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# Parasitic Infections of Some Freshwater Ornamental Fishes Imported in Pakistan

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**Abstract.-** This study was designed to investigate the parasitic infection in five species of freshwater ornamental fishes imported to Pakistan. A total of 178 fishes; goldfish, *Carassius auratus* L.; guppy, *Poecilia reticulata* (Peters); molly, *Poecilia sphenops* (Valemiemmes); platy, *Xiphophorus maculatus* (Gunther) and swordtail, *Xiphophorus helleri* (Heckel) were examined for parasitic infection. Three monogenean species (*Gyrodactylus turnbulli* Harris 1986;, *Gyrodactylus* VonNordmann, *Dactylogyrus extensus* Mueller and Van Cleave 1932); six protozoans (*Trichodina* Ehrenberg 1831, *Chilodonella* Stand, 1928, *Ichthyophthirius multifiliis* Fouquet, 1876, *Piscinoodinium pillulare* Schaperclaus 1954, *Tetrahymena* sp., *Epistylis* Ehrenberg, 1830.); one digenean (metacercaria of *Cryptocotyle* Lube,1899.) and two copepod arthropods (*Lernaea cyprinacea* L. and *Argulus foliaceus* Muller, 1785*)* were recorded from these fishes. The overall parasitic infection in these fishes was 69.10%. The infection and mean intensity in these fishes were; goldfish (75%, 31.24), molly (52%, 24.69), platy (66.66%, 22.25), swordtail (56.52%, 17.4) and guppy (75%, 15.86). Monogenean and protozoan were recorded from each fish species. Clinically the infected fishes were week, lethargic, with whitish to yellowish cysts on the skin. The scales were eroded and haemorrhages were present at the base of fins. The gills were damaged with hyperplasia on secondary lamellae. Monogenean parasitic load on goldfish was higher ($p < 0.01$) than other fish species. The intensity of metacercaria of *Cryptocotyle* sp. was higher ($p < 0.05$) in platy. The introduction of transmissible parasites through the import of ornamental fishes may cause serious disease problems in native fish species, which may have negative impact on emerging ornamental fish trade in Pakistan.

**Key words**: Freshwater ornamental fish, parasitic infection digenean parasites, monogean parasites.

## INTRODUCTION

Goldfish and its varieties (Ahilan *et al*., 2009) are the most popular ornamental fish kept as pet. Ornamental fish keeping is a popular hobby all over the world. The pet fish trade is a multimillion dollar industry. The vast majority of ornamental fishes in pet fish trade is of freshwater origin and farm-raised. Countries traditionally specializing in breeding and propagation of freshwater ornamental fishes are Japan, China, Singapore, Thailand, Indonesia and Malaysia. More recently ornamental fish are now cultivated in countries such as, Spain, Belgium, Czech Republic, Israel and Holland. Southeast Asia is the hub of ornamental fish trade, supplying up to 85% of the aquarium trade (Andras, 2012). In 2000 the global total wholesale value of live ornamental fishes both live freshwater and marine animals for aquarium only was estimated at US$ 900 million, with an estimated retail value of US$ 3 billion (Andras, 2012). Twenty species of ornamental fishes are imported into Pakistan from Southeast Asian countries (Ahmed, 1996) and are sold at the pet shops as healthy fishes to the hobbyists.

The occurrence of parasites on ornamental fishes and their transport to other countries has been reported worldwide; in Germany (Moravec *et al.*, 1999), Australia (Evans and Lester, 2001), Korea (Kim *et al.*, 2002), Sri Lanka (Thilakaratne *et al.*, 2003), Norway (Levsen *et al.*, 2003), in Brazil (Pizza *et al.*, 2005; Tavares-Dias *et al.*, 2010). Parasites on freshwater fishes are primarily protozoans, myxozoans, helminthes and crustacean. Monogeneans are typically parasites of the gills and skin of the fishes and are generally host specific (Woo, 2006). Dactylogyrids are gill parasites while gyrodactylids live on skin and fins. *Dactylogyrus extensus* is commonly found on goldfish, while *Gyrodactylus katherineri* is more often observed in koi, *Cyprinus carpio*. *Gyrodactylus turnbulli* and *G. bullatarudis* are parasites of guppy and are associated with stress for fish in the aquarium (Woo, 2006; Roberts, 2010).

