affinity with the genus *Cyclops* a well known live-food (Piasecki *et al*., 2004). A key to the genera of the family Lernaeidae was given by Kabata (1983). Fish parasites within this

family belong to 14 valid genera (Ho, 1998) and about 37 *Lernaea* species are valid (Kabata, 1979).

Lernaea species are globally distributed and common pests in freshwater fish particularly of cyprinids and other fishes (Piasecki *et al*., 2004). The only cosmopolitan species is *Lernaea cyprinacea* that can infect a wide range of fishes and even tadpoles (Lester & Roubal, 1995; Noga, 1996; Piasecki, *et al*., 2004). In central Europe, the numbers of *Lernaea* have recently declined and in north America, *Lernaea* species infect a number of cultured freshwater fishes (Piasecki *et al*., 2004). *Lernaea cyprinacea* was introduced into south America by importation of common carp *Cyprinus carpio* (Gabrielli & Orsi, 2000). Lernaeids are also infecting fishes in Australia (Hall, 1983; Rowland & Ingram, 1991). In southeast Asia *Lernaea polymorpha* (Yu, 1930) is an economically important parasite of bighead carp and silver carp (Shariff & Sommerville, 1986). In a tropical display aquarium in Malaysia, many species of fish became infected (Shariff *et al*., 1986). *Lernaea cyprinacea* is widely distributed in north America and southern Africa mainly via translocations of edible and ornamental cyprinids (Paperna, 1996; Hoffman, 1970). The majority of *Lernaea* species reported in Africa were with heavy infections and high prevalence especially in association with growing environmental stress in some areas (Oldewage, 1993; Piasecki *et al*., 2004).

Pathogenicity of lernaeids largely depends on the size of their host and attachment site preferences. Aggregated infections of the mouth and lips occur in *Barbus altianalis* with *Lernaea barinmiana* and in cichlids by *Lernaea cyprinacea*, fin infections caused by *Lernaea lophiara* in cichlids (Fryer, 1968; Paperna, 1996). *Lernaea polymorpha* aggregate around the eye and causes destruction of the lens and blindness (Shariff, 1981). *Lernaea* spp. can cause severe fin damage (Shariff & Roberts, 1989). Serious pathology of fish under crowded culture conditions can be caused by female *Lernaea* spp. that are highly metamorphosed vermiform ecto-parasites. Its head is equipped with antlers, the rest of the body and the egg sacs protrude into the water (Dzidziul, 1973; Khalifa & Post, 1976; Stoskopf *et al*., 1993; Piasecki *et al*., 2004). As a rule, *Lernaea* have been the cause of great economic losses of fish in many parts of the world (Shariff & Roberts, 1989). The adults of *L. cyprinacea* leave large holes with round openings in the muscle and skin allow the entrance of secondary microbial infections causing death of the fish (Van Duijn, 1973). Because of lernaeosis, heavy infections with *Lernaea* sp. led to mortality of gold perch *Macquaria ambigua* (Rowland & Ingram, 1991). Essa (2003) cited several references related to lernaeosis and reported that in different provinces in Egypt lernaeosis was caused by most notably *Lernaea cyprinacea* infections particularly in common carp with high prevalence and low one in tilapia species.

Although, few species of *Lernaea* were described in Africa such as those reported by Fryer (1959), anchor worms especially those infecting ornamental fishes still require further research. Herein the present study

represents an attempt to fill the gap in our knowledge of an economically important host that seems to be a burden of taxonomically valid species. Moreover, the intensive fish culture in commercial aquaria gave a great importance to study *Lernaea* parasites that subsequently lead to lernaeosis. Therefore, the aim of the present study is to investigate morphological description and subsequently taxonomic differentiation of postmetamorphic adult females of *Lernaea* species infecting goldfish *Carassius auratus*. Macroscopic alterations due to lernaeosis were occasionally observed. Thereafter, microscopic lesions on the host tissue resulted from present parasites were carefully investigated. Later on, notes on natural host resistance against infection were also included.

