

Investigating contamination with external and internal parasites in ornamental freshwater angelfish

(*Pterophyllum scalare*)

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Abstract

One of the main reasons the ornamental fish business suffers financial and health losses is parasitic diseases. Consequently, more than ever, research into the incidence and varieties of parasites in various regions of the country is required to identify parasites and prevent their spread across the country examining the ectoparasites and endoparasites of Angelfish (*Pterophyllum scalare*) was the goal of this study. 100 freshwater Angelfish specimens were checked for macroscopic and microscopic parasites. Wet smears were taken from the skin, fin and gills and then evaluated under a light microscope. Following the autopsy, the internal organs were examined with a light microscope. The parasites were stabilized, stained and identified using standard methods and diagnostic criteria. Among 100 fish samples, 71% were contaminated with parasites and 29% were not infected. 28% of the affected fish were concurrently contaminated with both *Hexamita* sp. and *Capillaria* sp. 6% of fish were contaminated with *Hexamita* sp., *Capillaria* sp. and *Ichthyophthirius multifiliis*. 1% were contaminated with *Ichthyosis* sp. and *Hexamita* sp. The other infected fish hosted a singular type of parasite. The parasites identified in this study were *Trichodina* sp. (3%), *Ichthyophthirius* (6%), *Monogenea* sp. (10%), *Camallanus* sp. (2%), *Capillaria* sp. (6%) and *Hexamita* sp. (9%). Research conducted in several countries shows that ornamental fish are infected with both ectoparasites and endoparasites. Considering the widespread commerce of these fish and their movement across borders, there is a risk of transmitting new parasitic fauna, which can pose a threat to local fish populations, particularly those that are valued, rare, or endangered.

Keywords: Ectoparasite, Endoparasite, Freshwater angelfish, Ornamental fish, *Pterophyllum scalare*

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Introduction

The aquaculture industry's most important sectors are food and ornamental fish cultivation. Aquaculture has emerged as a rapidly expanding food-production technology on a global scale (Seyrafi *et al.*, 2009; Sayrafi *et al.*, 2011; Sadeghinezhad *et al.*, 2015; Sasani *et al.*, 2016; Ahmadvand *et al.*, 2020; Rahmati Holasoo *et al.*, 2021, 2022, 2023, 2024). Ornamental fish farming is currently a significant global industry with many fans worldwide, known for its artistic and scientific aspects. Ornamental fish are distinctive due to their different visual effects and body coloration within their species. Ornamental fish excel in attractiveness, color variation and patterns within aquatic environment compared to other species. These fish are mostly kept for recreational purposes, biodiversity conservation, aesthetic appeal and tourism attraction. Aquarium fish care is a popular activity enjoyed by millions of people worldwide for many years (Rahmati-Holasoo *et al.*, 2010b). Ornamental fish can be categorized into two groups: saltwater and freshwater fish. Among the ornamental saltwater and freshwater fishes, the majority of the ornamental aquatic industry belongs to freshwater fish (Novák *et al.*, 2020). There are two main categories of pathogens in fish: infectious agents (bacteria, viruses, fungi and parasites) and non-infectious pathogens (environmental and nutritional illnesses). Parasitic diseases are among the significant harmful factors, such as infectious agents, in ornamental fish.

Fish parasite illnesses occur more frequently in unfavorable environmental circumstances. A low parasite load in fish is generally benign, but an excessive amount can lead to stress and subsequent infection (Bassey, 2011). Therapeutic techniques can help minimize the impact of illnesses and parasites. Specific stressors like fish overcrowding, poor water quality, or insufficient nutrition might accelerate the disease's advancement. Furthermore, parasites from non-native species can invade new habitats and negatively impact local native species. Although some of these factors have been identified as infections, the diseases affecting these fish species remain widely recognized. The *Pterophyllum* genus, a small genus in the cichlid family, is commonly referred to as Angelfish or Angel in Iran. The name *Pterophyllum* comes from the Greek words "Pteron," referring to "fins," and "Phylon," meaning "leaf" likely in reference to the distinctive shape of their fins. It inhabits only freshwater environments and comprises three species: *P. scalare*, *P. altum* and *P. leopoldi*. All fish of this genus belong to the South American *Cichlidae* family. *P. scalare* is the most readily accessible among these three species. *P. Scalare* can be easily bred in captivity. *P. scalare* is easily bred in captivity and financially feasible.