The most important ectoparasites of

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freshwater ornamental fishes are ciliate protozoans such as; *Ichthyophthirius multifiliis*, *Tetrahymena* sp. and *Piscinoodinium pilullare* (Thilakaratne *et al*., 2003). Trichodinids are also commonly found in freshwater fishes throughout the world (Woo, 2006). *Chilodonella* sp. can cause high morbidity and mortality in freshwater fishes by attacking skin and gills of the fish (Koyuncu, 2009). The larval stages of some trematodes infect fish gills and leads to high mortality (Tonguthai, 1997). *Lernaea cyprincea* and *Argulus foliaceus* has been reported parasitizing several freshwater fishes (Bond, 2004; Rasouli *et al.*, 2011). Iqbal *et al.* (2012b,c), Iqbal and Sajjad (2013a) and Iqbal and Mumtaz (2013b) have recently reported incidence of fungal genera such as *Saprolegnia*, *Achyla* (*C.idella* and *C.catla*); *Aspergillus*, *Rhizopus*, *Mucor*, *Penicillium*, *Alternaria* and *Blastomyces* in ornamental fishes (*C. auratus*, koi *Cyprinus carpio*, shubunkin and black moor two varieties of *C. auratus*) imported to Pakistan. The present study was aimed at investigating parasitic infection of some imported freshwater ornamental fishes and identify different fish parasites.

## MATERIALS AND METHODS

One hundred and seventy eight freshwater ornamental fishes were examined for parasitic infection. The fishes were obtained from local pet shops in Lahore and brought to laboratory in sterilized polyethylene bags containing aerated water and maintained in glass aquarium. Total length and body weight of each specimen was measured. For parasitological examination of the fish skin, fins and gills were examined for the ulcer and lesions. Stomach, intestine and liver were removed and placed into Petri plates containing distill water and examined. A single scratch was made between their caudal, pectoral fin for the quantification of parasites and a smear was prepared on glass slide. Body mucous and piece of organs were compressed between a glass slide and cover slip with a drop of 0.65% saline solution for microscopic observation. Parasites quantification was performed directly on wet mount of body mucous and gills under microscope. For histology the gills were aseptically dissected and then fixed in 10% neutral buffered formaline for 24h. Then dehydrated in gradient ethanol solutions and cleared in xylol, the specimens were embedded in paraffin wax and serial sections were cut (5µm thick) on rotary microtome. Then the sections were deparaffinized, and rehydrated and stained with hematoxylin and eosin (H&E). The stained slides were observed and photographed under microscope Swift M3300-d equipped with Canon EOS 500 Camera. The identification of parasites followed after Kabata (1985) and Hoffman (1999), Thilakaratne *et al*. (2003). Mean intensity and prevalence of parasites were determined. The statistical analysis of data was done on the program SPSS 13.

## RESULTS

A total of 178 fishes were examined out of which 123 fishes were infected. The overall infection was 69.10%. *Carassius auratus* was the most parasitized fish with 75% infection (Table I). The parasites were recovered from body surface, skin, fins and gills of these fishes (Table II). Six species of parasites were recorded from goldfish. These were two monogenean (*Gyrodactylus* sp., *D. extensus* Fig. 1D), two protozoans (*Trichodina* sp. and *I. multifiliis* Fig. 1C) and two copepods (*L. cyprinacea* and *A. foliaceus*). The parasitic load of monogenean dominated the infection (57.96%) followed by protozoans (37.48%) and crustaceans (4.56%) (Table III). *Dactylogyrus extensus* and *Trichodina* sp. were the most prevalent parasites, whereas *I. multifiliis*, *L. cyprinacea* and *A. foliaceus* had very low mean intensities. The caudal fin was the most affected site of infection (55%) as compared to other fins and body surface. Gills were white to yellowish in color, with excess mucus secretion; hyperplasia and hemorrhages were evident due to infection by *D. extensus*. Monogenean parasitic load on goldfish was significantly higher ($p<0.01$) than other fish species.

