

## Ectoparasitic species of the genus *Trichodina* (Ciliophora: Peritrichida) parasitizing Macedonian freshwater fish

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**Abstract** Trichodinids are probably the most commonly encountered protozoan parasites on fishes in marine and freshwater environments. They are typically found on the gills, skin and fins of fish. The aim of this study was to evaluate the prevalence and mean intensity of *Trichodina* sp. in freshwater fish from cyprinid aquaculture facilities in Macedonia. This study was carried out by seasons, in a period of three years. A total of 578 specimens of common carp (*Cyprinus carpio*, Linnaeus 1758), 76 specimens of grass carp (*Ctenopharyngodon idella*, Valenciennes 1844), 47 specimens of silver carp (*Hypophthalmichthys molitrix*, Valenciennes, 1844) and 53 specimens of bighead carp (*Hypophthalmichthys nobilis*, Richardson, 1845) from the most significant and larger cyprinid aquaculture facilities in Macedonia, including fish farms and reservoirs, were examined for parasitological investigations. Infestation with *Trichodina* sp. was found in 206 specimens of common carp in all aquaculture facilities examined, in all seasons, as well as, in 9 specimens of grass carp from two fish farms, only in autumn season. Total, the prevalence with *Trichodina* sp. was 11.84% (grass carp) and 35.67% (common carp), while the, mean intensity was 7.56 (grass carp) and 4.56 (common carp). The prevalence with *Trichodina* sp. in common carp by season was as following: spring – 11.82%, summer – 3.44%, autumn – 7.85%, winter – 7.05%, while the mean intensity was: spring – 4.55; summer – 4.67; autumn – 4.40; winter – 4.72. Our findings of *Trichodina* sp. in grass carp in the present study is considered as the first record in Macedonia. Also, this fish species is regarded as new host for *Trichodina* sp. in Macedonian waters.

### Pasożyty zewnętrzne ryb słodkowodnych Macedonii z rodziny *Trichodina* (Ciliophora: Peritrichida)

**Słowa kluczowe** pierwotniaki, *Trichodina* sp., amur biały, karp, akwakultura

**Streszczenie** Trichodinidae są prawdopodobnie najczęściej spotykanymi pierwotniakami pasożytującymi na rybach słodkowodnych i morskich. Zazwyczaj spotykane są na skrzelach, skórze i płetwach ryb. Celem artykułu jest określenie częstotliwości i intensywności zarażenia ryb karpiowatych przez *Trichodina* sp. Badania trwały trzy lata. Łącznie przebadano pod kątem parazytologicznym 578 osobników karpia (*Cyprinus carpio*, Linnaeus 1758), 76 amurów białych

(*Ctenopharyngodon idella*, Valenciennes 1844), 47 tołpyg białych (*Hypophthalmichthys molitrix*, Valenciennes, 1844) i 53 tołpyg pstrych (*Hypophthalmichthys nobilis*, Richardson, 1845). Zakażenie *Trichodina* sp. stwierdzono u 206 osobników karpia we wszystkich badanych stawach i okresach, oraz w okresie jesiennym u 9 amurów białych z dwóch stawów hodowlanych. Łącznie zarażonych *Trichodina* sp. było 11.84% amurów białych oraz 35.67% karpia, średnia intensywność zarażenia amurów białych wynosiła 7.56, a w przypadku karpia – 4.56. W okresie wiosennym zarażonych było 11.82% karpia, latem – 3.44%, jesienią – 7.85%, a zimą – 7.05%. Odpowiednio średnia intensywność zarażenia wynosiła wiosną – 4.55; latem – 4.67; jesienią – 4.40 i zimą – 4.72. Stwierdzenie *Trichodina* sp. pasożytującego na amurze białym jest pierwszym przypadkiem zaobserwowanym w Macedonii. W związku z tym gatunek ten jest nowym gospodarzem dla *Trichodina* sp. w wodach Macedonii.

## Introduction

The largest genus of the family Trichodinidae is the genus *Trichodina* (Ehrenberg, 1838), ciliate protists well known as the causative agent of trichodiniasis in numerous aquatic animals (Marcotegui et al., 2018; Martins et al., 2015), with more than 200 species described from fish (Asmat et al., 2005). According Tang and Zhao (2011; 2012), to date about 300 nominal *Trichodina* species have been reported from different environments in the world.

