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RESEARCH PAPER

Ectoparasite Fauna of Imported Ornamental Fishes in Oman

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الطفيليات الخارجية في أسماك الزينة المستوردة في عُمان

حنان البلوشي، منال الشرياني، ماجد الشحي، عادل السليماني، وجيلها يون

ABSTRACT. The movement of live aquatic animals crossing international borders is a regular activity for economic, social, and public resource development. A survey was carried out to investigate the prevalence of parasites translocated with imported ornamental fishes. Exotic diseases from imported fish have a potential hazard of biosecurity. A sample of 138 imported ornamental fish originating from Thailand and Sri Lanka were collected from pet shops around Muscat governorate and examined for ectoparasites. Parasitological examination was based on a visual macro and microscopic examination of fish's exterior. The findings of the current survey of imported ornamental fish revealed that 50.72% of the imported ornamental fish were infected by the one or more of ectoparasites species including *Ichthyophthirius multifiliis, Dactylogyrus* sp., *Gyrodactylus* sp., digenean metacercaria and *Trichodina* sp. Parasitic infection of the gills (55.795) was higher than the skin (42.75%). The most prevalent parasite in skin infection was *Gyrodactylus* sp. (14.49%) but this was absent from the gills which was dominated by digenean metacercaria (26.08%). The existence of such parasitic organisms could pose a threat to native fish populations and the aquaculture sector. It is recommended that a risk analysis method based on the detection and assessment of fish diseases found in live ornamental fish be established and used. Also, exotic pathogenic parasites, as well as their hosts, must be closely monitored to reduce the likelihood of infections being introduced into new areas.

KEYWORDS: Infection, Parasitic, White spot, Exotic, Diseases, Surveillance, Monogeneans

الخلاصة: تعتبر حركة الحيوانات المائية الحية عبر الحدود الدولية نشاطًا منتظمًا للتنمية الاقتصادية والاجتماعية وتطوير الموارد العامة. تم إجراء استطلاع للتحقيق في انتشار الطفيليات الخارجية في أسماك الزينة المستوردة. تمثل الأمراض الغريبة الناجمة عن الأسماك المستوردة خطرًا محتملاً على الأمن البيولوجي. تم جمع عينة من ١٣٨ سمكة زينة مستوردة من تايلاند وسريلانكا من متاجر الحيوانات الأليفة في محافظة مسقط وفحصها للطفيليات الخارجية. استند الفحص الطفيلي إلى الفحص البصري والمجهري للطبقة الخارجية للأسماك. أظهرت نتائج الدراسة الحالية لأسماك الزينة المستوردة أن ٥٠,٧٢٪ منها كانت مصابة بواحدة أو أكثر من أنواع الطفيليات الخارجية منها *Ichthyophthirius multifiliis, Dactylogyrus* sp., *Gyrodactylus* sp., digenean metacercaria و *Trichodina* sp. كانت الإصابة الطفيلية في الخياشيم (٥٥.٧٩٥ ٪) أعلى من الجلد (٤٢.٧٥٪). وكانت أكثر الطفيليات شيوعًا في إصابات الجلد هي *Gyrodactylus* sp (١٤.٤٩٪) ولكنها كانت غائبة في الخياشيم التي كانت مسيطرة عليها digenean metacercaria (٢٦.٠٨٪). يمكن أن تشكل وجود هذه الكائنات الطفيلية تهديدًا لسكان الأسماك المحلية وقطاع الاستزراع السمكي. يُوصى بإنشاء واستخدام طريقة تحليل المخاطر تعتمد على الكشف والتقييم لأمراض الأسماك في أسماك الزينة الحية. كما يجب مراقبة الطفيليات الخارجية الممرضة ومضيفيها عن كثب لتقليل احتمالية إدخال العدوى إلى مناطق جديدة

الكلمات الرئيسية: إصابة طفيلية، البقعة البيضاء، أمراض غريبة، مراقبة، أحاديات الخياشيم