Ornamental fish breeding has been regarded as a very lucrative industry in recent decades. The aquarium aquatic sector has been projected to be valued at over \$1,000 million based on studies undertaken by the pet industry. In

addition, the amount of trade in this type of fish increased significantly during the Covid-19 pandemic (Marlianingrum and Suprpta, 2022). Although such a large and significant business has the potential to support the sustainable development of aquatic resources and the economic prosperity of the respective nations, it will eventually encounter difficulties related to social and environmental issues. In such a way, the arrival of ornamental fish can increase the nation's susceptibility to new diseases (Rahmati-Holasoo 2010a, 2020), particularly parasitic ones and the importance of new fish as non-native species can spread numerous pathogenic elements. Therefore, with more familiarity with their diseases, it can be reduced by timely and principled treatment, which will lead to reduction of the import of these fish and will prevent and can be reproduced and bred in the country in a productive manner and the importation of generators from abroad, which is at a high cost, is prevented. Additionally,

disease and mortality may result from contamination with different internal and external pathogenic parasites. Imported ornamental fish may not undergo accurate and thorough sampling and testing during transfer, which could result in the spread of new parasite species to our priceless fish. Further study on both external and internal parasites in ornamental fish is required, based on the mentioned cases. This study aimed to explore the prevalence of parasitic contamination in Angelfish (*P. scalare*) in Tehran and identify the specific types of parasites affecting the fish.

Methods and materials

For the purpose of this study, a total of 100 angelfish were obtained from ornamental fish supply centers in Tehran throughout the spring and summer of 2022. These fish were then evaluated for both external and internal parasite contamination (Fig. 1).



Figure 1: Some fish samples that were analyzed for parasitic infestation.

Initially, the visible symptoms of the fish were examined to determine if there

were any lesions or tissue damage, as well as the presence of external

crustacean parasites like *Lernaea* sp. and *Argulus* sp. After examining the skin and gills, wet smears were prepared and examined with a microscope. To observe the gills, they were first euthanized using an anesthetic (PI22, Pars Imen Daru, Iran) and then dissected for microscopic examination. Following the examination of ectoparasites, the parasites were initially identified and then the light microscope was used to generate photographs and films of the parasites. Additionally, graphic images of the separated parasites were also created. Necropsy was performed and wet smears were made for the internal organs, including the intestines, spleen, liver, gonads, gallbladder, swim bladder and even feces. These samples were then examined for internal parasites using light microscopy. After an analysis of the internal parasites, they were quantified and visual documentation was created using a light microscope. Graphic images of the isolated parasites were generated, followed by capturing images of both external and internal parasites using a camera equipped with Microbin software version 2 and a German microscope camera (UI 2250). To stabilize and stain, protozoa were first prepared and if protozoa were observed, they were stained by Giemsa. In addition, an ammonium picrate fixative was used to stabilize and stain the monogenean parasites. The live parasite specimen was placed on a slide and a droplet of water was added to it. Then, three corners of the slide were fixed and a single droplet of the malemberg soluble was put onto the open side of the

specimen. Following the introduction of the soluble, the hooks and organs were clearly observed. Nematodes were also stained with carmine.

Results

This study involved the preparation and examination of 100 ornamental angelfish of varying sizes to determine the presence of external and internal parasites. Among the 100 cases, parasitic infection affected 71 (71%) while it did not affect 29 (29%) of them (Figs. 2-11). The parasites listed in Table 1 were obtained from the fish that were examined.

The prevalence of infection with each of the identified parasites in angelfish that were affected by a parasite was as follows: *Gyrodactylus* sp. 7%, *Dactylogyrus* sp. 3%, *Ichthyophthirius multifiliis* 6%, *Trichodina* sp. 3%, *Capillaria* sp. 6%, *Camallanus* sp. 2% and 9% for *Hexamita*. To clarify, 36 fish out of a total of 100 were found to have a singular kind of contamination. This information can be seen in Figure 3 and Table 2. 28% of fish that had multiple parasites including *Hexamita* sp. and *Capillaria* sp.. And 6% of fish had *Capillaria* sp., *Hexamita* sp. and *Ichthyophthirius multifiliis* all at the same time. Also, concurrence of *Ichthyouris* sp. and *Hexamita* sp. (1%) were observed. Out of 100 contaminated fish, 35 had a simultaneous infection with several parasites (Fig. 3 and Table 3). These fish exhibited the greatest incidence of parasite contamination from *Hexamita* sp., whereas the lowest occurrence of infection was related to *Ichthyouris* sp.

