

## Prevalence of *Argulus foliaceus* and Fungal Infections in Some Ornamental Fishes [*Discus (Symphysodon discus)*, Dwarf Gourami (*Trichogaster lalius*) and Guppy (*Poecilia reticulata*)] in Isfahan City of Iran

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### Summary

This study was aimed to determine the prevalence *Argulus foliaceus* and some fungal diseases of ornamental fishes (discus, dwarf gourami and guppy) in Isfahan, Iran. The 90 ornamental fish from 2 pet fish supply store in Isfahan city of Iran were randomly selected. The study demonstrated that only 2 out of those 90 samples were infested with *A. foliaceus*. The highest infestation rate was in Dwarf gourami (*Trichogaster lalius*) (23.31%), and the lowest infestation rate was in Guppy (*Poecilia reticulata*) (6.66%). A total of 90 fishes of 3 species were examined. The contaminant fungal species were identified as *Acremonium* sp., *Aspergillus* sp., *Alternaria* sp., *Penicillium* sp., and *Saprolegnia* sp.. Fungal infection was observed in discus, dwarf gourami and guppy. According to the presented study, it is clear that *A. foliaceus* can act as a potential risk factor for natural ecosystems and native fish population of Iran and other countries.

**Keywords:** Prevalence, *Argulus foliaceus*, Fungal infections, Ornamental fishes, Isfahan, Iran

## İran'ın İsfahan Kentinde *Argulus foliaceus* ve Mantar Enfeksiyonların Bazı Süs Balıklarında [*Diskus (Symphysodon discus)*, Cüce Gurami (*Trichogaster lalius*) ve Lepistes (*Poecilia reticulata*)] Yaygınlığı

### Özet

Bu çalışmanın amacı İran'ın İsfahan kentinde *Argulus foliaceus* ve mantar enfeksiyonların bazı süs balıklarında (Diskus, Cüce Gurami ve Lepistes) yaygınlığının belirlenmesidir. İran'ın İsfahan kentinde 2 pet balığı tedarik deposundan 90 süs balığı rastgele seçildi. Çalışma sadece 90 öreneğin iksinin *A. foliaceus* ile enfeste olduğunu göstermiştir. En yüksek enfestasyon oranı, Cüce Gurami (*Trichogaster lalius*) (23.31%) ve en düşük enfestasyon oranı Lepistes (*Poecilia reticulata*) (%6.66) olarak belirlendi. Üç türden toplam 90 balık incelenmiştir. Kontaminan mantar türleri *Acremonium* sp., *Aspergillus* sp., *Alternaria* sp., *Penicillium* sp. ve *Saprolegnia* sp. olarak belirlendi. Mantar enfeksiyonu Disk, Cüce Gurami ve Lepistes balıklarında gözlemlendi. Mevcut çalışmaya göre *A. foliaceus*'un İran ve diğer ülkelerde doğal ekosistemler ve yerli balık nüfusu için potansiyel bir risk faktörü olarak hareket edebileceği oldukça açıktır.

**Anahtar sözcükler:** Prevalans, *Argulus foliaceus*, Mantar enfeksiyonları, Süs balıkları, İsfahan, İran

### INTRODUCTION

Ornamental fish keeping has become an ever more common hobby global. The trade of ornamental fish is a multi-million dollar industry currently. Several species of ornamental fishes are imported from Southeast Asian countries into other countries such as Iran <sup>[1,2]</sup>. Fungal diseases of fish are considered to be a chief problem for both aquaculture and fisheries and happen in brood

stock and totally life stages of fish and eggs <sup>[1,3]</sup>. Among many aquatic fungi, *Achlya*, *Penicillium* sp., *Alternaria* sp., *Aphanomyces*, *Aspergillus* sp., *Dictyuchus*, *Fusarium solani*, *Protoachlya*, *Pythium*, *Saprolegnia*, and *Thraustotheca* were reported <sup>[1,3]</sup>. Fungus has been reported to cause serious diseases in estuarine and freshwater fishes in Australia, Japan and throughout South Asia <sup>[4]</sup>.