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Introduction

A study reported that around the world, 1 billion ornamental fish are sold annually, including 4,000 freshwater fish species accounting for roughly 90% of the trade and 1,400 marine fish species. The global wholesale value of live ornamental fish is 900 million dollars, with a retail value of US$ 3 billion (Fujimoto et al. 2020). The trading of live aquatic animals is a regular activity for economic, social, and public resource development for many countries. The European Union countries make up the world's largest ornamental fish market. The United States is the world's top importer of ornamental fish. Between 2000 and 2006, more than 90% of live exotic pets imported into the United States were ornamental fish, primarily from Southeast Asian countries. Each year, 187 million live ornamental fish are imported, with 99 percent of them being used for commercial sale in the pet market (Hossain and Heo 2021). Ornamental fish have received more attention in recent years because of growing local and global demand, as well as the resulting expansion of the export market and trade. Since 1985, the value of international trade in ornamental exports has expanded at a compound annual growth rate of around 14% and the value of ornamental fish transported into various nations across the world is estimated to be over $278 million (Tribuzy-Neto et al. 2021).

Ornamental fish cultivation has increasingly migrated to areas near consumer areas because producing fish near consumer regions is becoming more viable due to lower transportation costs (Pouil et al. 2020). Most freshwater aquarium fish are captured in the wild using artisanal fishing methods, such as small beach seines, dip nets, and a variety of small trap nets in rivers and streams. Divers (masks, snorkels, and SCUBA) are often used in the gathering of marine aquarium fish, along with hand nets, fish-holding buckets, and barrier nets to corral and fence the fish. Remote fishing locations are commonly used to catch wild fish destined for pet stores throughout the world. Fish may spend a few days to several weeks in fish camps after being collected before reaching importing and distribution facilities throughout the world. This time frame is determined by the logistics of capture, storage, and transportation. While waiting for a transport boat or a local dealer, the fish are usually divided by species, sized, and numbered in the fishing camps. A local dealer can gather fish from various areas and carry them in hauling boxes to a secondary holding facility or storehouse utilizing a transport boat, motorcycle, or even just a bicycle. The fish are then mass-consolidated after arriving from several regional storage facilities and sorted again at local exporting facilities, packed in oxygenated bags, and air-shipped to major retail stores throughout the world. Although the industry and enthusiasts raise certain marine aquarium fish and invertebrates, most marine ornamentals fish come from wild-caught fisheries and these fetch greater prices than freshwater species and usually come from tropical seas of Indonesia, Philippines, Sri Lanka, Maldives, and Central Pacific Islands are among the countries in the region (Biondo and Burki 2020). The increased and often reckless worldwide transportation of live aquatic animals has occasionally resulted in significant harm to aquatic food productivity and ecological function. These disturbances are brought on by a variety of exotic pathogens.

Both ecto- and endo-parasites can be found in abundance in ornamental fishes. These parasites are found in all ecosystems and have no impact on the fitness of healthy fish. Additionally, when fish are raised in captivity, parasites can become a concern under stressful conditions. Diseases are responsible for up to half of all output losses in aquaculture (Haenen et al. 2020). High stocking numbers combined with low water quality create ideal circumstances for parasite infection and reproduction, accelerating the pathogenic levels. The inevitable transfer of fish and equipment aids the spread of infectious diseases. Aerosol transfer can transmit parasitic diseases not just inside a system, but also to nearby culture units. These anthropogenic stressors can cause acute and chronic stress, and the requirement to cope with the allostatic load necessitates metabolic rearrangement, which lowers growth performance, suppresses immune system activity, and makes fish more susceptible to diseases (Lira et al. 2020). Many studies have shown that some serious diseases pose a danger to warm and cold-water fish production facilities, such as velvet diseaseor gold dust disease. This is caused by dinoflagellates from the genera *Amyloodinium* and *Piscinoodinium*, white spot disease, which is caused by *Ichthyophthirius multifiliis* in freshwater and *Cryptocaryon irritans* in marine waters and individuals of the genus *Myxobolus*. The high pathogenicity of these organisms is produced by histological alterations, such as degeneration and necrosis in cells near to the attachment point, as well as significant structural damage to the epithelium by the rhizoid itself. These alterations might be caused by toxins or irritants generated by parasites. The host is extremely susceptible to osmoregulatory dysfunction and subsequent infections by other pathogens such as bacteria or fungus under these circumstances. A study conducted by McDermott and Palmeiro (2020), reported that Koi herpesvirus (KHV) which infects koi and common carp (*Cyprinus carpio*) causes high rates of mortality. It was originally identified as a cause of widespread fish death in Palestine in 1998 and has since spread around the world. Furthermore, parasites are being carried around the world through the aquarium trade, according to the findings of many studies and treatment of fish for recognized parasites prior to export or upon arrival in an importing country reduces the likelihood of subsequent parasite invasion and dispersal.