MATERIALS AND METHODS

About 42 specimens of freshwater goldfish *Carassius auratus* (Linnaeus) distinguished by whitish spots on most of their body surface were collected from commercial aquaria immediately transported live into clean aerated ones during December 2005 and February 2006 for ectoparasitic examination. The fish of about 5 to 8 cm in length and of 5 to 15 g in weight were investigated macro-and microscopically. The worm-like body forms of lernaeid parasites were examined under light microscope for diagnosis of the infection. The prevalence and intensity of infection for collected samples were determined. *Lernaea* parasites were carefully detached from the infected parts of skin, fins and musculature tissues. Fresh *Lernaea* stages were investigated immediately after separation from host tissue. Each female was photographed, drawn and measured. For histological examinations, portions of different infected tissues were fixed in Bouin's solution, embedded in paraffin wax, cut in about 4 to 5 µm-thick sections and then were stained with haemotoxylin and eosin (H & E). The species identification was done according to literature of family Lernaeidae.

RESULTS

Six lernaeid adult females were isolated from the goldfish *Carassius auratus* during December 2005, with total prevalence of about 33.3 % and intensity of infection about 29 parasites/ fish. The early stages were observed as whitish spots by the naked eye on almost the entire fish body except abdomen particularly at the bases of pectoral, dorsal and anal fins. They were also found as well on the operculum, near to the eye and mouth. The infected fish with whitish spots were reared at about 15-24°C, where adult females were clearly discernible on the host body after about of 24 to 48 hours. Thereafter, all distinguishable postmetamorphic adult females were investigated, measured and described (Figs. 1-18 & 23-28). The body size of each stage was assessed by total body length that started from the tip of anterior extremity until the end, except appendages of posterior extremity that measured 9.25 to 11.07 mm.

Bodies of the present females include regions of cephalothorax that contains the holdfast organ (anchor), trunk, abdomen and posterior extremity. The neck and trunk carry four pairs of legs at definite positions.

Postmetamorphic adult female of *Lernaea* form 1. The general body color is white and its length about 10.23 mm. Small cephalothorax contains mouth surrounded by antennary processes. The head with four pointed dorsal horns that measure about 1.36 mm and ventral ones of 0.92 mm. The trunk is thick particularly at the abdomen somewhat broadens towards its posterior end and includes internal organs such as gut which appeared as a long narrow tube and gradually enlarges till the posterior region. The gut is notably reddish with black patches. At the posterior extremity of the parasite, a pair of caudal leg like-structures of about 0.73 mm long are clearly observable (Figs. 1, 2 & 23).

Postmetamorphic adult female of *Lernaea* form 2. Reddish female of *Lernaea* sp. has a short body of 9.25 mm length. Cephalothorax contained mouth, eyes and a head with 4 horns. Two short ventral horns with two chitinous short pointed pegs are clearly distinguished as sub-horn processes. The dorsal horns were more elongate, smooth and pointed. The trunk is thick gradually towards its posterior end. Portions of the ovary were observed in the posterior regions behind the gut. This seemed reddish with black patches. Legs located at different positions and situations were equipped with spines and setae. The posterior extremity terminated with two short caudal legs of length about 0.34 mm. (Figs. 3 to 5 & 24).

Postmetamorphic adult female of *Lernaea* form 3. This female brownish and had a body length of 11.07 mm. Cephalothorax included head, mouth, and a pair of leg at the neck region. The head has a characteristic holdfast organ. There are two discernible horns with atrophied ventral ones. The dorsal antlers distinguished by two different processes. The right horn is short with a curved tip including round mass, which terminated in a chitinized ridge of different size and shape. The left antler is long and rough including a characteristic rigid thumb measured 0.23 (0.16-0.28) x 0.21 (0.18-0.24) mm in size filling a socket like-shape (Fig. 7). Dorsal arms directed in parallel with the rest of the body. The trunk is narrower in the anterior region than in the posterior region including a distinguishable oviduct and a reddish gut with black patches especially near its tip. The abdomen of the parasite locates in the posterior region. The posterior extremity ends with a pair of caudal legs like-structures in a length about 0.46 mm (Figs. 6 to 8 & 25).

Postmetamorphic adult female of *Lernaea* form 4. The body length measured about 9.60 mm. Anchor with relatively two long dorsal horns and a short ventral arm were clearly discernible. The dorsal arms processed by characteristic two chitinized sub-horns emerged nearly from the middle of each arm. The trunk is clearly thick toward the posterior region of the parasite. The gut was dark brownish with a few pale patches. Legs located at approximately the same arrangement as previous forms. A pair of caudal legs at the posterior

extremity measured 0.72 mm. A rod like-structure of 0.39 mm at the center characterized the young egg (Fig. 10) that measured about 0.23-0.36 mm in size and released after pressing on the parasite specimen (Figs. 9 to 11 & 26).

