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doi:10.12980/JCLM.2.2014C1291

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First report of *Ichthyophthirius multifiliis* (Ciliophora: Oligohymenophorea) from *Aphanius dispar* (Cyprinodontidae) in Iran

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PEER REVIEW

Peer reviewer

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Comments

This is a good study in which authors surveyed parasitic infection of *A. dispar* by *I. multifiliis* for the first time in Hormuzgan Province, Iran. The findings are interesting and valuable. According to the results, firstly *A. dispar* is considered as a new host for the parasite. Secondly, a high infection rate (51.55%) is indicated that there is a danger for conservation of this indigenous species and also it is an alarm for authorities to take appropriate measures for control and prevention of the parasite.

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ABSTRACT

Objective: To determine the infection of *Aphanius dispar* (*A. dispar*) by *Ichthyophthirius multifiliis* (*I. multifiliis*) in Iran.

Methods: A total of 97 (22 males and 75 females) individuals were collected using dip net from Mehran River, Hormuzgan Province, South of Iran in January 2008. Total length, standard length and weight of the collected specimens were measured to using vernier caliper. Whole body of fish was examined macroscopically for the presence of any blister. For the detection of *Ichthyophthirius*, the mucus was scraped gently from skin onto a micro slide and stained by Azocarmine. Prevalence, intensity and abundance of parasite were analyzed.

Results: In this study 50 (51.55%) specimens including 12 males and 38 females were found infected with *I. multifiliis* which is an obligate protozoan parasite of freshwater fishes with a direct life cycle and it causes weight loss and mortality in fishes. The infection is known as ichthyophthiriasis or white spot disease, one of the most serious diseases in freshwater fishes. Prevalence, intensity and abundance of parasite in female and male fishes were 50.67%, 29.26, 14.83 and 54.55%, 18 and 9.82, respectively. No sex preference and predilection of body site as microhabitats for this parasite in *A. dispar*, as well as in three length groups of fish standard length ranging 18–23 mm, 23–28 mm and 28–34 mm. However, prevalence and abundance are significantly increased in larger fish than smaller one.

Conclusions: This is the first report of *I. multifiliis* (Fouquet, 1876) from *A. dispar* in Iran and this fish is considered as a new host for the parasite.

KEYWORDS

Fish, *Aphanius dispar*, Parasite, *Ichthyophthirius multifiliis*, Iran

1. Introduction

The only native representative of the family Cyprinodontidae (Teleostei, Cyprinodontiformes) in Iran, Europe, and the Persian Gulf area is the genus *Aphanius*.

Iran is a hot spot of *Aphanius* diversity, with eight species recognized at present and their distribution area include both freshwater and brackish water bodies[1–5]. Among the described *Aphanius* species, *Aphanius dispar* (Rüppell, 1829) (*A. dispar*), known as mahi gour-e-khari or mahi

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Foundation Project: Supported by the Young Researchers Club, Islamic Azad University, Babol Branch., (Grant NO.: BPJ1388).

Article history:

Received 18 Mar 2014

Received in revised form 25 Mar, 2nd revised form 6 Apr, 3rd revised form 16 Apr 2014

Accepted 20 May 2014

Available online 11 Jun 2014

domparchami, is the most widespread taxon. In Iran, it is distributed in several fresh water and brackish inland water bodies such as hot springs and endorheic drainage systems[3]. In spite of importance of these species as a biologic control of anopheles mosquito larvae from streams little attention is paid to the parasitic infections of these species[6], which actually will be enormously useful to conserve *A. dispar*. So far, the infection of *A. dispar* with *Ligula intestinalis* and *Clinostomum complanatum* has been reported[7,8]. The current study is the first record of *Ichthyophthirius multifiliis* (Fouquet, 1876) (*I. multifiliis*) from *A. dispar* in Makran Basin, south of Iran.