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The genus *Argulus* (Crustacea: Branchiura), or fish louse, are common parasites of freshwater fish [2,5]. Nearly 15 spp. are found on freshwater fishes and several of the species are parasitic on marine fishes [2,6]. *Argulus foliaceus* (*A. foliaceus*) have a direct life cycles that because of not need to the intermediate hosts for whole its life cycle and transports rapidly among the fishes [7]. Adults may live free from the host for up to fifteen days. *A. foliaceus* is an obligatory blood sucker and can survive for only a little day without the host fish, depending on size and ambient temperature [8]. A lot of researches have been done on prevalence of *A. foliaceus*, nonetheless the researches on prevalence of this ectoparasite in Iran are limited [2,5-7,9].

Consequently, this study was designed to find the prevalence *A. foliaceus* and some fungal diseases of ornamental fishes (discus, dwarf gourami and guppy) in Isfahan, Iran. This is the first study on prevalence of *A. foliaceus* in Isfahan, Iran. There is a lack of data on the characterisation and identification of fungal diseases of ornamental fishes in Iran. Such data are significant for fisheries management.

## MATERIAL and METHODS

The 90 ornamental fish [30 pieces discus (*Symphysodon discus*), 30 pieces dwarf gourami (*Trichogaster lalius*) and 30 pieces guppy (*Poecilia reticulata*)] from 2 pet fish supply store in Isfahan city of Iran (which are imported from Southeast Asian countries such as: Thailand, Malaysia and Singapore into Iran), in the summer 2013 were randomly selected. Then, the fish caught via hand tour and were transported to laboratory of mycology and parasitology in the School of Veterinary Medicine, Islamic Azad University Shahrekord Branch of Shahrekord, in sterile polyethylene bags in aerated aquarium/pond water. The fishes were kept separate in glass aquariums by continuous air supply at ambient temperature. Samples were examined macroscopically, microscopically, clinically for presence of *A. foliaceus* and fungal diseases.

### Identification of Fungi

For culturing of fungal specimens, three different types of media counting Malt extract agar (MEA), Sabouraud dextrose agar (SDA) and Potato dextrose agar (PDA) were prepared and streptomycin sulphate was supplemented to each preparation of media to avoid bacterial infection. The body surfaces of everything the fishes using in study were disinfected via dipping each fish in 1% formaldehyde for 1 to 5 min followed via 70% alcohol and lastly in sterile water in which it was thoroughly rinsed. The fungal isolates were collected from infected organs (skin, fins, gills, eyes) of fish with sterile needle and inoculated on MEA (Oxoid, UK), SDA (Oxoid, UK) and PDA (M096-India) agars. The agar plates were incubated at 28-30°C and fungal growth was observed after 4-7 days. The fungal

colonies of many colors were observed in the agar plates. For microscopic examination, slides were ready from each colony and stained with 0.05% trypan blue in lactophenol. The slides were observed under Olympus microscope and photographed. The fungi were identified by the help of available fungal identification keys and literature [10].

### Identification of *A. foliaceus*

In the macroscopic technique, contaminated fishes were carried out and their parasites were removed by forceps from different parts of the body. Via microscopic technique (using a compound light microscope at ×10 and ×40 magnification.), the fish were examined via dissecting microscope. The *Argulus* parasites were fixed in 70% ethanol and transferred to laboratory. Then, parasites were identified by the diagnostic key [11].