According to records, 8–10 million ornamental fish are imported into Australia each year, with similar quantities imported yearly for several decades. To reduce the spread of transboundary diseases, quarantine procedures are performed to be implemented for goods consignments by the government. Moreover, the principles of aquatic animal quarantine have been reviewed and closely follow the recommendations of the SPS agreement, such that disease exclusion is the primary justification for confinement, and the quarantine policy is based on a science-based risk analysis. Also, an environmental compatibility evaluation is performed to decide which species of live ornamental fish are allowed for import to limit the possibility of pest species establishing themselves in the wild. Thailand, Singapore, and Taiwan are the primary suppliers of live ornamental fish, accounting for approximately nine tons. To prevent the introduction and spread of aquatic diseases, an effective biosecurity program with thorough processes, practices, and regulations should be created. Even though quarantine is one of the primary requirements of the OIE (Office International des Epizooties), Sultanate relies only on the health certificate given by the country of origin. The detection of exotic infections in imported fish has resulted in a greater awareness and understanding of these diseases. In addition, it aids in reducing the danger of harmful viruses, bacteria, oomycetes, and parasites spreading to Oman's maritime environment. Increase the country's biosecurity by using a risk-analysis method that involves identifying and assessing fish diseases found in the live imported ornamental fish. In addition, the economics of the fisheries industry have been improved in Oman by implementing all biosecurity techniques and procedures in aquaculture.

The current data of the project assists aquaculture centers or farms by improving the health of their fish output while also preventing illness exacerbation or transmission in the surrounding environment. The objective of the present study was to evaluate the prevalence of parasitic pathogens in imported ornamental fish in Oman and study the ecological risks to the native fish species and implementation of biosecurity measures and restrictions.

Materials and methods

Fish sampling

Total of 138 fish representing 22 different species of ornamental fish were collected from different pet shops around Muscat governorate. The main two countries where the fish were im-

Table 1. List of the imported ornamental fish that were examined in the present study

Common name	Scientific name
Koi carp	*Cyprinus carpio*
Tiger Oscar cichlid	*Astronotus ocellatus*
Yellowbelly cichild	*Trichromus salvini*
Golden molly	*Poecilia sphenops*
Amazon molly	*Poecilia formosa*
Red zebra cichlid	*Maylandia estherae*
Tiger barb	*Puntigrus tetrazona*
Rose red discus	*Symphysodon aequifasciatus*
Clark’s anemonefish	*Amphiprion clarkii*
Dwarf groumi	*Trichogaster lalius*
Bala shark	*Balantiocheilos melanopterus*
Iridescent shark	*Pangasianodon hypophthalmus*
Walking catfish	*Clarias batrachus*
Giant snakehead	*Channa micropeltes*
Buenos Aries tetra	*Hyphessobrycon anisitsi*
Tetra negro	*Gymnocorymbus ternetzi*
Dalmatian molly	*Poecilia latipinna*
Rosy barb	*Pethia conchonius*
Tinfoil barb	*Barbonymus schwanenfeldii*
Angle fish	*Pterophyllum scalare*
Mexican tetra	*Astyanax argentatus*
Goldfish	*Carassius auratus*

ported were Thailand and Sri Lanka. The list of the ornamental fish that undergo a parasitological examination is shown in Table 1.

