



Microscopical and molecular detection of *Trichodina* spp. in fish in Mosul, Iraq

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Abstract

Trichodiniasis is a serious fish ectoparasitic infestation worldwide. The ciliate protist distributes it and infests the fish's skin, fins, and gills. Two hundred fish samples of five species (*Arabibarbus grypus*, *Cyprinus carpio*, *Chondrostoma regium*, *Luciobarbus xanthopterus* and *Mesopotamichthys sharpeyi*) were collected from local city markets in Mosul from August 2023 to the end of February 2024. For the detection of infestations with *Trichodina* spp., direct wet mount smears from the skin, gills and fins were done and confirmed the infestation by Polymerase Chain Reaction (PCR) and phylogenetical tree analysis by amplifying the 18S rRNA gene. Depending on morphological characteristics, microscopical examination results revealed that the total infestation rate with *Trichodina* spp. was 19.5% (39/200). *Trichodina* sp. appeared pear to round in shape with a distinct adhesive disc with a diameter of 37-60 μm and golden to brown. Molecular and gene sequencing analysis confirmed the infection with *Trichodina* spp., the reaction product 1700 bp. One positive isolate was recorded in the NCBI under accession number PQ012982. It showed a 100% match with other isolates for the same genus compared with other studies in South Korea registered in the GenBank.

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Introduction

The fish industry is one of the main economic sectors on which some countries increasingly rely to support agricultural resources (1). Scientists and researchers have increased their attention to this sector to develop, exploit, and maintain fish production as part of food requirements (2). Fish, like other animals, are exposed to many pathological agents like parasites during their different life spans, and these diseases increasingly appear in the intensive feeding system, which negatively affects their growth and production and may lead to mortalities, resulting in heavy losses in fish cultures (3,4). Ectoparasites infest the surface layer of the skin, fins, and gills (5). The spread of infection is rapid, accumulating very large numbers quickly due to rapid reproduction by binary fission and direct life cycle (6,7). Trichodiniasis (slime disease) is an essential global fish disease (8). The causative agent is a ciliate protist that

belongs to the family Trichodinidae, genus *Trichodina* (9). The *Trichodina* is an important ectoparasite-infested freshwater fish; this genus consists of about 270 species recorded worldwide (10). Microscopically, this parasite has distinctive features, for it has a basic saucer shape with a rigid lenticular ring, has a monogenic lifecycle on the host, and infestation occurs either by contaminated water or through direct contact (11). *Trichodina* parasite attaches to the epithelial surfaces of skin, fins and gills tissues and brows over with rotation movement, causing damage to host tissues by feeding on tissue debris. Finally, erosion of the epithelium will occur, which gives the infested fish a grayish appearance resulting from excess mucus production and frayed fin (5). Outbreaks with trichomoniasis often occur in poor water quality, crowded or stressed cultures with inappropriate environmental factors and nutritional deficiencies. Mortality occurs due to heavy skin damage (12-14). Acute Trichodiniasis causes loss of appetite, signs of irritation,

decreased growth, weight loss, abnormal coloration, lethargic movement, skin lesions, fin erosion, elevated mucus production, and localized lesions and gill necrosis disorders in normal respiratory and osmoregulatory processes of the fish. In addition, skin lesions and ulcers allow the development of secondary bacterial infections (15,16).

Hence, this study was organized to detect the *Trichodina* spp. Microscopically and molecularly by PCR technique and phylogenetic tree to approve the infection.

Materials and methods

Ethical approval

The institutional animal care and use committee in the College of Veterinary Medicine, University of Mosul, ethically permitted this study (UM.VET.2023.126) on 16/7/2023.

Fish samples

Two hundred samples of five fish species: 30 *Chondrostoma regium*, 54 *Cyprinus carpio*, 56 *Arabibarbus grypus*, 35 *Mesopotamichthys sharpeyi* and 25 *Luciobarbus xanthopterus* were brought from local fish markets in Mosul from Aug. 2023 to Feb. 2024.

Parasitological examination

Samples from different locations on fish skin, gills, and fins were separated and placed in Petri dishes. Then, direct wet smears were made by taking small samples on clean slides and examined by light microscope power 40X, 100X (17) with Lugol iodine-positive slides from *Trichodina* stained with Giemsa. Morphological characteristics of the parasite were identified depending on (18). Positive samples with microscopical examination were kept at -20°C until PCR analysis.

DNA Extraction

According to the manufacturer's instructions, DNA from fish tissue samples was extracted using the DNA extraction kit AddPrep genomic from tissue mini-Kit (Korea, Add bio). Then, the concentration and purity of extracted DNA were evaluated via the NanoDrop (Thermo Fisher, USA). The obtained DNA was stored at -20°C until further study.