Ichthyophthirius belongs to the phylum Ciliophora, the class Oligohymenophora, the suborder Ophryoglenina and has its own family Ichthyophthiridae. *Ichthyophthirius* is an obligate parasite of freshwater fishes with a direct life cycle and no intermediate host to reproduce. Ichthyophthiriasis (Ich) or white spot disease is one of the most serious diseases of fishes and as a devastating pathogen in aquaculture, in ornamental fish trade, and is responsible for substantial economic losses worldwide. Its pathogenetic effects are heavy damage to gill and skin tissues, resulting impairment of the osmotic balance and when they become excessive number, they cause weight loss, debilitation and massive mortality of fishes within a short time. This parasite is histophagus with a pre-buccal cavity opening into a cytostome. It is covered with cilia organized into kineties and the infective stage, the theront, also possesses a caudal cilium[1]. The life cycle is divided into three distinct stages. The trophont resides and feeds in the epidermis of the host where it can obtain a diameter of up to approximately 1 mm. The mature trophont escapes from the epidermis to the freshwater surrounding, where some of the parasites settle and develop into encysted tomonts. In this tomontocyst stage, numerous daughter cells (tomites) are produced[9]. The number of tomites resulting from one tomont varies between so and a few thousand. These swimming and infective ciliates escape the cyst as so-called theronts (length 20–60 µm) ready to penetrate the epithelium on gills, eyes, skin and fins of other fishes[10]. A number of studies have shown that all these life cycle stages are extremely temperature dependent. Consequently, infected fish typically develop small blister-like raised lesions along the invasive body parts. The mature parasite is very large, up to 1000 µm in diameter, very dark in color due to the thick cilia covering the entire cell and moves with an amoeboid motion[11].

In this paper, the first report of *I. multifiliis* from *A. dispar* in Makran Basin, south of Iran was recorded. Additionally, the parasite prevalence, intensity and abundance were determined, as well as observation of various fish body sites as microhabitats in both sexes to understand whether there is site predilection for invasive parasite.

2. Materials and methods

2.1. Study area

This study was conducted since January 2008 in Mehran river (27°28'24.1''N, 57°15'18.88''E, 211 m Altitude) from Makran Basin, near Rudan town, Hormuzgan Province, South of Iran. The bottom of this river is generally muddy with rubble, gravel and sand that *A. dispar* occurs in shallow water and among vegetation. Other fish fauna of this river are *Iranocichla hormuzensis*, *Nemacheilus*, *Garra* and *Cyprinion* and the flora of *Ziziphus* and *Tamarix* are also found in the region. The river water is clear and running slowly in summer but floody in winter[8].

2.2. Sampling

In this study, a total of 97 *A. dispar* were collected from the river, using dip net and preserved in 5% formalin solution in spot until examination. Total length, standard length and weight of the collected specimens were measured to the nearest 0.05 mm using vernier caliper; weight of specimens to the nearest 0.001 g and their sex was determined. Whole body of fish was examined macroscopically for the presence of any blister. In the case of infectivity, the locations and number of blisters were recorded. For the detection of *Ichthyophthirius*, the mucus was scraped gently from skin onto a micro slide and then spread the mucus carefully with a cover slip. *Ichthyophthirius* exposed in Azo-carmin for a few days and then washed for several minutes in alcohol containing a drop of added iodine solution. Next, the smears were mounted between a glass slide and a cover slip in Canada balsam after dehydration in accordance with Fernando *et al*[12]. Then, the parasites were investigated under light microscope, and based on the morphological characteristics were identified. A camera Lucida was also used to draw the parasite. Prevalence (%) was calculated according to the percent of infected fish divided by the total number of fish. Mean intensity was determined by dividing the total number of recovered parasites by the number of infected fish samples, while abundance was calculated by dividing the total number of recovered parasites by the number of (infected and uninfected) fish samples.