Parasitological examination

The procedures for diagnosing fish and identifying parasites was divided into two parts starting with a macroscopic screening for evident parasites to the human eyes and the overall external parts of the fish and its behavior using dissecting and compound microscopes with magnifications of x4 to ×1000 to visualize motile parasites. It was followed by a microscopic screening which was aimed to screen the non-visible parasites to the human eyes. In addition, skin scrapes and gill preparations were made. A skin scrape was performed by gently passing a slide glass through the mucus of the skin of the fish from random spots and suspected and infected areas. A drop of distilled water was added to the slide and observed under the light microscope. The gill preparation was done by cutting a tiny portion of the gill filaments from both sides.

Results

The results of the parasitological examination revealed that 50.72% of the examined imported ornamental fish were infected by one or more parasite species including *I. multifiliis, Dactylogyrus* sp., *Gyrodactylus* sp., digenean metacercaria and *Trichodina* sp. However, a parasitic skin infection was recorded in 42.75% of the examined imported ornamental fish while a parasitic gill infection was recorded in 55.79% of them. For the skin infection, there are some fish that recorded the highest aggregation of the parasitic infection among others such as golden molly (*Poecilia sphenops*), red zebra cichlid (*Maylandia estherae*), tetra negro (*Gymnocorymbus ternetzi*), and goldfish (*Carassius auratus*). Nevertheless, for the gill infection, fish that recorded the highest aggregation of the parasitic infection among others are Bala shark (*Balantiocheilos melanopterus*), Buenos Aries tetra (*Hyphessobrycon anisitsi*), and *G. ternetzi*. The observation of the skin scrapes performed for the sampled fish was recorded in Table 2 which shows the skin infection due to the founded parasites with the overall prevalence of these pa-

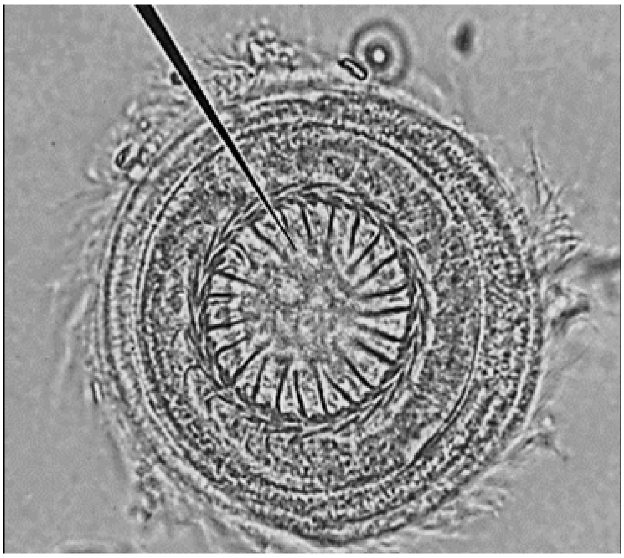
Figure 1. Shows an *Ichthyophthirius multifiliis* found in the skin mucus of red zebra cichlid (*Maylandia estherae*) at 10x magnification under the light microscope.

Table 2. Prevalence of skin and gill infestation of host species (S): skin, (G): gill

Parasites	Host	Prevalence
Ichthyophthirius multifiliis	Golden molly (*Poecilia sphenops)*	50% (S)
	Red zebra cichlid (*Maylandia estherae)*	33% (S)
	Iridescent shark (*Pangasianodon hypophthalmus)*	83% (S)
	Iridescent shark *(Pangasianodon hypophthalmus)*	100% (G)

