

Guppy (*Poecilia*) Poeciliidae fish naturally infected with *Lernaea cyprinacea* parasites (Linnaeus 1758) in KSA

Original
Article

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ABSTRACT

Background: The ornamental fish guppy (*Poecilia reticulata*) is a small colorful tropical cyprinid teleost fish. *Lernaea cyprinacea* (Anchor worm) are worldwide, crustacean copepod parasites that cause disease and mortality in several fish species of cultured or natural populations, especially wild-caught and pond-raised species of *Poecilia*. This study may be considered as a novel report from Kingdom of Saudi Arabia (KSA).

Objective: The present study is an investigation of *Lernaea* isolates infecting apparently healthy *Poecilia reticulata* from KSA.

Material and Methods: Guppy fish (*Poecilia reticulata*) were purchased two weeks prior to experiments. They were examined for parasitic infection and allowed to breed in the laboratory. They were observed for 15 days for appearance of infection by *Lernaea* spp. larva and adult parasites. The copepod specimens were removed by forceps, from different parts of the infected fish. Specimens were fixed in 70% alcohol, cleared in 90% lactic acid, mounted, and microscopically examined to identify the morphological features of *L. cyprinacea*.

Results: After 15 days, *L. cyprinacea* were detected in the ventral, anal and caudal fins of several *P. reticulata*. Intense focal inflammation and hemorrhage was easily observed at the attachment site, which appeared red and ulcerated. Total prevalence of infection was 68.1% (32/47). The prevalence of infection in females (29/38; 76.3%) was greater than in males (3/9; 33.3%).

Conclusion: *P. reticulata* may be considered as a newly recorded host of *L. cyprinacea* from KSA.

Keywords: copepoda, guppy, *Lernaea cyprinacea*, *Poecilia reticulata*.

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INTRODUCTION

Lernaea species are crustacean, copepod parasites that can infect and cause disease and mortality in many types of freshwater fishes, especially wild-caught and pond-raised species (both cultured and natural populations). *L. cyprinacea* (commonly known as Anchor worm), a parasitic cyclopoid copepod, is found worldwide; mostly in cyprinids. Several species (~110 species) of lernaeids (*Lernaea* and *Lernaea*-like parasites) have been described and *L. cyprinacea*, is one of the more common among them⁽¹⁾.

The economic importance of lernaeids has increased in several parts of the world as the cause of one of numerous diseases among most farmed fish species; especially fingerlings, leading to death even with only a small number of infested lernaeids⁽²⁻⁴⁾. Death of fish and/or associated damage is relative to the rate of parasitic infestation⁽⁵⁾.

Infestations occur more commonly in stagnant or slow-moving water bodies. *L. cyprinacea*, exhibits

little host specificity and has been reported previously from a variety of freshwater fishes, frogs, frog tadpoles, and adult and larval salamanders. Although infestations are common in cyprinids, including koi, goldfish, and other related carp, numerous other freshwater species are susceptible⁽⁶⁻⁸⁾. *Lernaea* species have nine stages in their life cycle, including three free-living naupliar stages, five copepodid stages and one adult stage. During development, the different stages live on and off the fish. After mating, the male copepod dies and the female bores into fish tissue, eventually using a large anchor anterior end "head" to permanently embed into the skin and muscle of the fish^(9,10). This direct life cycle can take from 18 to 25 days to complete and only a fish (or an amphibian) is necessary for *Lernaea* spp. to develop from egg to mature adult. Common sites of *Lernaea* infestation include the skin, fins, gills, and oral cavity. Large numbers of lernaeids in their copepodid stages can kill small fish by damaging their gills and interfering with their breathing. Fish can survive with *Lernaea* infestation, but chronic conditions frequently result in poor growth and debilitation; the fish become more susceptible to secondary infection by bacteria and fungus which ultimately kills them⁽¹¹⁻¹⁴⁾.

Guppy (*P. reticulata*) is a small and colorful tropical ornamental teleost. It is a member of the family Poeciliidae⁽¹⁵⁾ that undergo internal fertilization⁽¹⁶⁾. Guppies, whose natural habitat is in northeast South America, were introduced to many countries and are now found all over the world. They are highly adaptable and thrive in many different environmental and ecological conditions⁽¹⁵⁾. Male guppies, which are smaller than females, have ornamental caudal and dorsal fins, while females are duller in color.