The mounted parasites deposited in the Parasitology Collection of the School of Public Health, Tehran University of Medical Sciences, Tehran, Iran, and fish specimens deposited in the Zoological Museum of Biology Collection of Shiraz University (CBSU), Shiraz, Iran.

2.3. Statistics

The statistical analyses were carried out using SPSS version 21 (SPSS Inc., 2013). Univariate analysis of variance (ANOVA,

with Duncan’s post hoc test, $P < 0.05$) was used to test the significance of body site predilection among different fish length groups and also between the sexes and to determine specifically where significant differences occurred. Pearson *Chi*-square test was applied to find the correlation of prevalence with sex and between different length groups.

3. Results

A total of 97 *A. dispar* fish specimens (22 males and 75 females) (Figure 1a and 1b) were examined. Sex ratios of fishes were 3:1 in favor of females. Total and standard length of fishes ranged from 22.3–40.1 mm and 18.8–33.2 mm, respectively, and their weight ranged from 0.269 to 1.091 gram. Overall, 50 specimens (51.55%) including 12 males and 38 females had blister-like lesions namely white spot. The appearance of these fishes was disturbed with as many as external blisters on their bodies (Figure 1c). Removed parasites are identified *I. multifiliis* (Figure 1d and Figure 2).

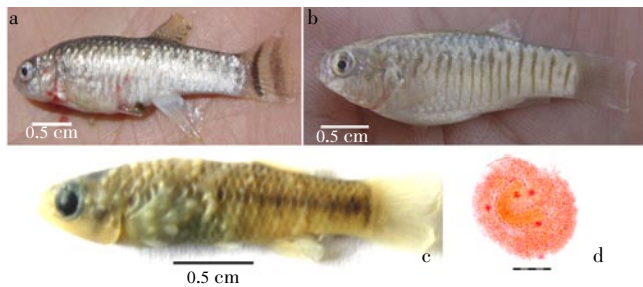


Figure 1. Image of *A. dispar* collected from Mehran River, Hormuzgan Province, South of Iran. a: male; b: female; c: heavily infected fish having blisters containing *I. multifiliis*; d: microscopic photo of stained *I. multifiliis* isolated from fish body.

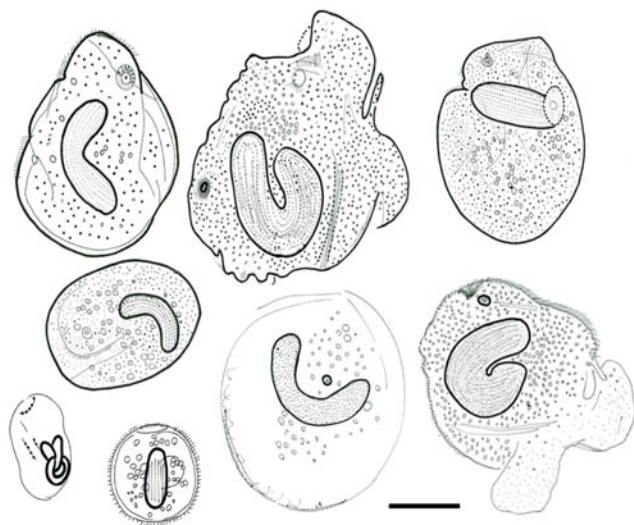


Figure 2. Camera lucida drawings of *I. multifiliis* isolated from *A. dispar*. Scale bar: 100 μ m

The trophonts of *I. multifiliis* has round body or ovate, extremely large, attaining size of 0.13–0.61 mm, small linear cytostome. Entire body ciliated by meridionally longitudinal

ciliary rows converging at anterior end near cytostome. Thick straight or horseshoelike macronucleus lies in the middle of body. There was a macronucleus dividing in some of them. Small micronucleus adheres to different areas of surface of macronucleus. Micronucleus is bilayer and there are two micronucleus in some samples (Figure 1d and Figure 2).