Dactylogyrus sp.	Tetra negro (*Gymnocorymbus ternetzi)*	67% (S)
	Angel fish (*Pterophyllum scalare)*	83% (S)
	Tiger Oscar cichlid *(Astronotus ocellatus)*	100% (G)
	Tiger barb *(Puntigrus tetrazona)*	67% (G)
	Rose red discus *(Symphysodon aequifasciatus)*	33% (G)
	Bala shark *(Balantiocheilos melanopterus)*	83% (G)
	Tetra negro *(Gymnocorymbus ternetzi)*	33% (G)
	Angel fish *(Pterophyllum scalare)*	67% (G)
	Goldfish *(Carassius auratus)*	75% (G)
Gyrodactylus sp.	Tiger barb (*Puntigrus tetrazona)*	67% (S)
	Buenos Aries tetra (*Hyphessobrycon anisitsi)*	67% (S)
	Tetra negro *(Gymnocorymbus ternetzi)*	50% (S)
	Goldfish (*Carassius auratus)*	75% (S)
Digenean metacercaria	Golden molly (*Poecilia sphenops)*	67% (S)
	Koi carp *(Cyprinus carpio)*	83% (G)
	Golden molly *(Poecilia sphenops)*	33% (G)
	Red zebra cichlid *(Maylandia estherae)*	67% (G)
	Buenos Aries tetra *(Hyphessobrycon anisitsi)*	83% (G)
	Bala shark *(Balantiocheilos melanopterus)*	83% (G)
	Tetra negro *(Gymnocorymbus ternetzi)*	67% (G)
	Rosy barb *(Pethia conchonius)*	83% (G)
	Tinfoil barb *(Barbonymus schwanenfeldii)*	33% (G)
	Mexican tetra *(Astyanax argentatus)*	67%(G)
Trichodina sp.	Yellowbelly cichlid (*Trichromus salvini*)	33% (S)
	Red zebra cichlid (*Maylandia estherae)*	50% (S)
	Dalmatian molly (*Poecilia latipinna)*	67% (S)
	Goldfish (*Carassius auratus)*	58% (S0
	Buenos Aries tetra *(Hyphessobrycon anisitsi)*	33% (G)

Figure 2. Shows the observed individuals of the parasitic *Trichodina* sp. found on the skin mucus of yellowbelly cichlid (*Trichromus salvini*) at 100x magnification under the light microscope.



rasites in the skin mucus of the ornamental fish, besides the preferred host of the parasites and the prevalence of each parasite by the host. The results in Table 2 showed that the most abundant parasite was *Gyrodactylus* sp., which recorded a 14.49% prevalence followed by *Trichodina* sp., which recorded an 11.59% prevalence and the *I. multifiliis* recorded a 7.24% prevalence. Whereas dactylogyrid monogeneans were recorded with a prevalence of 6.52% of the examined fish. The lowest prevalence was 2.89%, which was recorded for the digenean metacercaria. Figure 1 shows an *I. multifiliis* found in the skin mucus of *M. estherae* and Figure 2 shows the observed individuals of the parasitic *Trichodina* sp. found in the skin mucus of yellowbelly cichlid (*Trichromus salvini*).

Figure 3. Shows an infection of the parasitic digenean metacercaria within the gill filaments of a tiger barb (*Puntigrus tetrazona)* at 10x magnification.

