Isolation of *Ichthyophthirius multifiliis*, *Gyrodactylus* and *Copepoda: Lernaeidae* Species from Six Ornamental Fish Species for the First Time in Iraq

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Abstract

Ornamental fish species showed mucous hyperscretion, signs of irritation, slight shedding of scales and large areas of skin hyperaemia. They included brood Goldfish (*Carassius auratus*; varieties: Common, Fantail, Ryukin, Veiltail and Moor), Black molly (*Poecilia sphenops*), Gourami (*Trichogaster trichopterus*), Swordtail (*Xiphophorus helleri*), Guppy (*Poecilia reticulata*), Angelfish (*Pterophyllum scalare*) and brood Suckermouth Algae Eater (*Hypostomus plecostomus*). They were subjected to gross observation followed by microscopic examination of mucous scrapes from both sides of the body and from the fins. All the examined fish suffered mixed parasitic infestation. Four species of parasites were detected in mucous scrapes of *Carassius auratus*, *Poecilia sphenops*, and *Trichogaster trichopterus*, three of these parasites species in mucus scrapes of *Xiphophorus helleri*, whereas mucus scrapes of *Poecilia reticulata, Pterophyllum scalare* and *Hypostomus plecostomus* contained only two species of these ectoparasites. The results have indicated that three of the encountered parasites, namely *Ichthyophthirius multifiliis*, *Gyrodactylus* species and Copepoda: Lernaeidae species were recorded for the first time in Iraq. The binomial name of the fourth species could not be identified. The higher incidence percentage of infection 100% was for *Ichthyophthirius multifiliis* and the unidentified species, followed by 45.45% for *Gyrodactylus* species and then 36.36% for Copepoda: Lernaeidae species.

عزل *Ichthyophthirius multifiliis* و Gyrodactylus Species والطور اليرقي للدودة الكلابية من ستة أجناس من أسماك الزينة لأول مرة في العراق

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الخلاصة

فحصت عيانيا أمهات السمكة الذهبية *Carassius auratus* ، المولي الأسود *Poecilia sphenops*، الجورامي *Trichogaster trichopterus* ، ذنب السيف الأحمر *Xiphophorus helleri* ، Guppy Poecilia reticulata، سمكة الأنجل *Pterophyllum scalare* وأمهات الكناس *Hypostomus plecostomus* التي كانت تعاني من إفراط فرز المخاط، تخريش، مساحات احتقان كبيرة على الجلد وسقوط خفيف للحراشف. أخذت من الأسماك عينات من المخاط على سطوحها الخارجية وفحصت مجهريا. كانت جميع الأسماك المفحوصة تعاني من إصابة مختلطة. تم تشخيص أربعة أجناس من الطفيليات *Carassius auratus*، *Poecilia sphenops* و *Trichogaster trichopterus*، ثلاثة أجناس من الطفيليات في *Xiphophorus helleri* وجنسين من

الطفيليات في *Poecilia reticulata* ، *Pterophyllum scalare* و *Hypostomus plecostomus* . أظهرت نتائج هذه الدراسة أن *Ichthyophthirius multifiliis* ، Gyrodactylus species و copepodal Lernaea قد تأكد، ولأول مرة في العراق، وجودها في أجناس أسماك الزينة التي جرى فحصها. كانت أعلى نسبة حدوث للإصابة للطفيلي *Ichthyophthirius multifiliis* وجنس غير مشخص 100%، بينما كانت النسبة المئوية لحدوث الإصابة 45.45% *Gyrodactylus species* وأقل نسبة حدوث للإصابة كانت Copepodal Lernaea 36.36%.