Among 50 infected fishes, one of them was infected with most number 84 blisters and the lowest number was two blisters. The prevalence, intensity and abundance of infection by parasite of *I. multifiliis* were 51.55%, 26.56 and 13.69, respectively. Prevalence, intensity and abundance of parasite in female and male fishes were 50.67%, 29.26, 14.83 and 54.55%, 18, and 9.82, respectively.

Prevalence of parasite is higher in male than female, but there is not a significant difference between them ($\chi^2=0.102$, $P=0.74$). The abundance and intensity of parasites is higher in female than male, but the differences is not significant ($t=0.79$, $P=0.44$) ($t=0.79$, $P=0.44$) in turn.

The site predilection of the parasite was evaluated by counting the blisters in the different microhabitats (Figure 3) of which the lowest value of prevalence, intensity and abundance were correspondent to the parasites, located on the ventral fins of fish body. However, the highest values of these parameters are not exactly in concordance with each others, i.e. the values of prevalence in microhabitats are different from that of intensity and abundance, especially for lateral surface (Table 1). In fact, the parasite is more prevalent on pectoral fin and then lateral surfaces, but more abundant on dorsal surface (Table 1). The prevalence and abundance of parasites in different length groups are summarized in Table 2.

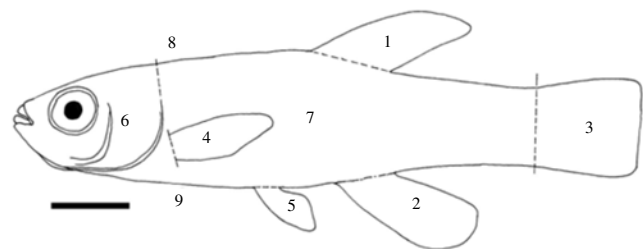


Figure 3. Body sites of *A. dispar* as microhabitats on which the number of blisters containing *I. multifiliis* was registered. 1: dorsal fin; 2: anal fin; 3: caudal fin; 4: pectoral fin; 5: ventral fin; 6: head part; 7: lateral surface; 8: dorsal surface; 9: ventral surface. Scale bar: 0.5 mm.

Table 1

Prevalence, intensity and abundance of *I. multifiliis* in different microhabitats.

Microhabitat	Prevalence %	Intensity	Abundance
Dorsal fin	27.84	3.00	0.84
Anal fin	40.21	2.95	1.19
Caudal fin	39.18	3.97	1.56
Pectoral fin	44.33	3.16	1.40
Ventral fin	20.62	1.70	0.35
Head part	37.11	4.97	1.85
Dorsal surface	28.86	5.25	1.52
Ventral surface	37.11	3.94	1.46
Lateral surface	43.29	8.17	3.54

Table 2. Variation in abundance and prevalence in different length groups of the fish.

Length group (mm)	Fish number	Prevalence	Abundance
18–23	30	53.33	11.6
23–28	54	46.30	13.57
28–34	13	69.23	19.15

The Univariate Analysis (ANOVA, Duncan Post Hoc test, $P < 0.05$) showed that there is not a significant difference for the predilection of parasites (blisters) in the microhabitats between males and females in three length groups of fishes ranging 18–23 mm, 23–28 mm and 28–34 mm of fish standard length, as well as no significant differences is present in abundance of parasites in these microhabitats. The variation of abundance and prevalence show significantly difference between length groups ($F = 41.28$, $P < 0.00005$) ($\chi^2 = 2.29$, $P < 0.00005$), respectively.