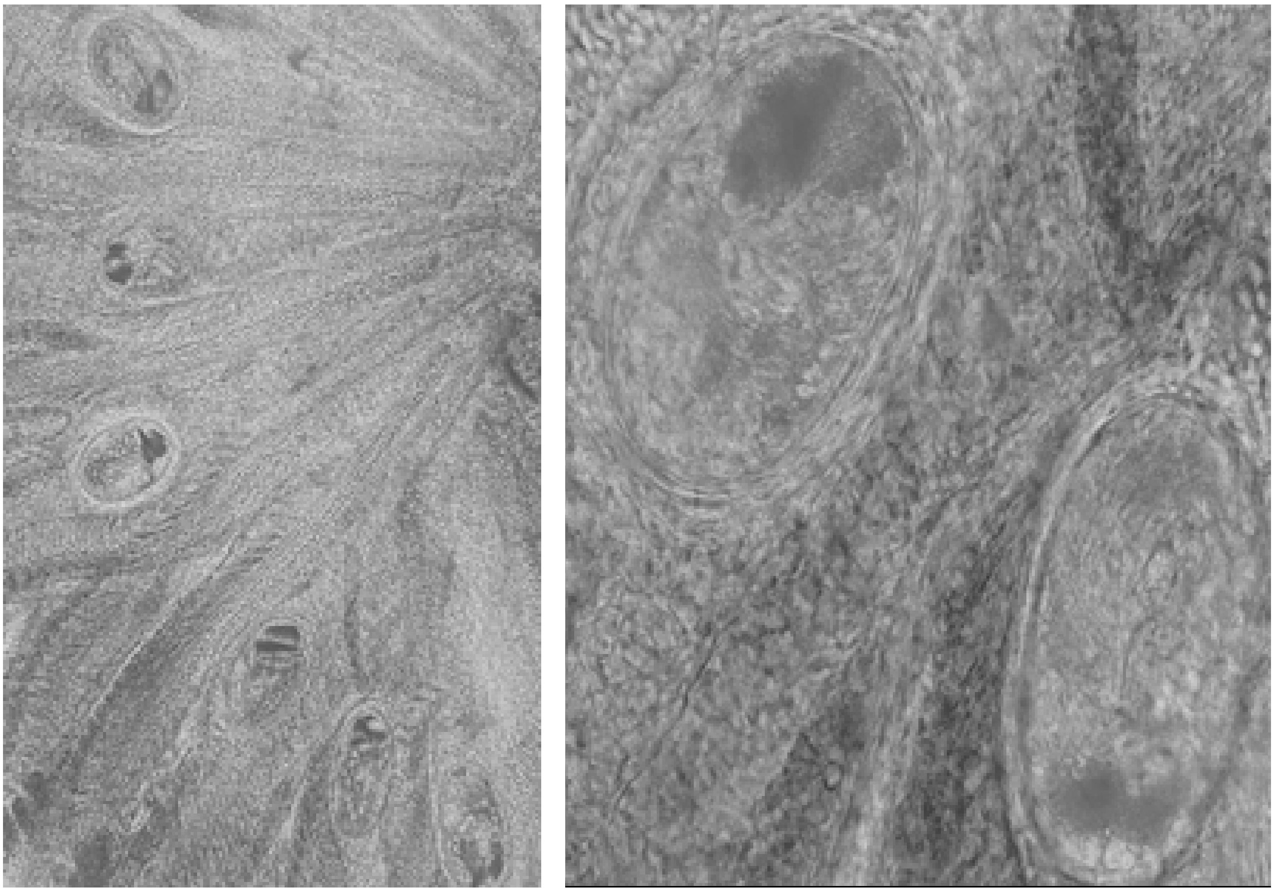


Figure 4. Shows individuals of parasitic digenean metacercaria found in the gill filaments of tinfoil barb (*Barbonymus schwanenfeldii*) at 10× magnification