Postmetamorphic adult female of *Lernaea* form 5. An adult female (Figs. 12 to 16 & 27a, b, Table 1) was with incompletely exposure its egg sacs. These were relatively short measured about 0.56 x 0.21 mm in size, suspended from body by a distinct membrane and contained eggs with dimensions about of 0.09-0.10 mm. The body length of this female was about 11.0 mm. Small head at the anterior tip included three black round spots (eyes). The latter surrounded by a thin membrane like-sheath (Figs. 15 & 27c). Anchor was with relatively two long dorsal arms and short pair smooth ventral ones. The dorsal arms are characterized by two chitinized processes (sub-horns). Both anterior and posterior dorsal arms are furcated before their ends into two branches; one smooth and one processed by a chitinized thumb like-shape. Thorax seems cylindrical connecting with the trunk at the anterior region. The trunk includes long irregular gut reaching posteriorally to the abdomen as well as the characteristic pre-genital prominence and legs are in definite sites of the body. The caudal legs are discernible as setae like-shape at the posterior extremity (Figs. 12 to 16 & 27a to c).

Postmetamorphic adult female of *Lernaea* form 6. The adult female with completely exposed egg sacs was measured (Table 1) and illustrated (Figs. 17, 18 & 28). Egg sacs were relatively short with a length about 1.80 mm and a width of 0.24 mm. Each egg sac contains approximately 32 to 50 spherical eggs with dimensions of about 0.03-0.08 mm and mean of 0.06 mm. A relatively thin membrane (Figs. 18) covers the two egg sacs. The body length measured 9.56 mm. Head distinguishes by two dorsal rough horns. Different shapes of chitinized thumbs, stud, peg and ridges processed antlers are either at the middle of the horn or at its terminal end. There is also difference in the size of anchors and their chitinized processes. Three distinct black spots seem to be eyes and measured about 0.01-0.03 mm with a mean of 0.02 mm and were located at the anterior tip of the head within a socket-like shape. The mean length of the cephalothorax is shorter than the trunk, measured about 1.50 mm with greatly modified anchors. The trunk was connected with the cephalothorax through a transverse construction like-appearance. Trunk is gradually thick toward its posterior region including gut, which is simple long tube. The gut was reddish and filled with black patches. At the posterior extremity, caudal legs seemed as two small plates' like-shape with mean length 0.13 mm (Figs. 18 & 28).

Table 1. Measurements of postmetamorphic adult females of *Lernaea* forms 1 to 6 in mm

Female	Body length	Body width Anterior	Body width Posterior	Horns Dorsal	Horns Ventral	Sub-horns	Egg sac Length	Egg sac Width	Caudal legs
1	10.23 (10.0-10.46)	0.45 (0.36-0.53)	0.50 (0.36-0.60)	1.36 (0.60-2.13)	0.92 (0.32-1.98)	-	-	-	0.73 (0.44-1.01)
2	9.25 (9.04-9.46)	0.65 (0.32-1.1)	0.49 (0.44-0.53)	1.83 (0.92-3.10)	2.66 (1.32-4.70)	0.56 (0.24-1.0)	-	-	0.34 (0.30-0.32)
3	11.07 (10.8-11.34)	0.34 (0.28-0.40)	0.64 (0.39-0.56$^{\Phi}$)	0.99 (1.16*-0.82)	**	0.28 (0.16-0.68)	-	-	0.46 (0.43-0.48)
4	9.60 (9.52-9.67)	0.32 (0.28-0.36)	0.56 (0.44-0.80$^{\Phi}$)	1.58 (1.26-1.96)	0.70 (0.52-0.88)	0.66 (0.64-0.72)	-	-	0.72 (0.64-0.80)
5	11.0 (10.76-11.30)	0.23 (0.16-0.30)	0.43 (0.20-0.80$^{\Phi}$)	0.65 (0.35-1.24)	0.47 (0.10-0.28)	0.19 (0.13-0.24)	0.56 (0.44-0.68)	0.21 (0.17-0.24)	0.23 (0.17-0.28)
6	9.56 (9.44-9.67)	0.24 (0.16-0.36)	0.33 (0.25-0.40$^{\Phi}$)	0.73 (0.50-0.96)	-	0.19 (0.07-0.39)	1.42 (1.03-1.80)	0.14 (0.08-0.24)	0.13 (0.09-0.16)