4. Discussion

According to the present results, the prevalence of *I. multifiliis* in *A. dispar* was 51.55% and it is relatively high. However, different prevalence in various fish species has been reported in previous literatures^[13,14]. Therefore, the diversity of hosts of this parasite and various prevalence are probably related to the innate fish resistance, different possible influences of environmental and nutritional factors. Regarding to Nigrelli *et al.* some species of fish are highly resistant to parasites or develop an acquired immunity and these attributes make low prevalence of parasites^[11], as it is seen in *Mastacembelus mastacembelus* (2.4%)^[15], but the reverse pattern can be supposed for *A. dispar* in the current study. Based on Wahli *et al.* there is positive phototaxis in theronts of *Ichthyophthirius*^[16], indicating the theronts prefer to meet pelagic host such as *A. dispar* species and it can be a reason to have high prevalence in this species. Moreover, temperature changes have main effect on each stage of development *e.g.* encystment, division and maturity of *Ichthyophthirius*^[11,17]. Thus it can suppose that fish species with high prevalence probably are living in a favorable temperature for parasite (not available data of habitat water). Because, considerable changes in temperature will kill these ciliates^[11]. Furthermore, great temperature changes in different seasons cause variations in fish parasite intensities^[18,19]. Buchmann and Bresciani demonstrated that *I. multifiliis* in traditional ponds was more prevalent at high temperatures^[20]. However, the temperature tolerance of the host species is also important to accept parasite. Therefore, further *in vivo* and *in vitro* studies will aid in clarifying this issue. Additionally, nutritional conditions are related to both host and parasite. On one hand, when there is not enough foods in the habitats, fish will become weak with low immunity system and these stressful conditions make fish

ready to accept parasites easily. On the other hand, Nigrelli *et al.* displayed that the young free-living ciliates must find a suitable host during the infective period, otherwise they starve to death^[11].

In the current study, the prevalence of infection was higher in males (54.55%) than females (50.67%) of *A. dispar*, while the intensity and abundance in females were higher than males (29.26, 18.00 and 14.83, 9.82 in turn), without significant difference. Nevertheless, it can presume that the males have more resistance to parasite infection than females and they will get more immunity than females when the parasites attack them or they change their feeding habit to be more powerful. On the other hand, females move slower than males because of spawning period (lengthy period due to short life), carrying eggs^[21], putting those sticky eggs on the water plants and staying among plant leaves for a few days to make sure about their eggs status (first author observations in aquaria). Such behavior leads to more contact between parasites and females body. In addition, females are generally larger than males therefore; invasive parasites have easier contact with the host and more opportunity for invasion and massive reproduction after a while. Meanwhile, the *Aphanius* females have smaller scale sizes than males, thus the penetration of parasite on the fish body of females would be much comfortable than males, as Leong *et al.* showed that fishes with smaller scales are generally more susceptible to infection than those with larger scales^[11]. Similarly, the body of males with larger scales discharges more mucus on epidermis, causing further barrier to prevent parasite penetration. Furthermore, there are usually secondary infections in infected fishes which make more weakness of fish body^[8]. So, condition of parasite for attack will be more favorable, and the intensity and abundance of other parasites in infected specimens will grow, as it was seen in this study. Likewise, the introduction of pathogenic organisms like *Gambusia* and some farmed cyprinids to native fish populations, which have no immunity against exotic species, impose much stress on the native fishes and predispose them to infectious diseases^[12]. However, further research is required to clarify more reasons for higher affinity of these parasites to the females.

Respect to the site predilection of parasite, the abundance, intensity and prevalence in lateral surface of fish body was high that might be due to the easier access of parasite to larger areas of fish body, while it is vice versa for the ventral fins which had the lowest values of all these parameters. Fish fins *e.g.* caudal, anal and pectoral fins usually have more important role in quick and reverse swimming and objects discarding than dorsal and ventral surfaces and head part of fish body. Therefore, parasites have more opportunity to attach on the fins infecting more fishes (higher prevalence); but likely the fins throw the parasite away due to the pressure of water current around fins (low intensity and

abundance). Buchmann and Uldal showed that in salmonid the fins, compared to other fish body parts, discharge higher mucus density, having more resistance against parasite penetration^[22]. The dorsal surface of fish body showed low prevalence and high intensity and abundance due to lack of intensive movements. Actually, once the objects and parasites inside the water bodies encounter with these sites of fish body, attach on the tissue and make their position tightly.