PCR analysis and gel electrophoresis

PCR was done by using specific primers obtained from (Macrogen, Korea) Tri-Forward (5'-AACCTGGTTGATCCTGCC-3') and Tri-Reverse (5'-TGATCCTTCTGCAGGTTTC-3') according to Mizuno *et al.*, 2016 (14). To amplify the 18SrRNA gene of *Trichodina* sp. A 25 µl PCR reaction included 8.5 µl of PCR grade water, 2 µl of DNA, 12.5 µl of master mix (2x AddTaq Master Mix), and 1 µl for each of the forward and reverse primers. Amplification was performed using a thermocycler in the

following conditions: one cycle at 94°C for 10 minutes, followed by 35 cycles of 45 seconds at 94°C, 45 seconds at 56°C, and 1 minute at 72°C. The final extension was set for one cycle of 72°C for ten minutes. At 4°C, the processes were finally cooled. Amplified PCR products were separated by gel electrophoresis using 1.5% agarose gel (Korea Addbio) was prepared and mixed with 3 µl with gel red staining solution (Korea Addbio) to verify the amplified products. The gel was examined under UV light using a gel documentation system (USA, Gel Doc EZ Image, Bio-Rad) to certify and determine expected bands. For each electrophoresis, a 100 bp DNA molecular weight marker was added.

DNA Sequencing and phylogenetic analysis

After processing PCR amplification, the PCR products of 12 positive samples were sequenced (Macrogen, Korea). The obtained gene sequences were compared with *Trichodina* spp using the Sanger sequencing method-18SrRNA gene sequences from other countries already registered in the GenBank. The sequencing results were obtained as FASTA format text files and then analyzed for genetic affinity.

Results

Results revealed that fish samples were infested with the ectoparasite *Trichodina* spp., where the infection rate was 19.5% by microscopic examination of a direct wet smear of skin, fins and gills depending on the morphological characteristics, where the *Trichodina* parasite appeared pear or round in shape having adhesive disc and golden to brown with size between 37-60 µm. as shown in (Figures 1-3).



Figure 1: *Trichodina* spp. in wet mount of the skin scrap 40X.



Figure 2: *Trichodina* spp. in wet mount of the skin scrap with Giemsa stain 100X.



Figure 3: *Trichodina* spp. in wet mount of the skin scrap. Parasite's binary fission 40X.

Molecular analysis results by PCR confirmed morphological identification of *Trichodina* spp. by amplification of 18SrRNA gene, paths 4, 5, 7 and 8 representing positive samples with the reaction product bp 1700 while 1, 2, 3, 6, 9 and 10 represent negative samples, and 11 are negative control (Figure 4).

The gene sequencing results also confirmed the presence of *Trichodina* spp. by using a partial 18S rRNA gene with the accession number PQ012982 when matched with isolates recorded in the NCBI Genbank. The results showed a 100% match with each of the isolates OQ940397, OQ940396, OQ940395, OQ940394, OQ940392, OQ940391, OP811527, OP811526, OP445708, OP445707, OP445706, and OP445703 in South Korea (Table 1).

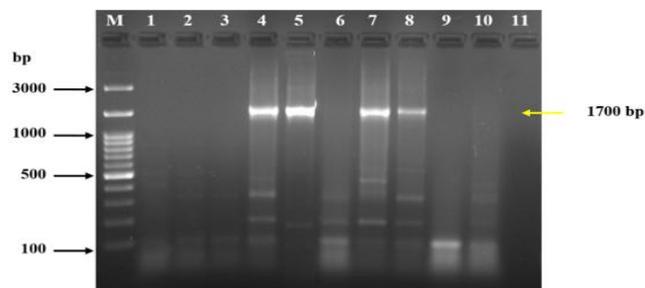


Figure 4: Agarose gel electrophoresis of PCR products for detecting *Trichodina* spp. Path M: The Marker represents a volume of 100 bp. Paths 4, 5, 7 and 8 represent positive samples with a product size of 1700 bp; Paths 1, 2, 3, 6, 9 and 10 represent negative samples; Path 11 represents negative control.

Table 1: Distribution of *Trichodina* spp. on partial 18SrRNA according to nblast in Genbank of NCBI

Sample	Query Cover %	Identic Number %	GenBank	Country
PQ012982	100	100	OQ940397	South Korea
	100	100	OQ940396	South Korea
	100	100	OQ940395	South Korea
	100	100	OQ940394	South Korea
	100	100	OQ940392	South Korea
	100	100	OQ940391	South Korea
	100	100	OP811527	South Korea
	100	100	OP811526	South Korea
	100	100	OP445708	South Korea
	100	100	OP445707	South Korea
	100	100	OP445706	South Korea
	100	100	OP445703	South Korea

The gene sequencing results of infested fish with *Trichodina* spp. were positive, and the isolation was recorded in NCBI under serial number PQ012982. At the same time, the genetic tree analysis of the parasite was compared with other isolates (Figure 5).