In several tropical countries they were used for biological control of mosquito larvae, the vectors of infectious malaria disease^(17,18) and filariasis⁽¹⁹⁻²¹⁾. The guppy became a model for biological studies because of its short generation interval, ease of breeding in laboratories, and the availability of many different strains⁽²²⁻²⁴⁾.

Although *L. cyprinacea* parasites infect a wide range of both fish culture and natural populations fishes, few species of *Lernaea* were described especially those infecting ornamental fishes that still require further research. This study investigates *Lernaea* infections in guppy (*P. reticulata*), the ornamental, small and colorful fish, in a sample from KSA.

MATERIAL AND METHODS

The present study was carried out in the laboratory of Zoological Research, Biology Department, Faculty of Science, Taif University, KSA. From a local breeder in El-Taif, KSA, 47 fish (38 females and 9 males) guppy (*P. reticulata*) specimens (only colored, active, healthy and sexually mature males and females) were purchased (Figure 1) and transferred to the aquaria two weeks prior to the examination. They were allowed to breed in the laboratory. The fish were daily examined for external copepod parasitic infection. All aquaria were provided with thermostats 100 W, thermometers, air pumps, air lines, and gravel cleaner or dip tube (Figure 2). The water in the aquaria was obtained from a header tank containing constantly aerated (dechlorinated) water composed of deionized water mixed with local tap water (5:1). The tap water was analyzed, and its specifications were as follows: pH 8.46; total dissolved saline 2.52 ppm; conductivity 0.0054 mS/cm. After 15 days, infection with the larvae and adult of copepod parasites was observed. The adult copepod specimens were removed by forceps from different parts of the infected fish; skin and pectoral, ventral and dorsal fins. Specimens fixed in 70% alcohol were cleared in 90% lacto phenol and mounted in dibutylphthalate polystyrene xylene

(DPX). The mounted specimens were examined and photographed using a Zeiss light microscope⁽²⁵⁾. The morphological identification of the *L. cyprinacea* specimens was performed according to Robinson⁽²⁶⁾.

Animal use followed a protocol approved and authorized by Institutional Animal Care and Use Committee (IACUC).

Statistical analysis: The copepod preference for attachment sites on the host was recorded. Infestation parameters, prevalence and intensity were calculated by QP3.0 (Quantitative Parasitology 3.0). This parasitology software provides statistically correct medians to analyze the highly aggregated (right-skewed) frequency distributions exhibited by parasites. QP3.0 describes parasitic infections within a sample of hosts and compares parasitic infections across different samples of hosts. Mann-Whitney test was applied to test the significance difference in parasite number at ≤ 0.05 using SPSS.

RESULTS

Apparently healthy guppy fish (*P. reticulata*), small colorful tropical ornamental teleosts, were purchased and examined for ectoparasites (Figures 1-3). They were kept under observation for signs of infection related to change in color and active movement. After an observation period of 15 days, natural infection was gradually noted in 32/47 fishes (29 females and 3 males) with a prevalence of 68.1%. By examination of fixed specimens, the body of adult *L. cyprinacea* female appeared long and tubular with an anchor on its anterior end and paired egg sacs on the posterior end. The anterior anchor was embedded into the fish's body while the posterior end, with its egg sacs, protruded out into the water (Figures 4-7). Adult females had a body length of 10.3-11.10 mm; body width of 0.36-0.40 mm to 0.50-0.56 mm from anterior to posterior respectively; length of horn ranged from 1.38 to 1.43 mm; egg sac length and width were 3.3-3.9.0 mm and 0.50-0.54 mm, respectively.

Adult females were anchored to host fins, feeding on the epithelial cells and mucus of the fish, characteristically raising blisters on the surface of the fins. The female parasites were found burrowed deep into the tissues with the anterior anchor embedded into the fish's body. Intense focal inflammation and hemorrhage were easily observed at the attachment site, making the area appear red and ulcerated. The scales of infected fish fell off.

Fish with severe infection (more than 5 parasites per fish) on their body surface showed dull colors and sluggish movement. Newly hatched nauplii observed



Fig. 1. Male and female Guppy.



Fig. 2. The aquarium with the optimum condition.



Fig. 3. Female Guppy carrying *Lernaea* on the anal fin.



Fig. 4. Number of *Lernaea* heads embedded in the fish tissue of ventral fin (X10).



Fig. 5. An adult female of *Lernaea* anterior part of the body full of blood (X40).



Fig. 6. Caudal fin of Guppy with attached *L. cyprinacea* (X40).