$^{\Phi}$At abdomen, *left, **atrophied

Histology

Internal structures of young and adult specimens were histologically investigated (Figs. 19 - 22). Cuboid cells with dark patches are distinguished the gut of most stages. The gut starts from the buccal to the end of posterior region. In this situation, the parasite ends with a telson and caudal appendages at its extremity (Figs. 19 & 21). The reproductive organs particularly ovary and cement gland connected with oviduct are clearly discernible. The ovary is surrounded by irregular thick wall. Around ovary, peripheral masses of small spherical vacuoles, probably early oogonia like-spheres are noticed. Developing egg sac seems within abdominal region (Fig. 20). On the other hand, the growth of oocytes may lead to increase the mass of cytoplasm (Fig. 22).

Host-parasite interactions

Gross pathology. The postmetamorphic adult females penetrate most of the body surface where the parasite changes gradually into dark reddish or brownish color causing inflammation (of nearly 5 mm bloody ulcers) in skin, fins and muscles at raised temperature to 24°C. The intensity of infection was about 29 parasites/ fish during winter. The infected fish were nervous with dropping scales and bleeding spots particularly at the bases of fins. They were also stressed, exhausted and stopped feeding.

Microscopic lesions. In the present study, the anterior most region of *Lernaea* embedded in the host tissue caused severe histological alterations. These are oedema along the way of penetration, haemorrhages in tissue surrounding the infection site (Fig. 29) and a fibroid sheath around the head. This caused disruption of the infected tissues in the ulcerative area particularly at the fin base (Fig. 30). The tongue like-process ending with the holdfast organ

induced congestion and tissue proliferation (Fig. 31). In addition, the holdfast organ of the parasite extended into myofibril tissue of the host trunk that became necrotized and sloughed beside a lot of fat tissue around the attachment site of infection (Fig. 32).

Immune response. Fish resistance against infection was occasionally observed. In February, fish of about 5 cm length and 5-10 g weight were infected with white spots that were extruded after approximately two weeks by the host.

DISCUSSION

Although *Lernaea* species such as those reported by Kabata (1979), Shariff and Sommerville (1990), Lester & Roubal (1995) and Ho (1998) were recorded from various hosts and localities allover the world, no *Lernaea* species was so far recorded from freshwater goldfish in Egypt. So, the present material was recorded for the first time in this study. A description of six adult females of various forms of *Lernaea* species was widely different morphologically and hence the description based on shape beside size of the parasite. To enable accurate identification of species, several taxonomical characters were considered. Species in the genus *Lernaea* based mainly on morphology of anchors that their growth greatly affected by the consistency of the infected tissue. Paperna (1996) reported that differentiation to lernaeid genera and species in the genus *Lernaea* was based mainly on the morphology of anchors and according to the number of horns of the parasite females, head with 2-3 (rarely 4). The head of the present females has 2-4 horns even this was differentiated according to shape and size of sub-horn processes. In agreement with Harding (1950) and Fryer (1956) the posterior region is characterized by a double pregenital prominence that is also distinguished the present females with additional appendages of the posterior extremity. Moreover, the color of the parasite body seems also to be an important characteristic. The body of present stages was whitish to dark brown colour. Shariff & Roberts (1989) reported that the transparent body of *Lernaea polymorpha* became milky and finally an ivory color. In addition, the host and infection site were also taken into consideration.

All the present forms were isolated from the skin, fin and muscles of goldfish *Carassius auratus* despite *Lernaea cyprinacea* found in different hosts including the present host and sometimes found on carp co-existing with *L. barbicola* and *Lernaea barnimiana* (Van Duijn, 1973; Van As & Basson, 1984; Paperna, 1996). Moreover, Lester and Roubal (1995) reported that *Lernaea elegans* a morphologically similar parasite that is found on a range of fishes (Bauer, 1991). It is worthy to mention that, optimum temperature of flourishing parasites of the present *Lernaea* females was of 15-24°C. On the other side, *Lernaea* sp. flourishes and reproduces more utilizing a number of fish cultured for food and ornamental purposes at temperature of 25-28°C (Shields & Tidd, 1968). In addition, *Lernaea cyprinacea* flourishes at temperature 20-30°C but

21-25°C is suitable to hatch *Lernaea barnimiana* as reported by (Molnár & Scakolczai, 1995; Paperna, 1996). Moreover, Noga (1996) reported that *Lernaea cyprinacea* does not reproduce at less than 14°C.