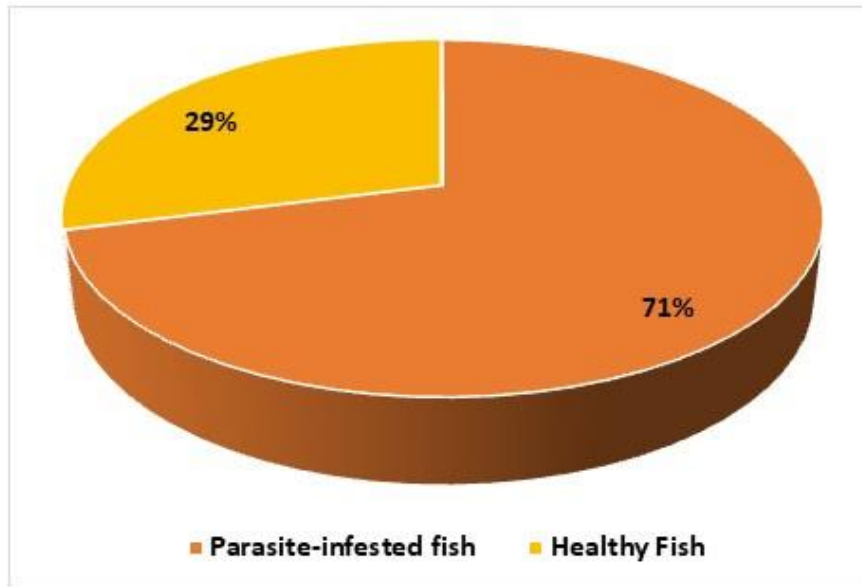


Figure 2: 71% of samples were infected with ectoparasites and endo parasites.

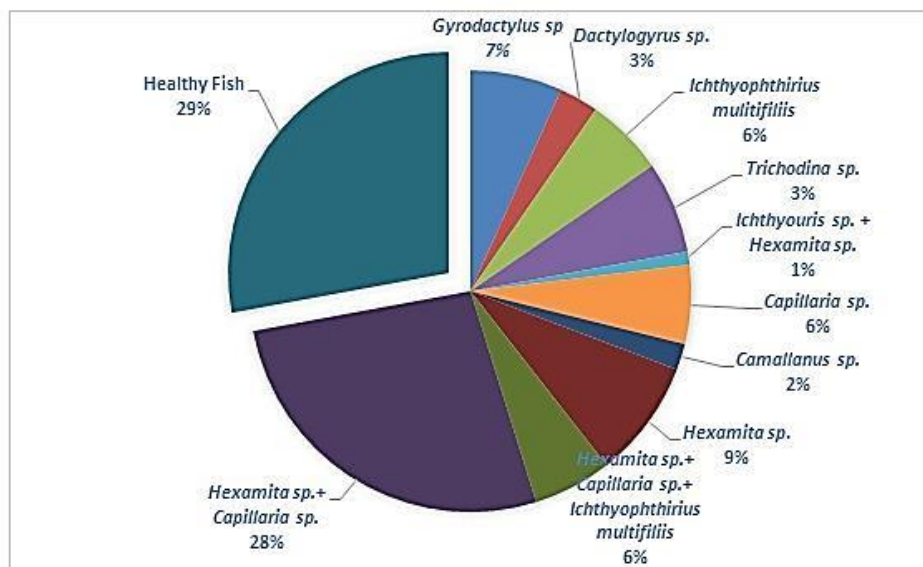


Figure 3: Frequency of all parasite infections, both simultaneous and non-simultaneous, in angelfish (*P. scalare*).



Figure 4: *Ichthyophthirius multifiliis* with a horseshoe-shaped core is seen.



Figure 5: Two pairs of eye spots on the *Dactylogyrus sp.* parasite are seen.

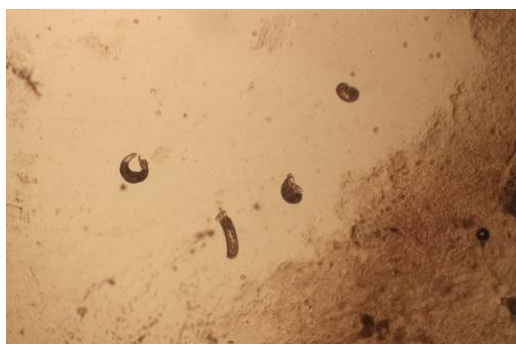


Figure 6: Wet smear shows *Dactylogyrus* sp. parasite.