In the current study, prevalence and abundance of infection in larger fish were significantly higher than those of small fish. This is most probably due to more exposure of the parasites with the larger fish as a result of their wider body surface and older age. This result adds support to the results of Clayton and Price who found direct correlation between fish size and infection of *I. multifiliis* in common carp, poeciliid and goodeid fishes^[23,24].

The mean intensity of parasite has significant and positive correlation with fish unhealthy condition, high stocking density of fish, fish nutritional deficiencies, low values of water conductivity and organic dry matter that favor parasite development^[19,25–27]. Moreover, studies clearly showed that the development of *Ichthyophthirius* was inhibited at lower temperatures and increased at higher temperatures and especially during summer in temperate regions with optimum temperature for reproduction (24–26 °C)^[11,19]. Moreover, the impact of parasite relates to the small size, the large number and continuous release of infectious stage into the water column^[28,29].

Severe infection of fish by ciliate *Ichthyophthirius* has considerable impact on growth, behavior and fish resistance to other stressing factors, susceptibility to predation, high mortality due to epithelial hyperplasia and leukocyte mobilization, predominantly neutrophils and inflammation, reduced market ability of fish and concomitant economic loss^[1,30].

Since these parasites are dangerous and cause a strong negative impact on fish population, it is necessary to identify such parasites and take control measures, especially for important fish species such as *A. dispar* and other endemic species inhabiting in Mehran River. Also, more urgent action is to prevent the introduction of exotic fishes like *Gambusia* and cyprinids to this river.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors greatly acknowledged Professor Hamid Reza Esmaeili (Shiraz University, Iran) for his kind help to provide

the fish specimens for this study. This study was supported by the Young Researchers Club, Islamic Azad University, Babol Branch., (Grant NO.: BPJ1388).

Comments

Background

Aphanius is a genus of pupfishes that belongs to Cyprinodontidae and more than thirty species of this genus have been known. Recently some species of this family play a major and prominent role as biological control method of mosquito larvae particularly for anopheles larvae. Several species in the genus have very small distributions and are threatened seriously in the world. One of these species is *A. dispar* which is indigenous fish in Iran and conservation of this fish is a matter of high importance.

Research frontiers

The study was carried out on 97 *A. dispar* that were collected using dip net from Mehran River, Hormuzgan Province, South of Iran. Total standard length and weight of the collected specimens were measured by employing vernier caliper. Body of collected fishes macroscopically was surveyed for any blister. Next, the mucus was scraped from skin onto a micro slide and finally the mucus was stained by Azo–carmine for the purpose of *Ichthyophthirius*.

Related reports

The paper mentioned the findings of Buchmann and Bresciani about pathogenicity of *I. multifiliis* at high temperatures. And also mentioned the prevalence of *Ichthyophthirius* in different conditions such as size of host.

Innovations and breakthroughs

The novelty in the study is that though *A. dispar* is native and has a considerable distribution in Iran there is limited information about parasitic infection of the fish. Meanwhile, the study has chosen a protozoan which can impose a noticeable economical damages and losses to the fish breeding.

Applications

The high infection rate of the fishes shows that this species is at danger of the parasite and serious measures should be considered to protect this valuable and indigenous fish. And owing to acting as a larvivores fish which lead us to employ as a biological control, monitoring and surveying are significant.

Peer review

This is a good study in which authors surveyed parasitic infection of *A. dispar* by *I. multifiliis* for the first time in Hormuzgan Province, Iran. The findings are interesting

and valuable. According to the results, firstly *A. dispar* is considered as a new host for the parasite. Secondly, a high infection rate (51.55%) is indicated that there is a danger for conservation of this indigenous species and also it is an alarm for authorities to take appropriate measures for control and prevention of the parasite.

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