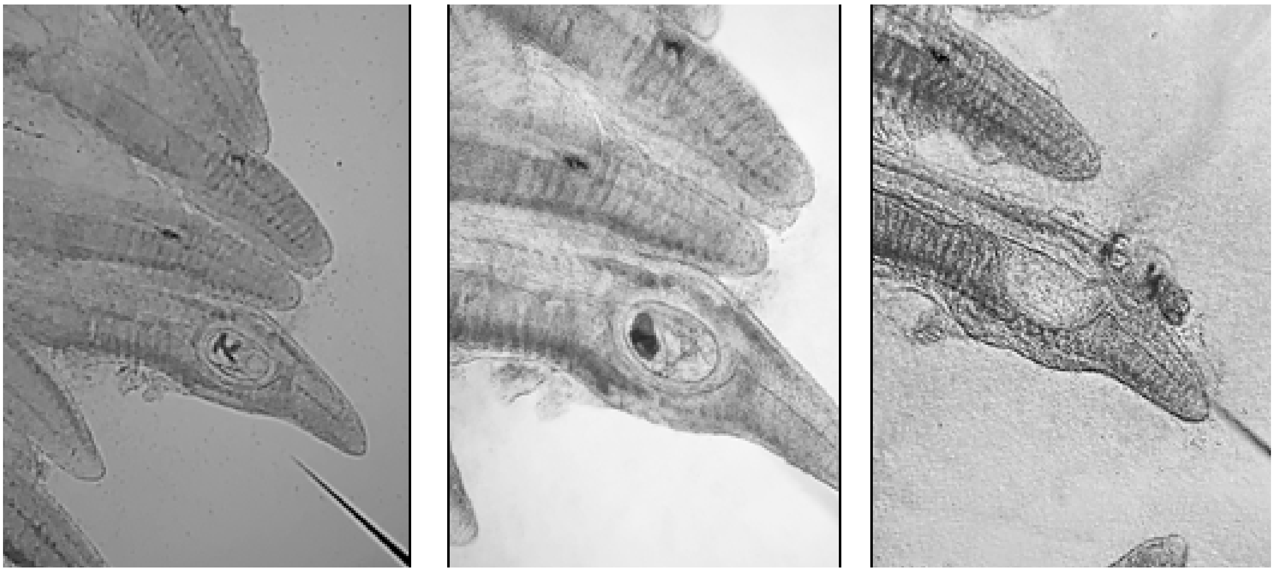


Figure 5. Shows an infection caused by a parasitic dactylogyrid monogenean observed on the gills of a tiger oscar cichlid (*Astronotus ocellatus*) at 10x magnification.

Figure 6. Shows individuals of *Dactylogyrus* sp. attached to the gill's filaments of goldfish (*Carassius auratus*) at 10x magnification.

Figure 3 shows an infection of digenean metacercaria within the gill filaments of *P. tetrazona*. Figure 4 shows individuals of parasitic digenean metacercaria found in the gill filaments of tinfoil barb (*Barbonymus schwanenfeldii*). Figure 5 shows a heavy infection caused by a parasitic dactylogyrid monogeneans observed in the gills of tiger oscar cichlid (*Astronotus ocellatus*). Figure 6 shows individuals of *Dactylogyrus* sp. attached to the gill filaments of *C. auratus*. Table 2 summarises parasitic infections in the gills and the prevalence of each. The results in Table 2 show that the most abundant parasite in the gill infection was digenean metacercaria that recorded 26.08% prevalence followed by dactylogyrid monogeneans, with a prevalence of 23.91%. While the prevalence of *I. multifiliis* in the gills was recorded only in 7.24% of the examined fish. The lowest prevalence was recorded for *Trichodina* sp. which was 1.44%. There are, however, no records of *Gyrodactylus* sp. on the gills of the examined fish.

Discussion

This study found that some common parasite species such as *I. multifiliis*, *Dactylogyrus* sp., *Gyrodactylus* sp., digenean metacercaria and *Trichodina* sp. infected almost half of the examined imported ornamental fish. A scientific study reported that ornamental fishes were found infected with three different ectoparasite genera: monogenean, digenean, and protozoan ciliates, according to the survey that is strongly agreed by the current study (Al-Rawahi et al. 2019). Furthermore, fish gills perform a variety of critical processes such as breathing, osmoregulation, and excretion, and they are constantly in contact with the outside world, making them highly sensitive to changes in water quality. Gills provide an excellent indication of water quality, and they could be served as a model for environmental impact research (Mohammadi et al. 2012). The prevalence of gill infections was found to be higher than those found on the skin (i.e., 55.79% and 42.75% respectively). The reason for this occurrence is that the attachment organ (i.e., haptors) of most parasites is attached to the inner surface of the proximal area of the gill filament, which is located between the outer and inner hemibranchs of the same gill arch. This type of attachment to the gill filament might give some protection from the host's gill ventilating current (Arafa et al. 2009).