The most dominant parasites in the *P. reticulata* were monogenean, *G. turnbulli* (Fig. 1B) and *D. extensus* (64.70%). Second most abundant parasite was metacercariae of *Cryptocotyle* sp. (25.21%) followed by protozoans *Trichodina* sp. and *Chilodonella* sp. (10.08%, Table III). The highest infection in *P. sphenops* was of monogenean

**Table I. - Fish species examined and infected**

| Fish species | Sample size (n) | Infected fish | % Infection | TL(cm) (Mean ± SD) | BW (g) (Mean ± SD) |
|---|---|---|---|---|---|
| *C. auratus* | 60 | 45 | 75.0 | 10.36±1.76 | 15.38±0.76 |
| *X. maculatus* | 30 | 20 | 66.6 | 4.80±0.89 | 2.20±0.76 |
| *X. helleri* | 23 | 15 | 65.2 | 5.38±0.65 | 2.38±0.65 |
| *P. reticulata* | 40 | 30 | 75.0 | 4.90±1.02 | 2.80±0.41 |
| *P. sphenops* | 25 | 13 | 52.0 | 4.20±1.50 | 2.75±0.44 |
| | 178 | 123 | 69.10 | | |

(59.1%) followed by metacercariae of *Cryptocotyle* sp. (37.3%) followed and *Trichodina* sp. was the lowest (3.4%, Table III).

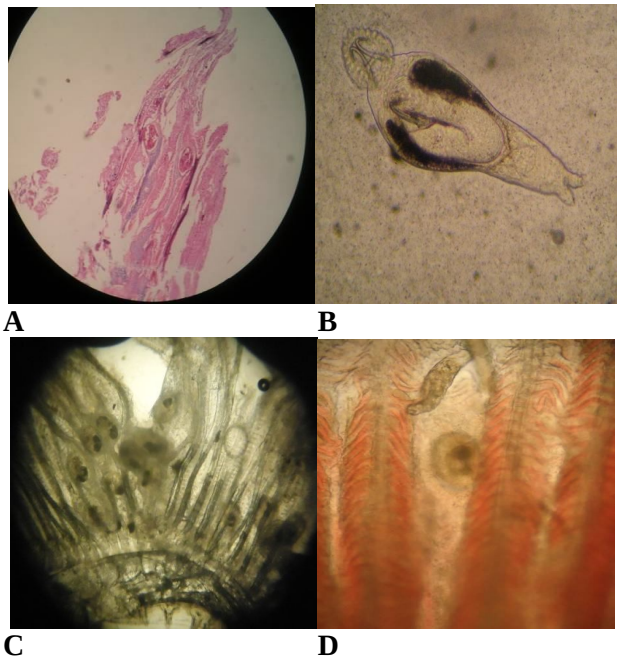


Fig 1. (A) Light photomicrograph of metacercarea (Cysts) of *Cryptocotyle* sp. on the gills of *X. maculates* (H&E x100) (B): *Gyrodactylus turnbulli* removed from the caudal fin of the *Poecilia reticulata* (C) *Ichthyohthirius multifiliis* infection on the gill of *C. auratus*. (D) Concurrent infection of *I. multifiliis* and *Dactylogyrus extensus* on gill filament of the *C. auratus*.

In *X. maculatus* the parasitic lead of both monogenean (44.0%) and the metacercaria of *Cryptocotyle* species (43.5%) infection were almost equal (Fig. 1A) and protozoan were 12.3% (Table III). The mean intensity of metacercareae of *Cryptocotyle* sp. was higher ($p<0.05$) in *X. maculatus* than in other fishes. *Epistylis* sp. was observed in *X. maculates* only. In *X. helleri* the parasitic load of monogenean was 49.0%, and of metacercaria of *Cryptocotyle* sp. 35.2%. Three species of protozoans, *Trichodina* sp. *Tetrahymena* sp. and *P. pillulare* had low intensities (Table III).

**Table II.- Location of parasites in various fish species.**

| Parasites | Host species | Location |
|---|---|---|
| *G. turnbulli* | guppy, molly, platy, swordtail | Fins, body surface |
| *Gyrodactylus* sp. | Goldfish | Fins |
| *D. extensus* | goldfish, guppy, molly, platy, swordtail | Gill lamellae |
| *Trichodina* sp. | goldfish, guppy, molly, platy, swordtail | Fins, body surface, gills |
| *Chilodonella* sp. | Guppy | Fins |
| *I.multifiliis* | goldfish | Gills |
| *P.pillulare* | Swordtail | Fins, body surface |
| *Tetrahymene* sp. | Swordtail | Fins |
| *Epistylis* sp. | Platy | Gills |
| *Cryptocotyle* sp. | guppy, molly, platy, swordtail | Gills |
| *L .cyprinacea* | Goldfish | Fins, gills |
| *A. foliaceus* | Fins | Goldfish |