Figure 10: Eggs of *Capillaria* sp. parasite are seen in wet smears of angelfish intestine.

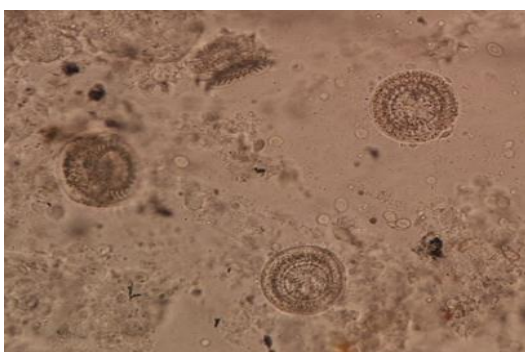


Figure 7: Wet smear of *Trichodina* sp. Protozoa.

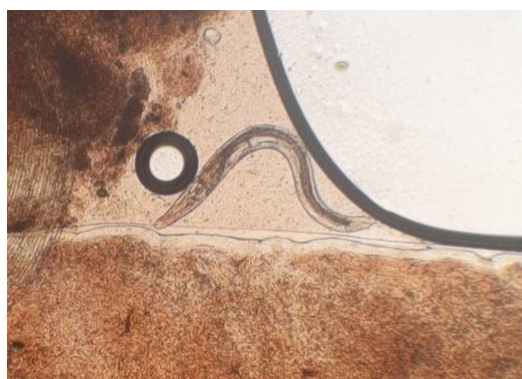


Figure 11: *Ichthyouris* sp. nematode is seen in wet smear of angelfish intestine.

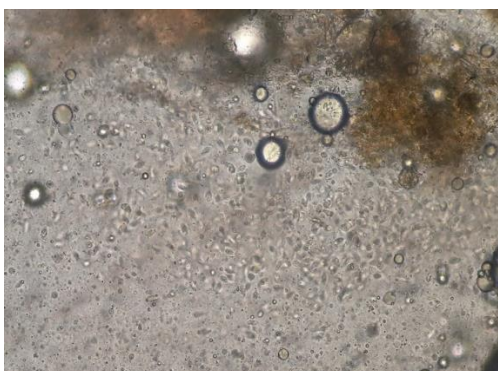


Figure 8: *Hexamita* sp. in the intestine of angelfish.



Figure 9: Angelfish with white feces due to simultaneous contamination with *Hexamita* sp. and *Ichthyouris* sp.

Table 1: Isolated parasites from angelfish.

Affected organ	Type of parasite
Skin	<i>Gyrodactylus</i> sp.
Skin, Gills	<i>Dactylogyrus</i> sp.
Skin, Gills	<i>Ichthyophthirius multifiliis</i>
Skin	<i>Trichodina</i> sp.
Digestive system	<i>Capillaria</i> sp.
Digestive system	<i>Camallanus</i> sp.
Digestive system	<i>Ichthyouris</i> sp.
Digestive system	<i>Hexamita</i> sp.

Table 2: Prevalence of parasitic infection of angelfish without simultaneous infection.

Type of parasite	Number of infected fish	Parasite abundance (%)
<i>Gyrodactylus</i> sp.	7	7
<i>Dactylogyrus</i> sp.	3	3
<i>Ichthyophthirius multifiliis</i>	6	6
<i>Trichodina</i> sp.	3	3
<i>Capillaria</i> sp.	6	6
<i>Camallanus</i> sp.	2	2
<i>Hexamita</i> sp.	9	9

Table 3: Prevalence of parasitic infection of angelfish (simultaneous infection).

Type of parasite	Number of fish	Parasite abundance (%)
<i>Hexamita</i> sp., <i>Capillaria</i> sp. & <i>Ichthyophthirius multifiliis</i>	6	6
<i>Hexamita</i> sp. & <i>Capillaria</i> sp.	28	28
<i>Ichthyouris</i> sp. & <i>Hexamita</i> sp.	1	1

Discussion

Establishing commercial units for the reproduction and breeding of ornamental aquaculture is one of the suitable solutions for the aquaculture sector's commercialization, competitiveness and active participation in international markets. If we exclude the entire aquarium industry from our analysis and only look at the revenue from the export of ornamental fish, we can see that since 1975, the global ornamental fish export market has grown from 21 million dollars to over 360 million dollars and this trend is still going strong. When we include the aquarium industry as a whole, however, the total revenue is over 20 billion dollars, which can have a substantial impact on a nation's ability to grow economically. The aquarium industry and the breeding of ornamental fish are expanding well in Iran due to the country's population's interest in ornamental fish (Akbari *et al.*, 2014). Although freshwater fish make up the bulk of ornamental fish nowadays, the majority of marine species are still captured in the wild (Lecchini *et al.*, 2006).