Nevertheless, the results in Table 2 showed that the most abundant parasite in skin infections was *Gyrodactylus* sp., which was recorded 14.49% while there were no records on the presence of *Gyrodactylus* sp. in the gills of the examined fish species. A study showed that *Gyrodactylus* was principally found on the fins of most diseased fish, although where it lives depends on the severity of infection (Chanda *et al.* 2011). Furthermore, the lowest prevalence was recorded for *Trichodina* sp. (Fig. 2) which showed 1.44% prevalence in the gills of the examined fish. This is confirmed by a previous study that showed that the protozoan *Trichodina* sp. had the lowest frequency of parasitic infection (11 %) in the examined ornamental fish (Al-Rawahi et al. 2019).

The results showed that tiger oscar cichlid (*Astronotus ocellatus*) recorded a huge gill infection by *Dactylogyrus* sp. as shown in Figure 5. This was also reported by a previous study that *Dactylogyrus* sp. was a common parasite in Oscar and oviparous dactylogyrids are predominantly gill parasites, although they can also be found in the skin. In addition, clinical signs of *Dactylogyrus* sp. infection included lethargy, unilateral swimming, gill filament erosion, and scale loss in the affected fish (Mohammadi et al. 2012). Moreover, for the skin infection, some fish recorded the highest aggregation of the parasitic infection among others such as *P. sphenops*, *M. estherae*, *G. ternetzi*, and *C. auratus*. A study conducted by Evans and Lester (2001), showed that poeciliids frequently interbreed, and the parasite population of all family members may be extremely similar. The trade of fish enables parasitic dispersion among poeciliids reared in Asia across ponds for breeding reasons.

Nevertheless, for the gill infection, the fish that recorded the highest aggregation of the parasitic infestation among others are *B. melanopterus*, Buenos Aries tetra (*Hyphessobrycon anisitsi*), and *G. ternetzi*. A heavy infection of parasites on *B. melanopterus* as reported that assemblages could be chronic and life-threatening, thus leads to economic losses as well as increases the risk of transmission to other species including native species. As shown in Figure 1, a huge aggregation of *I. multifiliis* individuals observed in the fins of *P. hypophthalmus* at 10× magnification (Morine *et al*. 2012). *P. hypophthalmus* recorded a 100% prevalence of parasitic *I. multifiliis* on its gills known to be a highly susceptible to this protozoan ciliate (Mamun et al. 2020). Nabi et al. (2015) reported that the protozoan parasite *I. multifiliis* was shown to be highly susceptible in fry of *P. hypophthalmus* imported from Thailand to Europe as ornamental fish. In addition, ichthyophthiriosis is a disease caused by *I. multifiliis* that causes considerable financial losses in the aquaculture sector, especially the ornamental fish trade (Tange et al. 2020). However, there is a variation on the type of the detected parasites found in each examined fish which might be connected to the occurrence's seasonality and water temperature (Brzezinski et al. 2021).

Conclusion

The current survey of imported ornamental fish found that *I. multifiliis*, *Dactylogyrus* sp., *Gyrodactylus* sp., digenean metacercaria and *Trichodina* sp. infected almost half of the imported ornamental fish. Some species such as *I. multifiliis* may be life-threatening if left unmanaged and allowed to proliferate. The identity of other parasite species remains to be determined and they may pose a threat to native fish populations and the aquaculture sector. The level of risk will increase with the lack of pre- and post-border controls, such as permitted health certifications, sophisticated inspection tests, and quarantine detention. The lack of knowledge about pathogenic parasites found in imported ornamental fish, the function of carrier or reservoir species in the spread of exotic diseases, hence, the introduction of exotic diseases into native fish might worsen the problem. It is recommended that a risk analysis method based on the detection and assessment of fish diseases found in live ornamental fish be established and used to enhance national biosecurity. In addition, exotic pathogenic parasites, as well as their hosts, must be closely monitored to reduce the risk of parasite introduction into Omani waters.

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