## DISCUSSION

In the present study five fish species were examined for parasitic infection. Four groups of parasites; monogenean, protozoans, digenean and copepods were recorded. High mean intensity of monogenean; *D. extensus,* and *Gyrodactylus* sp. was observed in *C. auratus.* Mean intensities of G. *turnbulli*, *D. extensus* were high in *P. reticulata* and *P. sphenops* as compared to *X. maculatus* and

**Table III.- Number and mean intensity of parasites recovered from ornamental fishes.**

| Parasites recovered | Number of infected fish, number of parasites and mean intensity | | | | |
|---|---|---|---|---|---|
| | ***C. auratus*** | ***P. reticulata*** | ***P. sphenops*** | ***X. maculatus*** | ***X. helleri*** |
| **Monogenean** | **57.96%** | **64.30%** | **59.16%** | **44.04%** | **49.04%** |
| *G.turnbulli* | 0 | 15(123,8.2) | 10(98, 9.8) | 15(82,5.46) | 10(72,7.2) |
| *Gyrodactylus* sp. | 20(229,11.4) | 0 | 0 | 0 | 0 |
| *D. extensus* | 42(586,13.9) | 17(185,10.8) | 5(92,18.4) | 12(114,9.5) | 8(56,7.0) |
| **Protozoans** | **37.48%** | **10.08%** | **3.42%** | **12.35%** | **15.70%** |
| *Trichodina* sp. | 45(464,10.3) | 10(42, 4.2) | 9(11,1.2) | 10(35 ,3.5) | 5(20,4.0) |
| *Chilodonella* sp. | 0 | 3(6, 2.0) | 0 | 0 | 0 |
| *I. multifiliis* | 15(63,4.2) | 0 | 0 | 0 | 0 |
| *P.pillulare* | 0 | 0 | 0 | 0 | 3(15,5.0) |
| *Tetrahymena* sp. | 0 | 0 | 0 | 0 | 2(6,3.0) |
| *Epistylis* sp. | 0 | 0 | 0 | 5(20,4.0) | 0 |
| **Digenean** | **00** | **25.21%** | **37.38%** | **43.59%** | **35.24%** |
| *Cryptocotyle* sp. | 0 | 15(120, 8.0) | 7(120, 17.1) | 9(194,21.55) | 10(92,9.2) |
| **Crustacean** | **3.72%** | **00** | **00** | **00** | **00** |
| *L. cyprinacea* | 1(2, 2.0) | 0 | 0 | 0 | 0 |
| *A. foliaceus* | 25(62,2.48) | 0 | 0 | 0 | 0 |
| Total parasites | 1406 (48.33%) | 476(16.36%) | 321(11.03%) | 445(15.28%) | 261(8.97%) |
| Mean intensity | 31.24 | 15.86 | 24.69 | 22.25 | 17.4 |

(Note: Figures in column are: infected fish 20 (total parasites 229, mean intensity 11.4)

*X. helleri*. The high infection of these parasites in these fishes is due to their high reproductive rates. Moreover, monogenean has high host specificity. Gyrodactylids are viviparous and dactylogyrids are oviparous, their short direct life cycle facilitates them to spread rapidly in fish farms. Even their transmission is increased under poor pond management conditions (Woo, 2006, Thilakaratine *et al.,* 2003). Our results are comparable to the findings of Martins *et al.* (2002), Thilakaratine *et al.* (2003), Tavares-Dias *et al.* (2010) and Chanda *et al.* (2011).

The protozoan, *Trichodina* sp. was found in all the five fishes. This parasite is neither host nor site specific (Thilakaratine *et al.,* 2003). The high infection of *Trichodina* sp. is associated with its reproduction by binary fission, which is facilitated under high organic content of the pond (Ogut and Palm, 2005). Thilakaratine *et al,* (2003) and Tavares-Dias *et al.* (2010) reported *Trichodina* sp. infection in ornamental fishes in Sri Lanka and Brazil. *Chilodonella* sp. was found only in guppy with low mean intensity. This is highly pathogenic parasite of skin and gills, especially of goldfish. Infected skin is vulnerable to secondary infection under influence of water pollution (Hoole *et al.*, 2001). *Ichthyophthirius multifiliis*, the causative agent of white spot disease, was only found on the gills of *C. auratus*. This parasite is wide spread and well adapted to different environmental conditions and has low host specificity. Heavy gill infection result in sever gill damage and respiratory stress (Tavares-Dias *et al.,* 2010). Low prevalence and high mean intensity of *I. multifiliis* in freshwater fishes have been reported earlier (Thilakaratne *et al.,* 2003; Tavares-Dias *et al.,* 2010; Chanda *et al*., 2011).