Fig. 7. Larval *Lernaea* attached to the rays of the caudal fin (X40).

on the caudal fin of the infected fish (not parasitic), develop through naupliar stages (Figure 6), after which they became parasitic, and attached to the host. The copepod stages were found on the gills but were not permanently embedded in the tissue.

The infection was high in the female guppy reaching 76.3 % (29/38) and decreased to 33.3% in male guppy (3/9). Number of parasites was significantly higher in females than males ($P < 0.05$) (Table 1), fixed especially on ventral and anal fins (Table 2).

Table 1. Prevalence of infestation, mean abundance and intensity, median r of the parasites *Lernaea cyprinacea* in relation to sex.

Sex	Range*	Prevalence	Mean abundance	Median	Mean intensity	r
Female	1-12	76.7%	0.97	4	4.33 ± 0.50	2.99
Male	2-5	33.3%	0.33	2	3.0 ± 0.58	3

* Range of parasites number on each infested host.

Table 2. Parasite sites and attachment-preference on Guppy (*Poecilia reticulata*).

Sex	Skin (n)	Pectoral fin (n)	Ventral fins (n)	Dorsal fins (n)	Anal fins (n)	Caudal fins (n)
Female	6	7	12	2	8	5
Male	2	0	5	0	0	2

n: Number of copepod specimens

DISCUSSION

The ornamental guppy (*P. reticulata*) fish, though apparently healthy at time of purchase proved to be naturally parasitized by ovigerous female *L. cyprinacea* on their skin and fins. Other records in the literature show that *Lernaea* infestations are particularly common in fish of the carp family (*Cyprinidae*), including koi, goldfish, and other related carp, as well as other numerous susceptible freshwater species. About 64 species of fish were recorded as host for *L. cyprinacea*^[27]. The same researchers stressed the importance of careful examination of all aquatic species for anchor worms to determine parasite infestation by *L. cyprinacea*. In 2017, Baliga *et al.*,^[28] isolated copepods that had different anchor shapes, from freshwater fishes including *P. reticulata*; and proved that they were genetically identical belonging to the same *L. cyprinacea* species.

The present study is the first record of infection by *L. cyprinacea* (68.1%) in this sample of guppy fish examined from KSA. Although *L. cyprinacea* seems to have a random choice for attachment on the body host, it was shown that the parasites were fixed especially on the fins and skin^[29]. The same observations were previously reported in many other literatures^[30-34]. This may be because fins offer greater protection against currents and tissues and the base of the fins are more easily penetrated^[33,35,36]. In our study there is enough evidence to conclude that there is a significant difference ($P < 0.05$) in number of parasites between infected males and females (Table 1). The study revealed that the parasites have preference for fixation in descending order for: ventral, anal, and pectoral fins; skin and caudal fins came last. Females were significantly more susceptible to infection than males and even the parasite load of females doubled that of

the males. It was reported that severe focal damage to affected tissues caused by parasites, increases the risk of infection by bacterial and fungal pathogens present in the environment. In flowing rivers and streams, anchor worm infections are usually limited to a single parasite per fish host, causing little damage, but in closed environments severe infestations often result^[29,37]. Suggested differences in susceptibility of fish species to the parasite could be due to differences in ecological, behavioral and physiological mechanisms, and morphological variations. Some fish species such as those that are without scales might produce hormones or secrete mucous which renders them unacceptable to the copepod or makes them immune^[10,38].

The tightly packed structure and arrangement of scales in some fish species might not allow for easy implantation of the parasite's anchor. This also explains the higher parasite intensity in smaller and younger fish which are easily accessible to the parasite, or in whom the defense mechanisms are less well developed compared with larger or older fish^[34]. Parasitic crustaceans harm the fish in several ways: they cause tissue damage due to permanently attached adults, result in stunted growth of fish and retarded or inhibited reproduction. This is probably because of the nutritional drain, though also it may be because of hormonal changes and feeding. Attachment of parasites to fish is frequently associated with anemia of the fish^[39]. Previous studies^[9,14,16,36,40-66] recorded other fish hosts from all over the world susceptible to *L. cyprinacea* (Table 3).

In conclusion, the parasite in this host was reported in previous studies in other fish species. *P. reticulata* may be considered as a newly recorded host of *L. cyprinacea* in KSA.

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Table 3. List of *L. cyprinacea* records on different host species from all over the world.