### Statistical Analysis

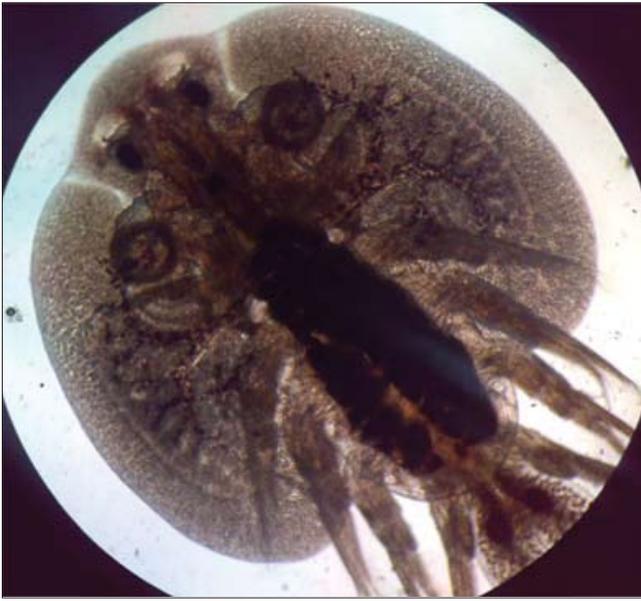
Prevalence of infection was calculated by using the following formula:

$$\text{Prevalence of infection (\%)} = \frac{\text{No. of fungal affected fish}}{\text{Total no. of examined fish}} \times 100$$

## RESULTS

The study demonstrated that only 2 out of those 90 samples were infested with *A. foliaceus*, meaning 2.22% prevalence. The highest infestation rate was in guppy (6.66%) and no detected in discus and dwarf gourami. Under the light microscope, these parasites were identified at *A. foliaceus* according to the rounded lobes of abdomen and the posterior emargination not reaching the mid-line and posterior lobes cephalothoracic carapace not extended beyond the beginning of abdomen (Fig. 1). In this study, all fish had generalized symptoms including lack of appetite and abnormal swimming. Bloody spots were also observed on skin and fins. In the present study, the distribution of *A. foliaceus* infection on different body regions was examined.

Results of fungal infection by any details showed in Table 1, 2, and 3. The fungal isolates were collected from infected organs (skin, fins, gills, eyes) of fish and the contaminant fungal species were identified as *Acremonium* sp., *Aspergillus* sp., *Alternaria*, *Penicillium* sp., and *Saprolegnia* sp.. The highest infestation rate was in Dwarf gourami (*Trichogaster lalius*) (23.31%), and the lowest infestation rate was in Guppy (*Poecilia reticulata*) (6.66%). A total of 90 fishes of *Acremonium* sp., *Aspergillus* sp., *Alternaria*, *Penicillium* sp., and *Saprolegnia* sp.. were examined (Table 1). Fungal infection was observed in discus, dwarf gourami and guppy. However, no *Saprolegnia* sp. infection was observed in discus and dwarf gourami; no *Acremonium* sp. infection was no observed in guppy; no *Aspergillus* sp.



**Fig 1.** *Argulus foliaceus* magnification (x4)

**Şekil 1.** *Argulus foliaceus* büyütme (x4)

**Table 1.** The number and percentage of healthy and fungi infected fishes

**Tablo 1.** Sağlıklı ve mantarlarla enfekte balıkların sayı ve yüzdesi

| Fish Species                                    |             | Number | Ratio (%) |
|---|-------------|--------|-----------|
| Discus<br>( <i>Symphysodon discus</i> )         | Infected    | 6      | 20        |
|   | No Infected | 24     | 80        |
| Dwarf gourami<br>( <i>Trichogaster lalius</i> ) | Infected    | 7      | 23.31     |
|   | No Infected | 23     | 76.69     |
| Guppy<br>( <i>Poecilia reticulata</i> )         | Infected    | 2      | 6.66      |
|   | No Infected | 28     | 93.34     |

infection was no observed in dwarf gourami and guppy; no *Alternaria* infection was no observed in guppy (Table 3).