*Piscinoodinium pillulare* was only recorded from fins and body surface of *X. helleri* with low mean intensity. This is potentially a serious pathogen of commercial fishes in Sri Lanka and Brazil (Thilakaratne *et al.,* 2003; Pizza *et al.,* 2005; Tavares-Dias *et al.,* 2010) even in aquarium, hatchery and intensive culture condition. The infection of *P. pillulare* in fish causes "Velvet disease". The gills infection of fish by *P. pillulare,*

induce hyperplasia and obstruction of respiratory surface and cause mortality. Low infection of *Epistylis* sp. was observed in *X. maculatus*. *Epistylis* sp. is very common in South Asian countries in aquarium fishes (Kabata, 1985).The site of attachment on fish by *Epistylis* develop lesions that become inflamed and necrotic and eventually ulcerated under heavy infection. (Hoole *et al.,* 2001). De Padua *et al.* (2013) reported that *Epistylis* infection in cultured catfishes in Brazil is an emerging disease. Low infection of *Tetrahymena* sp. was found in *X. helleri*. Low *Tetrahymena* sp. infection was reported from six ornamental fishes from Sri Lanka (Thilakaratine *et al.,* 2003) and Brazil (Tavares-Dias *et al.*, 2010) and also from guppy, *P. reticulata* imported from Singapore (Leibowitz and Zilberg, 2009). *Tetrahymena* infection is known as guppy disease, which causes serious economic losses in commercial guppy farms (Thilakaratne *et al*., 2003).

Metacercariae of *Cryptocotyle* sp. was found in *P. reticulata, P. sphenops*, *X. maculatus* and *X. helleri* and its intensity was low. This is important fish parasite because of its mode of attachment in fish. Low level infection of *L. cyprinacea* was recorded from *C. auratus* only. Although, this parasite is not host specific but is common in goldfish and other carps (Kabata, 1985; Thilakaratne *et al*., 2003). *Lernaea cyprinacea* has also been reported in major and Chinese carps under culture conditions (Iqbal *et al.*, 2012a). *Argulus foliaceus* commonly infect cyprinids and its low infection was observed in *C. auratus* in present study. Both *Argulus* sp. and *L. cyprinace* infection produce ulceration and haemorrhages on the skin of the host and facilitate secondary infections of bacteria, fungi and viruses (Woo, 2006). High parasite load of *A. foliaceus* in *C. carpio* and other carps cause high mortality (Pekmezic *et al.,* 2009; Chanda *et al.*, 2011).

The variations in the infection levels of different species of parasites in these fishes may be attributed to, the susceptibility of host to respective parasite species. Moreover, high infection of monogenean and moderate to low infection of protozoans on these ornamental fishes may be associated with the presence of these parasites on the host fish before shipment. High level of infection of digenean, *Cryptocotle* sp. in four fish species may be because of its predilection for these fish species. Low infection of *L. cyprinacea* and *A. foliaceus* may be associated with two factors; 1) either the ornamental fish farmers purged and removed the heavily infected fishes and ignored the less infected specimens from stock before shipment or 2) the infected fishes accidentally escaped into the shipping consignment and remained unnoticed by the inspection staff. Furthermore, checking of fishes for transport does not normally include conscientious efforts to locate fish parasites. Moreover, the basic health management practices might be easily over looked due to shortage of trained personal at fish farms. This is the first report of introduction of parasites with ornamental fishes imported into Pakistan. According to Ferraz (1999) due to economic and social significance of pet fish industry, it seems necessary to improve the fish health management practices at fish farms before shipment to overseas importers. In Pakistan, strict regulations on import of live fish need to be implemented. Health certification for imported live ornamental fishes into the country may be made mandatory. The high risk species must meet rigorous clearance criteria. Quarantine of imported ornamental fish is an important bio-security measure. These steps may surely help to save the emerging ornamental fish industry in Pakistan.

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*Conflict of interest declaration*

There is no conflict of interest or otherwise.

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