Some of the major elements that contributed to the growth of this pastime were the advent of plastic bag transportation, easier access to more affordable air travel and advancements

in aquarium equipment in the years following World War II (Monticini, 2010). In ornamental fish, parasites are the most frequent cause of concern. Some parasites are host-specific, meaning they only infect a single genus or a few particular species. They can also vary in size and form. Global spread is another feature of several of these parasites (Roberts and Smith, 2011). Apart from their direct role in fish mortality, parasites can also significantly affect fish development and their ability to withstand additional stressors. In addition, the marketability and salability of fish that have parasites may decrease. In addition, parasite infections can alter the behavior and sexual features of host fish and cause decreased growth, weight loss, emaciation, lower reproductive success, or sterility (Overstreet, 2021).

Examining 100 angelfish in the current study discovered that the fish had parasite infections in their skin, gills, fins and gastrointestinal system. The parasites included *Gyrodactylus* sp., *Dactylogyrus* sp., *Ichthyophthirius multifiliis*, *Trichodina* sp., *Ichthyouris* sp., *Capillaria* sp., *Camallanus* sp. and *Hexamita* sp.

The majority of parasitic fish species do not represent a threat to people, as they are a naturally occurring part of the

environment. However, to complete their life cycle, parasites with an indirect life cycle require an intermediary host, which may be problematic for both humans and other living things. The amount of harmful parasites due to fish is directly correlated with their density. Stated differently, low parasite density fish populations show minimal symptoms, while high parasite density fish populations experience fatal illnesses and sometimes even mortality. In fish, parasites can result in physiological harm including altered behavior, stunted development and decreased fertility together with mechanical harm such tissue and gill damage (Buchmann and Lindenstrøm, 2002; Al-Dulaimi, 2010; Notash, 2012).

Goldfish had the highest prevalence of parasite contamination, with *Gyrodactylus* sp. and *Ichthyophthirius* sp. having the highest frequency, according to one study in 2009 investigation of the contamination of ten species of ornamental fish in Iran (Mousavi *et al.*, 2009). This fish contained 1.5% of *Dactylogyrus extensus* 0.9% of *Dactylogyrus Baueri* and 2.1% of *Gyrodactylus kobayashii*, all of which were imported fish. High density, malnourishment and low water quality are the main reasons that mortality occurs for these fish. In a study conducted in 2006 (Meshgi *et al.*, 2006), parasitic contamination was examined in 1008 fish. Of these, 98.2% of Goldfish had *Dactylogyrus vastator*, 60.5% had protozoan parasites of *Ichthyobodo necator*, *Chilodenella* sp. and *Trichodina* sp. and 16.9% had

crustacean parasite infection. The ciliated *Trichodina* sp., was observed in the skin of angel fish in the current study. *Capillaria* sp. have been reported in ornamental discus fish in Iran (Rahmati-Holasoo *et al.*, 2010b). Also, in the present study *Capillaria* sp. was observed in angelfish. Mehdizadeh Mood (2009) isolated parasites such as *Ichthyophthirius multifiliis*, *Trichodina* sp, *Gyrodactylus* sp. and two species of *Argulus* spp. from the skin of goldfish. Moreover, *Myxobolus longisporus*, *Dactylogyrus vastator*, *Dactylogyrus Baueri* and *Centrocestus formosanus* were found to have contaminated the water (Mehdizadeh Mood, 2009).

In investigating ornamental fish pollution from Sri Lanka, one of the largest exporters of ornamental fish, the parasites *Dactylogyrus* sp. and *Gyrodactylus* sp were isolated from goldfish (Thilakaratne *et al.*, 2003). Koyuncu and Tokşen (2010) investigated six species of ornamental fish in Turkey and discovered two species of *Argulus* in goldfish, the monogeneans *Dactylogyrus extensus* and the parasitic crustaceans *Lernaea* sp. and the protozoa *Ichthyobodo*. Regarding this, Kayış *et al.* (2013) in Turkey reported on the monogeneans of *Gyrodactylus* sp. and *Dactylogyrus* sp. as well as the protozoa from goldfish, including *Trichodina* sp., *Epistylis* sp., *Ichthyophthirius multifiliis*, *Ichthyobodo necator*, *Hexamita* sp. and *Chilodenella cyprinus*. Regarding this, one study reported the monogeneans, *Trichodina* sp., *Epistylis* sp., *Ichthyophthirius multifiliis*, *Ichthyobodo necator*,

Hexamita sp. and *Chilodenella cyprini* from goldfish, as well as the monogeneans of *Dactylogyrus* and *Gyrodactylus* (Kayış *et al.*, 2013). This article's conclusion is as follows, the frequency of parasite species has grown as a result of rising fish densities and increased imports.