On the other hand, these parasites exhibit gregarious behavior and tend to attract other larvae to the site of infection as noted by Fryer (1995). Consequently, different forms probable co-exists together on one host as presented in the current study. As regards to previous situation, the present females probably are females of different species unless they belong to one or two species. In addition, Shariff & Roberts (1989) and Lester & Roubal (1995) reported that *Lernaea cyprinacea* and *L. polymorpha* require only one host to complete their life cycle. Moreover, Noga (1996) reported that marine aquarium fish are rarely infected with morphologically similar but taxonomically unrelated species. Although, Poddubnaja (1973) emphasized that the validity of *Lernaea* species required following the different immature stages for established valid species by producing different phenotypes resembling different described species of *Lernaea* from a single matured specimen. In this study, the description was on a fresh adult female specimen as far as it seems to be not enough criteria to distinguish species.

In agreement with Lester & Rubal (1995), the taxonomy of the new *Lernaea* species also based on the shape of horns, which still an important taxonomical character in differentiation of the *Lernaea* genus as well as species. Although they reported that the shape of anchors, a different character not only because of their inherent variability but also because their shape is modified by bone or other resistance the anchors encounter during development in the host (Fryer, 1961; Thurston, 1969). It was possible to compare the present females with previous described species from other different hosts. Thus far, six females (Table 1) were compared with related species (Table 2) that their majority were with considerably differences in shape and minor similarities in size. Body lengths of the present *Lernaea* were measured about of 9.25-11.07 mm, though Piasecki *et al*. (2004) reported that female *Lernaea* reach lengths of 12-16 mm. *Lernaea cyprinacea* and particularly *L. polymorpha* were the most related species with the present females.

The present females appear very close to the shape of the adult stage of *Lernaea cyprinacea* and that of *Lernaea polymorpha*, with minor differences in size (Tables 1 & 2) particularly body length and horns. Female of *Lernaea* form 5 was characterized by the T-shape holdfast organ and relatively short ventral arms approaching to the shape of *Lernaea polymorpha* (Shariff & Sommerville, 1990). This female seemed widely different from *Lernaea cyprinacea* although some similarities in measurements. By comparison of female form 5 with *Lernaea polymorpha* body lengths and dorsal horns, were with minor differences. The body width was relatively smaller in female form 5 than in *Lernaea polymorpha*. The egg sac in this femaleis was shorter and thinner than

in *Lernaea polymorpha*. Therefore, female form 5 more closed to *Lernaea polymorpha*.

Table 2. Measurements of previous related species in mm

Species	Body length	Body width Anterior	Body width Posterior	Horns Dorsal	Horns Ventral	Sub-horns	Egg sac Length	Egg sac Width	Caudal process
***L. hardingi*[Δ] (Fryer 1959)**	**10.23**	**0.28**	**0.28 0.41[Φ]**	**1.04 (0.83-1.24)**	**1.17 (1.11-1.24)**	**-**	**1.24 (.97-1.52)**	**0.17 (0.14-0.21)**	**0.28**
***L. cyprinacea*[I] (Shariff & Sommerville 1986)**	**10.75**	**0.38**	**0.63**	**1.38 (1.25-1.50)**	**1.25 (1.0-1.50)**	**0.38 (0.25-0.50)**	**3.63 (3.50-3.75)**	**0.50**	**0.25**
***L. polymorpha*[I] (Shariff & Sommerville 1986)**	**11.50**	**0.38**	**0.75**	**2.19 (2.13-2.25)**	**0.19 (0.13-0.25)**	**0.13**	**3.69 (3.50-3.88)**	**0.57 (0.50-0.63)**	**-**
***L. cruciata*[I] (Kabata 1988)**	**8.40**	**0.40**	**0.80**	**1.90 (1.80-2.0)**	**-**	**1.75 (1.70-1.80)**	**-**	**-**	**0.20**

[Δ, I]Approximate measurements from drawings of species by Fryer (1959) and Lester & Roubal (1995) respectively. [Φ]At abdomen.