Introduction

Most parasites cause little pathology under normal condition, and wild fish may harbor a variety of parasites without any associated morbidity or mortality (1, 2). In cultured fish populations, however, parasitic infestations often cause serious outbreaks with remarkable economic loss. The presence of dense populations of fish kept under stressful environmental conditions may favour certain parasite species leading to the establishment of very high noxious parasites populations. The numbers of parasites that could cause harm to a fish varies considerably with the size of the host and its health status (2, 3, 4). Beside their own insult, parasites can act as vectors of bacteria, viruses, or parasitic protozoa that further endanger the affected fish (5, 6, 7). Perhaps the most important holotrichian ciliate parasite is *Ichthyophthirius multifiliis*, the causative agent of white-spot disease. *Ichthyophthirius* causes great losses in both warm-water and temperate fish culture and may even cause losses in wild fish as well as in domesticated aquarium fish (3, 8, 9, 10). The parasite normally infects the epidermis, fins and gills but in epizootics may even be found in the corneal, bucal cavity and esophageal epithelia, *I. multufillis* may reach 0.5-1.0 mm in diameter and has the appearance of a white spot within the integument. It is easily recognized microscopically by its size and characteristic horseshoe-shaped macronucleus, although in the juvenile stage (tomite) nucleus is round, when mature, *I. multifillis* ruptures the skin and escapes into the water causing to the host epithelial erosion and cuticular thickening. Once free, the parasite encysts on the substrate and divides internally to produce up to 2000 oval ciliated tomites, then leave the cyst to reinfect fish. Ichthyophthiriasis is a cold water fish disease and increasingly encountered in winter (3, 11, 12). *Gyrodactylus* species are significant in both fresh-water and marine fish culture. Gyrodactylid trematodes are about 0.3 – 1.0 mm in length with two points at the anterior end. An anterior sucker is present, but no eyespots. An attachment organ (haptor) with two large hooks, surrounded by up to 16 hook lets is located at the caudal end. IT is found on skin, gills and fins. Unapparent infections are common. The parasites fed on blood and epithelium by scraping and sucking. Lesions can include localized hemorrhagic areas, excessive mucus, and localized ulcerations. Gyrodactylids are viviparous. (13,14). In one *Gyrodactylus* four Generations may be performed (11). *Lernea*, commonly called anchor worms, are elongated copepods that attach to the skin of fish. Larval stages are free-swimming and have a typical crustacean form with segmented body and legs (15). Free-swimming immature forms of *Lernea* species fed on superficial mucus and debris, undergoing a number of molts while growing and differentiating into smaller males and larger females. When the parasites reach maturity, copulation occurs and the females penetrate the skin of the host fish. The life cycle is temperature dependent. Heavy infestation of *Lernea* can be fatal, affected fish show irritation and localized hemorrhagic reactions at the point of entry. These areas may become secondarily infected with bacteria (2, 16, 17).

Materials and Methods

- **The Fish:** The fish were brought from an earth pond which was supplemented with water coming from carp ponds. Number of the employed fish was as the following: 12 brood Goldfish (*Carassius auratus*) (Common, Fantail, Ryukin, Veil tail, and Moor) with a length of 9.5-14 cm, four red Swordtail (*Xiphophorus helleri*) with a length of 7-7.5 cm, one Gourami (*Trichogaster trichopterus*) with a length of 6.5 cm, three Black molly (*Poecilia sphenops*) with a length of 4-5 cm, seven Guppy *(Poecilea reticulata*) with a length of 2.75-4 cm, nine deep Angel fish (*Pterophyllum scalare*) with a length of 2-2.5 cm and eight brood Algae eater (*Hypostomus plecostomus*) with a length of 6-11cm. At the beginning, they were held in 120 liters glass aquaria supplemented with dechlorinated tap water, oxygenated with coarse air bubbles and fed powder fish feed twice a day. Water temperature was 17 $\pm$ 2C°. After the clinical signs started to appear, the fish were transferred into 260 liters aquarium supplemented with dechlorinated tap water, oxygenated with fine air bubble and fed pellets and crushed shrimp. Water temperature was 20 $\pm$ 2C°.
- **Fish examination:** clinical examination was carried out on the living fish in the aquarium. They were grossly examined for the detection of any gross lesion. Microscopical examination was performed by taking mucus scrapes from both sides of the fish and from fins and transferred onto glass slides. The mucus scrapes were examined under a compound light microscope with magnification power of X10 and X400 following steps outlined by Lucky (18) and Brown *et al*. (19). Identification of the parasites was according to (2, 3). Incidence percentage of infection was calculated by the division of the number of infected fish with the specific parasite on the number of examined fish as described by Margolis *et al*. (20).