Iqbal and Haroon (2014) conducted studies on ornamental fish in Pakistan and found that *Ichthyophthirius multifiliis* was only present in the gills of goldfish. This parasite causes serious damage to fish gills and disturbs their respiratory system since it has a wide range, adapts to diverse environmental circumstances and has few specialized hosts. It has also been observed that Goldfish from Pakistan and Iraq include species of *Dactylogyrus* sp., *Gyrodactylus* sp. and *Lernea* sp. (Iqbal and Hussain 2013; Iqbal and Haroon, 2014). In 2015, *Amyloodinium ocellatum* was isolated from *Amphiprion xanthurus* imported into Iran (Manjili, 2015). Mirzaei (2015) searched for the presence of *L. cyprinacea* among ornamental fish in Kerman, Iran, between September 2011 and 2012. Only 5.3% of the 3520 fish were infected, leaving 94.7% uninfected. In addition, issues such as tissue atrophy, blockage of the digestive tract and lack of natural food can result from parasites like *Tetrahymena* sp. and *Dactylogyrus* sp. in ornamental fish (Ahmadi *et al.*, 2021). In a study conducted in 2022 to evaluate the parasitic fauna of ornamental fish from seven farms, focusing on six common breeding species in East Azerbaijan province in

the northwest of Iran, a total of 600 freshwater ornamental fish were sampled and examined for parasites, which led to the identification of ciliated protozoa, monogenean trematodes, crustacean arthropods and a fish-infecting nematode. The parasite was discovered in fish from five of seven farms, with a total incidence of 26.33%. The results indicated that different parasite families had varying contamination rates, with protozoa having the greatest contamination rate (16.83%), followed by monogenean trematodes (13.17%), crustacean arthropods (4%) and nematodes (0.33%). *X. helleri* and *P. scalare* had the highest infection rates with *Gyrodactylus* sp. and *Trichodina* sp., respectively. Goldfish had much greater infection rates with *A. japonicus* and *L. cyprinacea* compared to other species (Rahmati-Holasoo *et al.*, 2022).

Rahmati Holasoo *et al.* (2023) examined 140 ornamental fish, including 70 Sutchi catfish (*P. hypophthalmus*) and 70 silver dollar fish (*M. hypsauchen*), between January and February 2021 to investigate the contamination status of parasitic pathogens in freshwater catfish and silver dollar fish in Alborz province. The findings revealed the presence of six parasite species in fish, including five protozoan species (*Nyctotherus piscicola*, *Trichodina heterodontata*, *Ichthyophthirius multifiliis*, *Protoopalina* sp. and *Hexamita* sp.) and one monogenic species (*Ancyrocephalus* sp.). The total percentage of identified

parasites among fish was 46.43% (65/140) (Rahmati Holasoo *et al.*, 2023).

According to research by Dominguez *et al.*, (2023), 333 ornamental fish in five Brazilian states had at least one parasite infection, accounting for 70.6% of the fish. Eight farms in different municipalities supplied the fish. Anesthesia was administered to each fish before euthanasia. Twelve different parasite species including *Trichodina* sp., *Lernaea cyprinacea*, *monogeneans*, *digenean metacercariae*, *cestodes*, *nematodes*, *Ichthyophthirius multifiliis*, *diplomonad flagellates*, *Ichthyobodo* sp. and *Tetrahymena* sp. were detected by Dominguez *et al.* (2023).

Conclusion

Numerous studies have been carried out on parasitic infections in ornamental fish, both locally and internationally, demonstrating the relevance of investigating these diseases. Ornamental fish have extensive trade worldwide and imports and exports of these fish among different countries can lead to the spread of infections. Importance of these fish should be handled with extreme caution and quarantine steps to prevent the entry of new parasite fauna into the nation, thereby minimizing possible harm. Investigations and various studies have shown that the entry of these parasitic infections into the country was due to not observing quarantine measures and inadequately conducting initial tests before entrance.

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