Postmetamorphic adult female of *Lernaea* form 6 seems to be a new *Lernaea* species. Although, Poddubnaja (1973) emphasized the validity of the species of *Lernaea* may become important only to follow the different immature stages for established valid species by producing different phenotypes resembling different described species of *Lernaea* from a single matured specimen. Female 6 may also be compared with previous related species from different hosts (Tables 1 & 2) such as *Lernaea hardingi* reported by Fryer (1959). In addition, Lester & Rubal (1995) redrawn and reported species from Kabata (1988) and Shariff & Somerville (1986). Female 6 was widely different from previously described forms particularly in shape of the holdfast organs. This parasite seems similar to *Lernaea polymorpha* although there are minor differences in shape and size (Shariff & Sommerville, 1986; Lester & Rubal, 1995) particularly of body length, width at the posterior region, dorsal horns, chitinized processes, egg sac dimensions and probable posterior extremity. There is also similarity in body width at the anterior region. *Lernaea* form 6 seems with two horns beside sub-horn processes. This female is widely different from *Lernaea cruciata* (Kabata 1988) except lacking ventral horns. *Lernaea* form 6 was characterized by relatively short egg sacs measured of 1.8 mm although Piasecki *et al*. (2004) reported that egg sacs of female *Lernaea* reach of 6 mm. In addition, each egg sac of *Lernaea* form 6 contained about 32-50 eggs different from some previous species, such as those in *Lernaea barnimiana* that produces 75-205 eggs (Paperna, 1996). Finally, consider shape and size of twin egg sac, holdfast organ, mouthparts, and appendages of the posterior extremity of postmetamorphic females in the systemic status of *Lernaea* species as basic taxonomical characters. Therefore, female 6 might be cited as a new species infected goldfish *Carassius auratus*. Boxshall *et al*. (1997) described a new

species of *Lernaea*, closely related to *L. cyprinacea* in Brazil and concerned on its appendages therefore form 6 still need further study.

Other points on morphology as observed in fresh specimens and histological sections of present females. The shape and length of the caudal legs at the posterior extremity varied in size or modified in shape (Figs. 2, 5, 8, 11, 16 & 18). As a rule, wide differences were in cephalothoraxes particularly in shape and size of horns with their chitinized processes (sub-horns) beside caudal appendages at the posterior extremity.

Majority of the present females has well developed gut in agreement with Fryer (1959) may be to obtain sufficient food to produce a continual succession of eggs and maintain itself. Herein, the gut is mostly characteristic organ located within body like-tube of the parasite started by mouth. Moreover, in majority forms the gut appeared with dark patches may be due to metamorphosed females ingest tissue debris and erythrocytes released by mechanical change from their mouthparts and possibly from the secretion of digestive enzymes (Khalifa & Post, 1976; Shariff & Roberts, 1989).

The present parasites are hazard to goldfish *Carassius auratus* of different sizes in commercial aquaria with prevalence about 33.3%. Also Dempster *et al*. (1988) reported that the anchor worms are pathogenic to small fish in commercial aquaria in spite of Gabrielli & Orsi (2000) reported in fish farms 100% prevalence of infection with *Lernaea cyprinacea* found in *Cyprinus carpio*.

Pathogenicity of the present *Lernaea* largely evokes ulcers and inflammation around the attachment site of infection and probably economic loss occurs especially in case of heavy infection. The intensity of infection reached about 29 parasites/ fish in spite of Faisal *et al*. (1988) reported that, six or more females of *Lernaea cyprinacea* fingerlings slowed the growth of cultured *Cyprinus carpio* and *Ctenopharyngodon idella*. Although, the infection by a single female or more was highly damage to young or small fish Paperna (1996) reported that heavy infections of *Lernaea barnimiana* recorded up to 106 per fish.