## DISCUSSION

In the current study, some ornamental fishes were infected by fungus and *A. foliaceus*. The propagating and rearing of ornamental fish have a remarkable situation in the globe and plays a valuable role in exchange income of several nations and in Occupation. The world trade share of these fish which was estimated about 900 million dollar, has given a vital insight into it [12]. Since of the economic importance of these aquatics, several researchers have paid helpfulness the different factors threatening their health. *Argulus* sp. have been reported from various fish species global [2,6,7,9,13-15].

Does research carried out on Koi and goldfish checks the *Argulus* as the greatest prevalent parasite [14]. A small number of parasites species like protozoa are apparently non-host specific species and have been found in a large-scale of brackish water or fresh fish species all over the

**Table 2.** Frequency percentages of the identified fungi species

**Tablo 2.** Tespit edilen mantar türlerinin sıklık oranları

| Isolated Fungi         | Ratio (%) |
|------------------------|-----------|
| <i>Penicillium</i> sp. | 40.0      |
| <i>Acremonium</i> sp.  | 26.6      |
| <i>Aspergillus</i> sp. | 20.0      |
| <i>Alternaria</i>      | 6.7       |
| <i>Saprolegnia</i> sp. | 6.7       |

**Table 3.** The percentage of different fungal species isolated from fishes

**Tablo 3.** Balıklardan izole edilen farklı mantar türlerinin oranları

| Identified Fungi Species | Fish Species |                     |             | Total n (%) |
|--------------------------|--------------|---------------------|-------------|-------------|
|                          | Discus n (%) | Dwarf gourami n (%) | Guppy n (%) |             |
| <i>Penicillium</i> sp.   | 2 (33.3)     | 3 (42.8)            | 1 (50)      | 6 (40)      |
| <i>Acremonium</i> sp.    | 1 (16.7)     | 2 (28.6)            | 0 (0)       | 3 (20)      |
| <i>Aspergillus</i> sp.   | 1 (16.7)     | 0 (0)               | 0 (0)       | 1 (6.7)     |
| <i>Alternaria</i>        | 2 (33.3)     | 2 (28.6)            | 0 (0)       | 4 (26.6)    |
| <i>Saprolegnia</i> sp.   | 0 (0)        | 0 (0)               | 1 (50)      | 1 (6.7)     |
| Total                    | 6 (100)      | 7 (100)             | 2 (100)     | 15 (100)    |

world. *A. foliaceus* were reported from different fish species global and reported by some of the goldfish in Turkey [8,13,16]. In the present study, *A. foliaceus* was reported on guppy (*Poecilia reticulata*) which this was first recorded in Isfahan, Iran and findings of the present study are comparable to the findings of Mirzaei and Khovand [2].

Freshwater ornamental fish in Sri Lanka examined. Three species of copepod arthropods (*Lernaea cyprinacea*, *Ergasilus ceylonensis*, *Argulus foliaceus*), were identified [5]. The lice fish is a risky parasite lacking specificity, therefore that it can perhaps infested all freshwater fishes and happening in coastal or estuarine and marine water habitats [16]. In the other study, tiger Oscar (*Astronotus ocellatus*) species taken from a local pet shop with symptoms were examined for bacterial, fungal and parasitological infections. The parasites observed on the skin and fins of fish were identified as *A. foliaceus* [13].

Another study in Iran, lionhead goldfish (*Carassius auratus*), taken from a goldfish aquarium with symptoms, were examined for ectoparasites. The parasites collected from the skin and fins of fish were identified as *A. foliaceus*. This is the first report of infection with *A. foliaceus* of lionhead goldfish (*Carassius auratus*) in Iran [6]. Al-Dulaimi [7] in Babylon province of Iraq reported cases of the infection with *A. foliaceus* lice in various goldfish species in the earthen ponds. Notash [15] studied on the goldfishes in east Azerbaijan province of Iran and reported that they were infested by at least one species of crustacean. Results showed that of 300 samples, 85 (28.33%) samples were positive and 215 (71.67%) samples were negative from existence of *Argulus*. Too, Ebrahimzadeh Mousavi et

al.<sup>[9]</sup> studied on the 10 various ornamental fishes farm in Iran and reported that 230 pieces of the goldfishes were infested by 3 species of this ectoparasite. Other study, the concurrent fungal and parasitic infection of *Argulus foliaceus*, was observed in 3 fishes (12.5%). The parasitic infection of *A. foliaceus* is discussed elsewhere. Five fishes (20.83%) did not show any clinical signs<sup>[4]</sup>. In the new study in Iran, *A. foliaceus* was reported on goldfish and Koi which this was first recorded in Kerman, southeast of Iran<sup>[2]</sup>.