Results

The closed microscopic examination revealed presence of *Ichthyophthirius multifiliis* in the mucus scrapes preparations from Goldfish (Common, Fantail, Ryukin, Veiltail, and Moor), Gourami, red Swordtail, Guppy, deep Angel fish, and Algae eater. Similarly, *Gyrodactylus* species have been observed in Gold fish Common and its relatives, Gourami, Black molly, and red Swordtail. Presence of the Copepodal stage of Lernaea, has been confirmed in Gold fish Common and its relatives, Black molly, and Gourami, (Prof. Furhan T. Mhaisen: personal communication). *Carassius auratus, Poecilia sphenops,* and *Trichogaster trichopterus* were infested with four species of parasites. *Poecilia sphenops* was infested with three species of parasites. While *Poecilia reticulata*, *Pterophyllum scalare*, and *Hypostomus plecostomus* were infested with two species of parasites as show in Table (1).

Table (1) Parasites species detected in the ornamental fish

Fish species	Parasites			
	Ichthyophthirius multifiliis	***Gyrodactylus* Species**	**Unidentified Species**	***Copepodal Lernaea***
Goldfish	*	*	*	*
Black Molly	*	*	*	*
Gourami	*	*	*	*
Swordtail	*	*	*	
Guppy	*		*	
Angelfish	*		*	
Algae eater	*		*	

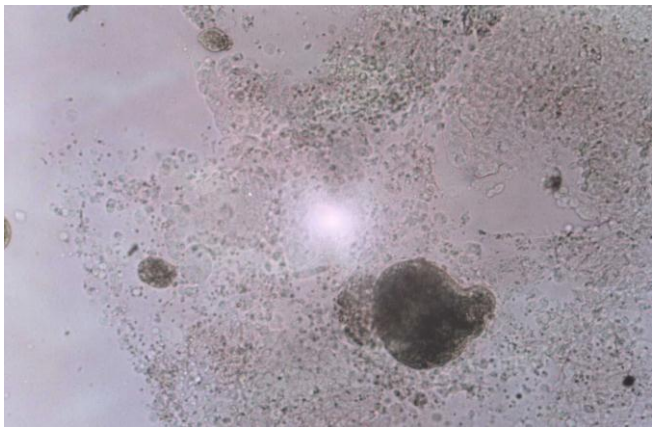

Fig. (1) *Ichthyophthirius multifiliis*. The parasite was microphotographed while it was moving. (X 40)

The gross lesions noticed in infected fish were large hyperemic area of the skin, light shedding of the scales, most fish were restless and showed signs of irritation like scraping of the skin with the inner surface of the aquarium, mucus hypersecretion of the skin, swallowing of the air was noticed in some fish which rise to the surface. After several days from the beginning of the clinical signs some mortality occurs.

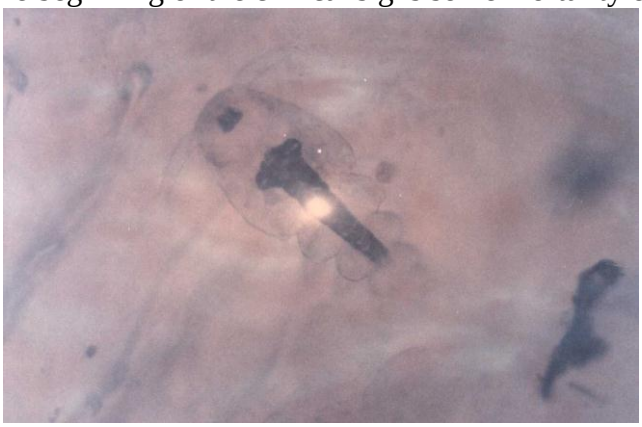

Fig. (2) Copepoda: Lernaeidae species. (X 10)

The higher incidence percentage of infection was for Ichthyophthiriasis and an unidentified parasite in which they infected all the examined fish 100%, 45.45% for Gyrodactylasis, and the lowest incidence percentage of infection was for copepodal lernaeasis 36.36% as shown in Table (2).