As a rule, large numbers of *Lernaea* can cause serious problems resulted from severe wounds. In agreement with Khalifa & Post (1976) penetration of lernaeid females associated with punctuate hemorrhages reach to approximately 5 mm across as the parasite growth. In similarity with findings of Lester & Roubal (1995), that muscle necrosis is evident at the anterior end of penetrating parasites, epithelial tongues extend into the muscles surrounded the head with leukocyte infiltrating, after several changes a thick sheath envelopes the anterior end within the host tissue. The tissue around the anchor turns into a granuloma or necrotic tissue beside localize hemorrhagic lesions may stress the host fish then allow the entrance of secondary, bacterial and fungal infections (Kabata, 1970; 1985; Shariff & Roberts, 1989; Stoskopf *et al*., 1993; Barnham, 1998; Piasecki *et al*., 2004).

Herein, goldfish *Carassius auratus* might stimulate its immune response against infection with whitish spots that extruded after approximately two weeks may be due to resistant fish contained more eosinophilic granule cells and lymphocytes (Shariff & Roberts, 1989). As well as, Lester & Roubal (1995) reported that experimentally infected goldfish with *Lernaea cyprinacea* that rejected by the host week after maturation of the first egg sacs and wounds left by rejected females healed rapidly (Shields & Goode, 1978). Moreover, Noga (1986), Shariff *et al*. (1986) and Woo & Shariff (1990) reported that in several fish species had acquired resistance against *L. cyprinacea* and few new infections of *L. cyprinacea* developed in *Helostoma temmincki* that had recovered from an earlier infection. Finally, experimental and molecular evidences for the present forms may be the subject of further study.

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EXPLANATION OF FIGURES

Figs. 1 to 13. Photomicrographs of fresh preparations of postmetamorphic adult females of *Lernaea* species infected body surface of freshwater goldfish *Carassius auratus*. Scale bars 0.1 mm except Fig. 15 about 0.02 mm.

Fig. 1. Anterior region of body of female form 1 surrounded by soft tissue (h) and showing head and anchor. The arrow shows site may become sub-horn process.

Fig. 2. Posterior region of same previous specimen indicates discernible caudal legs (arrow).

Fig. 3. Anterior region of the body of female of *Lernaea* form 2 showing smooth dorsal horns (arrows). Note ventral horns with dark chitinized processes (sub-horns).

Fig. 4. Higher magnification of the female form 2 shows ventral horns terminated with chitinized peg like-shapes (arrows).

Fig. 5. Posterior region of the previous female shows caudal-legs (arrow) at the posterior extremity.

Fig. 6. Anterior and middle regions of the female form 3. Note different characteristic chitinized processes (arrows) of holdfast organ and second pair of leg (arrowhead).

Fig. 7. In the former specimen, other position of a chitinized thumb like-appearance on the antler (arrowhead) established within socket like-shape (arrow).

Fig. 8. Posterior region of same previous specimen shows the caudal leg (arrow).

Fig. 9. Anterior region of the female of *Lernaea* form 4 showing characteristic sub-horns of dorsal arms (arrows).

Fig. 10. A young egg released from the above former specimen showing a characteristic rod like-shape at the center (arrow).

Fig. 11. Posterior region of female form 4 shows the caudal-legs (arrow).

Fig. 12. Anterior region of adult female form 5 shows chitinized processes of dorsal horn (arrow) and smooth ventral arm (arrowhead).

Fig. 13. Other view of the female form 5 shows bifurcation (arrowhead) of dorsal horns into smooth and dark chitinized sub-horns (arrow).

Fig. 14. Higher magnification of lateral view of the posterior region of the former specimen shows abdomen and pregenital prominence (arrow) and incompletely exposure egg sac (arrowhead).

Fig. 15. The anterior tip of head of the female form 5 shows three black round spots seem to be eyes (arrow) surrounding by membranous sheath like-shape.

Fig. 16. Posterior extremity of former specimen shows caudal appendages similar to shape of setae (arrow).

Fig. 17. Anterior region of postmetamorphic adult female of *Lernaea* form 6 shows characteristic holdfast organ. Note shape and number of sub-horns with terminal chitinized thumbs like- shape (vertical arrows) and note other emerged chitinious processes from the arms (arrows).

Fig. 18. Lateral view of posterior region of female form 6 showing egg sac (arrow). Note atrophied caudal appendages (arrowhead).

Fig. 19 to 22. Histological sections of parasitic females of *Lernaea* infected host tissues show some their internal structures. Stained with H & E, Scale bar 0.1 mm.