There is a lack of information on the identification and characterization of fungal diseases of ornamental fishes in Iran. Such information is very important for fisheries management. Five fungi, *Aspergillus* spp., *Fusarium* spp., *Mucor* spp., *Penicillium* spp., and *Rhizopus* spp., were reported from 8 edible smoked-dried freshwater fishes via Fayioye et al.<sup>[17]</sup>. Junaid et al.<sup>[18]</sup> isolated seven fungal species from stock fish in Nigeria and these included *A. flavus*, *A. fumigatus*, *A. niger*, *Trichophyton verrucosum*, *Rhizopus* spp., *Mucor* spp., and *Penicillium* spp. and between these *Mucor* spp., displayed the highest occurrence. In another study, fungi of 8 various genera; *Saprolegnia*, *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, *Scopulariopsis* and *Curvularia* were isolated from 2 fish species, *Oreochromis* spp., and *Claris gariepinus*<sup>[19]</sup>. Shahbazain et al.<sup>[20]</sup> isolated *Penicillium expansum*, *Penicillium citrinum*; *Aspergillus terruse*, *Aspergillus clavatus*; *Alternaria* spp.; *Saprolegnia parasitic*, *Saprolegnia lapponica*, *Saprolegnia ferax* and *Saprolegnia hypogyna* and seven other species of fungi from infected eggs of rainbow trout, *Oncorhynchus mykiss* in Iran. Fadaeifard et al.<sup>[21]</sup> isolated eight species of fungi from eggs and brood stock of rainbow trout *O. mykiss*. These isolates were *Penicillium* spp., *Acremonium* spp., *Alternaria* spp., *Fusarium solani*, *Aspergillus* spp., *Mucor* spp., *Saprolegnia* spp., and *Cladosporium* spp. In another study, 5 fungal species viz. *Aspergillus* sp., *Penicillium* sp., *Alternaria* sp., *Blastomyces* sp., and *Rhizopus* sp., were isolated from 4 species of carps, *C. auratus* L.; *Hypophthalmichthys molitrix* Richardsons; *Labeo rohita* Hamilton and *C. idella*<sup>[1]</sup>. The fungal genera like *Penicillium* spp., *Fusarium* spp., *Mucor* spp., and *Saprolegnia* sp., were isolated from *Acipenser persicus* eggs, where they caused 22% mortality of these eggs<sup>[3]</sup>. Too, fungal species like *Branchiomyces* sp., *Saprolegnia* spp., and *Aphanomyces* spp., have also been reported to be pathogenic to fish<sup>[3]</sup>.

Absence of good aquarium keeping in pet shops and fish farms increases the chances of fungal infection in fishes. The basic health management practices may be easily overlooked by reason of lack of trained personal or resources. In the current study, *A. foliaceus* was reported on guppy (*Poecilia reticulata*) which this was first recorded in Isfahan, Iran. According to the presented study, it is clear that *A. foliaceus* can act as a potential risk factor for natural ecosystems and native fish population of Iran and other nations, that should be mentioned to prevent the burst of new parasitic fauna to Iran and various nations as well as stop direct economic losses caused via mortality

derived from infestation with this ecto-parasite. Too, lack of good aquarium keeping in pet shops and fish farms increases the chances of fungal infection in fishes. The basic health management practices may be easily overlooked due to dearth of trained personal or resources.

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