Trichodinids are ciliate protozoans widely known as ectocommensals and are probably the most commonly encountered protozoan parasites on fishes in marine and freshwater environments (Martins, Ghiraldelli, 2008). According Huh et al. (2005) and Khan (2004) trichodina can serve as a facultative ecto-parasite and can proliferate and invade hosts during unfavorable conditions in environments, such as poor water quality and food deficiency. Lom (1995) concluded that the trichodinid are able to invade their hosts within a short period because of their direct transmission, especially fish with less condition. Lom and Dykova (1992) considered that the determination of *Trichodina* species is difficult because many species are morphologically variable and show low host specificity. In the taxonomy of trichodinids, the exact number, shape and arrangement of the cytoskeletal denticles is critical for determining taxonomic relationships. These characters are usually revealed by silver nitrate staining of microscope slides, which stains the cell cytoplasm black and leaves the denticles white. Trichodinids are best known as ecto-parasites of skin, fin and gill of the fish with most of the species reported from freshwater environments.

According to data from parasitological research in Macedonia, Hristovski et al. (2006; 2012) determined *Trichodina* sp. at common carp (*Cyprinus carpio*) from Prespa Lake, with a prevalence of 1.32%. In addition, the same authors also established *Trichodina* sp. in *Leuciscus cephalus prespensis*, *Chondrostoma nasus prespensis*, *Alburnus alburnus belvica* and *Rutilus rubilio prespensis* from Prespa Lake.

According to literary reviews from the Balkan countries, *Trichodina* sp. at *Cyprinus carpio* in Bosnia and Herzegovina was first established by Žitnan et al. (1969) in the fish farming “Vučijak” – Prnjavor. Data on the presence of this parasite in waters in Serbia is published by Cakić (1992), while in Croatia, Tomec et al. (1995) in the fish farms “Narta” and “Blatnica” and Valić et al. (2005) in a fish population in the Krka River.

In the cyprinid fish farms in Romania, *Trichodina* sp. was established on gills of *Cyprinus carpio* by Lazar (2009), while on the fins, gills and body cavity by Radu et al. (2008). In the cyprinid fish farms in the Czech Republic, Ondračková et al. (2012) determined the presence of *Trichodina* sp. in two years old carp. In Turkey, data on the presence of *Trichodina* sp. at *Cyprinus carpio* in the Seyhan River was reported by Cengizler et al. (2001).

According to literary reviews from the world, Ali et al. (1988) and Mansoor & Al-Shaikh (2010) determined the presence of *Trichodina domerguei* in waters in Iraq, while Subasinghe (1992) established *Trichodina* sp. in juveniles and adult carp in cyprinid fish farms in Sri Lanka. Tantry et al. (2016) reported first findings of trichodinid fauna at *Cyprinus carpio* collected from various fish ponds and farms in Jammu division of Jammu and Kashmir state.

Regarding grass carp (*Ctenopharyngodon idella*), species of the genus *Trichodina* are identified by Ali et al. (1988) in fish farms in Iraq and Bozorgnia et al. (2012) in fish farms in Iran.

## Materials and methods

The aim of this study was to evaluate the prevalence and mean intensity of *Trichodina* sp. in freshwater fish from cyprinid aquaculture facilities in Macedonia. This study was carried out by seasons, in a period of three years. A total of 578 specimens of common carp (*Cyprinus carpio*, Linnaeus 1758), 76 specimens of grass carp (*Ctenopharyngodon idella*, Valenciennes 1844), 47 specimens of silver carp (*Hypophthalmichthys molitrix*, Valenciennes, 1844) and 53 specimens of bighead carp (*Hypophthalmichthys nobilis*, Richardson, 1845) from the most significant and larger cyprinid aquaculture facilities in Macedonia, including fish farms and reservoirs, were examined for parasitological investigations. These aquaculture facilities included fish farms Zhabeni, Bukri, Dolneni and Zhelezara, as well as fish cage culture systems on reservoirs Tikvesh, Mladost, Globochica and Gradche. The specimens were placed in plastic containers and transferred alive to the laboratory.

Gills, fins and skin were examined using stereomicroscopes “Zeiss” – Stemi DV4 and „MBS 10”, as well as microscopes “Zeiss” – PrimoVert and “Reichart” were used. All parasites found in each fish were identified and enumerated. Parasites on native smears were observed under a light microscope and magnification  $\times 200$  and  $\times 400$ .