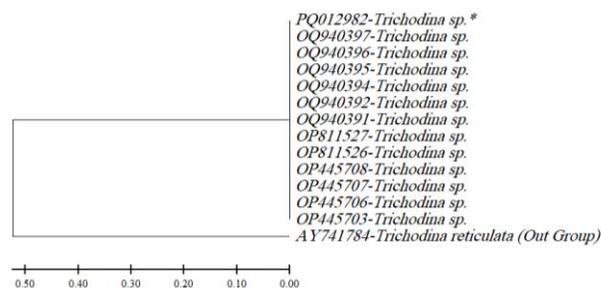


Figure 5: Phylogenetic tree of *Trichodina* spp.

Discussion

A total of 200 fish samples were examined microscopically. The total infestation rate was 19.5%, recorded depending on morphological characteristics and key identification. The *Trichodina* parasite appeared pear or round in shape, having an adhesive disc, and golden to brown with a size between 37-60 μm (19). The parasite *Trichodina* usually dwells in small numbers on the gills and skin, but when the host, parasite and environment relationship is broken due to bad water quality, nutritional deficiency, infections and other parasitic diseases, the parasite multiplies quickly, leading to outbreaks (14,20).

The infestation rate in this study, 19.5%, is higher than in Kirkuk (21), which recorded 13.8% in *Silurus glanis* fish. It is similar to recorded 17.2 and 18.2% (22,23), but were disagree recorded 25, 31 and 64.1%, which is more than the rate of this study, these high and low differences in the infestation rates may be attributed to species of fish, other stress factors such as environment, management, nutrition, overcrowding, temperature and PH of water (24-28). The researcher mentioned that *Trichodina* is commensals ectoparasite protozoa but under bad quality water and/or suppresses fish's immune system, causing serious diseases (28). This variation in the infestation rates may also be attributed to other reasons about the host, like his age, food, health and immune status, species of and numbers of parasites and numbers of fish examined throughout the study period in addition to seasons of the year, geographical location, depth of water, also direct contact is one of the most essential ways of transmission of infection, especially in crowded farms. It is considered the most pathogenic fish parasites where death causes severe injuries (29-31).

PCR technique is considered a sensitive method in identifying protozoa infecting fish due to the similarity of the external morphological characteristics and the difficulty of identifying by traditional methods; molecular biological techniques are very important to obtain accurate diagnosis (32). The results of the polymerase chain reaction using the 18S rRNA gene with a reaction product of 1700 bp matched the researchers' results (13), confirming infection with the parasite *Trichodina*. Molecular analysis is used as a diagnostic method to achieve quick and accurate detection of parasitic infections in fishes, helping accurate treatment, particularly with intensive fish farming systems (33).

Conclusions

This study recorded and confirmed the presence of *Trichodina* spp. in Mosul, with an infestation rate of 19.5%. Further studies are needed to evaluate, control, and treat this parasite. Molecular analysis confirmed the infection of *Trichodina* spp. with the accession number PQ012982, according to the analysis of the parasite's genetic tree, which matches 100% with other studies recorded in GenBank.

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Conflict of interest

The authors announced that there are no matches of concern pertaining to the publication of this manuscript.

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التحري المجهري والجزيني عن طفيلي الترايكودينا في الأسماك في مدينة الموصل

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الخلاصة

داء الترايكودينا من الإصابات الطفيلية الخارجية الشديدة واسعة الانتشار في جميع أنحاء العالم ويسببه طفيلي من الأوالي الهدبية الذي يصيب الجلد والزعانف والغلاصم في الأسماك. تم جمع ٢٠٠ عينة خمسة أنواع من الأسماك (الشبوط، الكارب الاعتيادي والزولي والكتان والبنّي) من الأسواق المحلية في الموصل للفترة من شهر آب ٢٠٢٣ إلى شهر شباط ٢٠٢٤. استخدمت الطريقة المجهرية باستخدام المسحة المباشرة الرطبة للجلد والغلاصم والزعانف ولتأكيد الإصابة بطفيلي الترايكودينا، تم استخدام طريقة تفاعل البلمرة المتسلسل وتحليل الشجرة الجينية بتضخيم الجين ١٨ rRNA. S. مجهرياً بينت نتائج الدراسة وبالأعتماد على الصفات الشكلية للطفيلي أن نسبة الإصابة الكلية بطفيلي الترايكودينا وبنسبة ١٩,٥ % (٢٠٠/٣٩) حيث ظهر الطفيلي بأشكال كمتريه أو مستديرة ذات قرص تثبيت مميز وبقطر ما بين ٣٧-٦٠ ميكرومتر وبلون ذهبي إلى بني. أثبتت نتائج الدراسة الجزيئية وتحليل تعاقب تسلسل الجينات أكدت الإصابة بطفيلي الترايكودينا وبناتج تفاعل بحجم ١٧٠٠ كيلو دالتون وتم تسجيل العزلة الموجبة تحت الرقم التسلسلي PQ012982 وبنسبة تطابق ١٠٠% مع العزلات الأخرى لكوريا الجنوبية المسجلة في بنك الجينات العالمي ووفقاً لتحليل الشجرة الجينية للطفيلي.