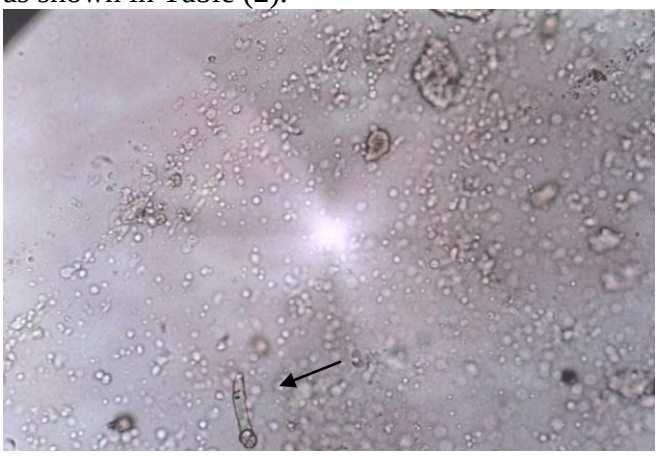

Fig. (3) Gyrodactylus Species. (X 40)

Table (2) Incidence percentage of infection of the detected parasites

Parasite	Number fish		Incidence Percentage of Infection
	Examined	Infected	
Ichthyophthirius multifiliis	44	44	100
***Gyrodactylus* species**	44	20	45.45
Unidentified species	44	44	100
Copepodal Lernaea	44	16	36.36

Discussion

Results of the present study showed that *Ichthyophthirius multifiliis*, *Gyrodactylus* species, and *copepodal Lernaea* have been firstly recorded in Goldfish, Black molly, Gourami, red Swordtail, Guppy, deep Angel fish, and Algae eater. All the examined fish were infected with mixed infestation of several parasites species. There were four species of parasites on the same fish in *Carassius auratus*, *Poecilia sphenops*, and *Trichogaster trichopterus*, three species of Parasites on the same fish in *Xiphophorus helleri*, and two species of parasites on the same fish in *Poecilia reticulata*, *Pterophyllum scalare* and *Hypostomus plecostomus*. *Ichthyophthirius multifiliis* has a worldwide distribution and affects all fresh water fishes. Under aquarium conditions, it is particularly virulent. *Ichthyophthirius* is one that is readily identified by most aquarists. Predominant signs include small spots over the body. Diagnosis can be confirmed by microscopic examination of biopsy materials from skin or gills. Theronts appear round to oval and may be from 30 to 100 mm in diameter. The horseshoe-shaped nucleus is often visible and aids in identification. *Ichthyophthirius multifiliis* is one of the few fish parasites with cilia surrounding the entire organism (8, 12, 19). *Gyrodactylus* infect a broad range of hosts, including most freshwater tropical fishes and aquarium fishes (13, 14, 21). Each species is host-specific and may even be specific to a particular site on the host. The occurrence of Gyrodactylids in epizootics in cultured fish populations is generally a sign of poor husbandry conditions. They are often found in conjunction with one or more species of ectoparasitic protozoa (4, 22). Members of the genus *Lernaea* are highly specialized parasitic copepods, with a high degree of structural departure from ancestral free-living crustaceans. *Lernaea* is a very successful freshwater genus, capable of fully exploiting mesoparasitism and adapting to a large variety of habitats offered by different hosts and by different sites on these hosts (11, 16, 23). *Lernaea* infestations are very common in Koi and Goldfish from production ponds, but any species can be affected (3, 24, 25). The parasites may be present on the fish surface, Fins, gills or in the internal organs without harmful consequences to the host. But some conditions, such as changes in the aquatic parameters, climate, stress, introduction of some pathogen that was not present in the environment, are factors that can increase the host susceptibility to parasites and provoke an unbalance of the host/parasite/environment system (14). Overcrowding of fish which was first observed increased the chances of contact between parasites and potential hosts. Crowding led to changes in the organic load of the water, which promotes parasitism by enhancing the microenvironment for the parasites. Feeding powdered fish feed additionally increased the organic matter of the water, by the dissolving of an eating feed (6, 14). Mixed infestation was higher for *Carassius auratus*, *Poecilia sphenops*, and *Trichogaster trichopterus* with four species of parasites, and for *Xiphophorus helleri* with three species of parasites, the larger fish species within the examined fish. This could be due to the increased susceptibility of these fish to the infestation with these parasites, (26) reported that the infestation percentage and virulence increase by increasing the fish length. In addition to unknown reasons. investigation is needed to obtain broad spectrum information on species of parasites that infest pet fish, their pathogenecity and their coexistence. Estimation of the possible economic loss should not be overlooked.

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