Fig. 19. Longitudinal section of the anterior region of female embedded within host tissue (arrows) showing buccal region (Br), anterior region of the gut (Ag).

Fig. 20. Posterior region of adult female showing ovary (O), cement gland above oviduct (Ov), a probable portion of hidden egg sac (S) within wide abdominal region (arrowhead).

Fig. 21. Free posterior region of same previous specimen. Note black patches in gut tissue (Pg). In addition, posterior appendages include telson like-shape (T) with caudal legs (Ca) at the posterior extremity of the parasite.

Fig. 22. High-magnified portion of the posterior region of female shows characteristic ovarian wall (ow) around the ovary (O) and probable developing oogonia (arrowhead).

Fig. 23 to 28. Schematic illustration of postmetamorphic adult females of *Lernaea* forms 1 to 6 infected the host fish *Carassius auratus*. Scale bar 0.1 mm.

Fig. 23. Female of *Lernaea* form 1. A: mouth surrounding by antennary processes, B: holdfast organ, C: antlers, D: second pair of legs, E: gut, F: trunk, G: third leg, H: abdomen, I: caudal leg.

Fig. 24. Female of *Lernaea* form 2. A: eyes, B: mouth, C: dorsal horn, D: ventral horn terminated with chitinized peg like-shape, F: first pair of legs, G: trunk, H: gut, I: ovary, J: caudal legs.

Fig. 25. Female of *Lernaea* form 3. A: holdfast organ, B: antler, C: chitinized process (sub-horn) or thumb like-shape, D: mass terminated with chitinized ridge (E), F: first pair of legs, G: one of second pair of legs, H: trunk, I: gut, J: one of the third pair of legs, probable ovary (O), K: oviduct, L: abdomen, M: caudal legs.

Fig. 26. Female of *Lernaea* form 4. A: dorsal arm, B: ventral arm, C: process of dorsal arm, D: one of second pair of legs, E: trunk, F: gut, G: probable portion of ovary or oviduct, H: one of third pair of legs, I: abdomen, J: caudal legs.

Fig. 27. a. Adult Female of *Lernaea* form 5 showing incompletely exposure egg sacs. A: eye, B: anterior branch of dorsal arm of anchor with chitinized process, C: ventral arm of anchor, D: first pair of legs, E: gut, F: second pair of leg, G: trunk, H: site of the third pair of legs, I: abdomen and pre-genital prominence, J: egg sac in lateral view, K: caudal legs. b. Anterior region of previous form shows posterior branch of dorsal arm (N) that furcated into smooth terminal horn (L) and chitinized process (M). c. anterior tip indicates 3 round dark spots or eyes (O).

Fig. 28. Postmetamorphic adult female of *Lernaea* form 6. A: mouth, B: anterior branch of the dorsal arm terminated with chitinized thumb like-shape (C) and other stud at nearly the middle (D), posterior branch of dorsal arm processed with chitinized thumb (E), chitinized peg (F) and chitinized ridge (G), junction of neck (H) with trunk (I), J: gut, egg sacs (K), L: posterior extremity.

Fig. 29. Longitudinal section of female parasite at the bases of tail fin (arrowheads) induced tunnel like-appearance leading edema along mostly way of penetration in the host tissue. Hemorrhages (h) distinguished in tissue around the site of infection. Note also the free posterior region outside the host tissue (arrow).

Fig. 30. L. S. of the anterior region of *Lernaea* parasite showing mostly of its body embedded within tissues of the anal fin (arrows). Note fibrotic capsule like-structure (arrowhead) around holdfast organ (h). In addition, some horns are visible. Note severe damage, disruption (d) and broken rays (b) probably occurred by the parasite in ulcerative contour tissue near to holdfast organ (h) different from control tissue (c).

Fig. 31. Connective tissue (a) around the holdfast organ (arrowhead) and tongue like-shape (arrows) at the site of infected tissue of the pelvic fin are clearly discernible. Note mild oedema (b), congestion (c), disruptive (d) and tissue proliferation (e).

Fig. 32. Anterior region shows anchor (arrowhead) embedded in white muscular tissue of the host. Note sloughed myofibril (s), necrosis (lower arrow) and fat tissue (f, upper arrow) may similar to spongiotic tissue.