Host(s) of <i>L. cyprinacea</i>	Country	References
<i>Silurus asotus</i> , <i>Arapaima gigas</i> , <i>Anguilla japonica</i> , <i>Carassius auratus</i> , <i>Carassius auratus langsdorfii</i> , <i>Carassius auratus subsp.</i> , <i>Carassius cuvieri</i> , <i>Ctenopharyngodon idellus</i> , <i>Cyprinus carpio</i> , <i>Gnathopogon elongatus suwae</i> , <i>Hemibarbus labeo</i> , <i>Oncorhynchus mykiss</i> , <i>Pseudobagrus tokiensis</i> , <i>Plecoglossus altivelis</i> , <i>Pungitius pungitius</i> , <i>Lepomis macrochirus</i> , <i>Micropterus salmoides</i> , <i>Pterophyllum scalare</i> , <i>Chaenogobius macrognathos</i> , <i>Gymnogobius tridentiger obscurus</i>	Japan	[9,40]
<i>Odontesthes bonariensis</i>	Argentina	[14]
<i>Lepomis gibbosus</i> , <i>Pseudorasbora parva</i>	Romania	[16]
<i>Labeo altivelis</i> , <i>Oreochromis macrochir</i> , <i>Ctenopharyngodon idella</i> , <i>Oreochromis mossambicus</i> , <i>Oreochromis placidus</i> , <i>Tilapia rendalli</i>	Zimbabwe	[29]
<i>Alburnus alburnus</i> , <i>Barbus lacerta</i> , <i>Capoeta aculeate</i> , <i>Capoeta damascina</i> , <i>Capoeta aculeate gracilis</i> , <i>Carassius carassius</i> , <i>Chalcalburnus chalcoides</i> , <i>Chalcalburnus mossulensis</i> , <i>Chondrostoma orientalis</i> , <i>Ctenopharyngodon idella</i> , <i>Cyprinus carpio</i> , <i>Leuciscus cephalus</i> , <i>Hypophthalmichthys molitrix</i> , <i>Mastacembelus mastacembelus</i> , <i>Pseudorasbora parva</i> , <i>Aphanius vladkovi</i> , <i>Aristichthys nobilis</i> , <i>Chondrostoma regium</i> , <i>Gobio sp.</i> , <i>Leuciscus persidis</i> , <i>Schizothorax sp. A. stellatus</i>	Iran	[32, 41-44]
<i>Barbus graellsii</i> , <i>Barbus haasi</i> , <i>Cyprinus carpio</i> , <i>Leuciscus cephalus</i> , <i>Chondrostoma toxostoma</i> , <i>Parachondrostoma miegii</i>	Spain	[35,45]
<i>Ctenopharyngodon idella</i> , <i>Catla catla</i> , <i>Labeo rohita</i> , <i>Hypophthalmichthys molitrix</i> , <i>Cirrhinus mrigala</i>	Pakistan	[36,46-48]
<i>Carassius carassius</i> , <i>Poecilia latipinna</i> , <i>Chondrostoma nasus</i> , <i>Gambusia affinis</i> , <i>Pseudophoxinus burduricus</i> , <i>Gambusia holbrooki</i> , <i>Oxynoemacheilus anaticolicus</i>	Turkey	[49-54]
<i>Carassius auratus</i> , <i>Carp</i> , <i>Clarias lazera</i> , <i>Tilapia sp.</i> , <i>Hypophthalmichthys molitrix</i>	Egypt	[55-58]
<i>Barbus altianalis</i> , <i>Lates albertianus</i>	Uganda	[59-60]
<i>Cyprinus carpio</i> , <i>Carassius carassius</i> , <i>Idus idus</i> , <i>Rutilus rutilus</i>	Britain	[61]
<i>Gambusia affinis</i>	Italy	[62]
<i>Natropis girardi</i> , <i>Macrhybopsis tetranema</i>	Mexico	[63]
<i>Astyanax bimaculatus</i>	Brazil	[64]
<i>Barbus barbulus</i> , <i>B. grypus</i> , <i>B. luteus</i> , <i>Cyprinus carpio</i> , <i>Leuciscus lepidus</i>	Iraq	[65]
<i>Galaxias occidentalis</i> , <i>Edelia vittata</i> , <i>Bostockia porosa</i> , <i>Tandanus bostocki</i> , <i>Carassius auratus</i> , <i>Gambusia holbrooki</i> , <i>Phalloceros caudimaculatus</i>	W. Australia	[66]
<i>Poecilia reticulata</i>	KSA	Present study

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