Classical epidemiological variables (prevalence and mean intensity) were calculated according to Bush et al. (1997).

The parasite specimens were identified using reference keys of Lom and Dykova (1992)

## Results and discussion

A total of 578 specimens of common carp, 76 specimens of grass carp, 47 specimens of silver carp and 53 specimens of bighead carp from the most significant and larger cyprinid aquaculture facilities in Macedonia were examined for parasitological investigations. Infestation with *Trichodina* sp. was found in 206 specimens of common carp in all aquaculture facilities examined, in all seasons, as well as, in 9 specimens of grass carp from two fish farms, only in autumn, as following:

- on gills in common carp from fish farm Zhabeni, in summer and autumn,
- on gills in common carp from fish farm Bukri, in spring and summer,
- on fins in common carp from fish cage culture system on Tikvesh reservoir, in autumn,
- on gills in common carp from fish cage culture system on Mladost reservoir, in spring, autumn and winter,
- on fins and gills in common carp from fish cage culture system on Globochica reservoir, in spring and winter,
- on fins and gills in common carp from fish farm Dolneni, in autumn,

- on gills and liver in common carp from fish cage culture system on Gradche reservoir, in spring and winter,
- on gills in common carp from from fish farm Zhelezara, in spring,
- on fins in grass carp from fish farm Zhabeni, in autumn,
- on gills in grass carp from fish farm Bukri, in autumn,

Data on fish examined, fish infected, as well as the prevalence and mean intensity with *Trichodina sp.*, total and by seasons, are given in Table 1 and Table 2.

Table 1. Prevalence and mean intensity with *Trichodina sp.* in common carp (*Cyprinus carpio*) and grass carp (*Ctenopharyngodon idella*) from cyprinid aquaculture facilities in Macedonia

Fish species	Number of examined fish	Number of infected fish	Mean intensity	Prevalence (%)
Common carp ( <i>Cyprinus carpio</i> )	578	206	4.56	35.67
Grass carp ( <i>Ctenopharyngodon idella</i> )	76	9	7.56	11.84

Table 2. Prevalence (E) and mean intensity (I) with *Trichodina sp.* in common carp (*Cyprinus carpio*) from cyprinid aquaculture facilities in Macedonia, by seasons

Parasite species	Spring		Summer		Autumn		Winter	
	I	E (%)	I	E (%)	I	E (%)	I	E (%)
<i>Trichodina sp.</i>	4.55	11.82	4.67	3.44	4.40	7.85	4.40	7.05

Total, the prevalence with *Trichodina sp.* in common carp was 35.67 %, while the mean intensity 4.56. The prevalence with *Trichodina sp.* in common carp by season was as following: spring – 11.82%, summer – 3.44%, autumn – 7.85%, winter – 7.05%, while the mean intensity was: spring – 4.55; summer – 4.67; autumn – 4.40; winter – 4.40.

The prevalence with *Trichodina sp.* in grass carp was 11.84 %, while the mean intensity 7.56.

Our findings of *Trichodina sp.* in grass carp in the present study is considered as the first record in Macedonia. Also, this fish species is regarded as new host for *Trichodina sp.* in Macedonian waters.

Trichodinids are usually found on the skin, fins and gills of marine and freshwater fish. The presence of a ring of interlocking cytoskeletal denticles is their characteristic and it provide cell support and adhesion to surfaces including fish tissue. There are two surfaces, one of them is cytostome which is on the part that faces away from the host (oral surface) and the other is aboral surface, attaches to the skin of the host or other substrate.

Trichodinids have a simple direct life cycle. They have a single host and do not use alternation of generations or mass asexual replication of the host. Transmission occurs by direct contact between infected and uninfected hosts, and also by active swimming of trichodinids from one host to another.

According to Abowei et al. (2011), the representatives of the genus *Trichodina* are uniformly ciliated protozoan parasites whose body has the shape of a plate. Trichodinids are attached to the body of the fish by special fixative apparatus – an adhesive disc. The parasite moves through the water with the help of lashes that are arranged in two rows along the edges of the upper and lower side of the body. These parasites infest the skin, gills and fins of fish, causing tissue irritation. One of the symptoms is a general impairment of fish health, anxiety, loss of appetite and dyspnea.

According to Basson and Van as (2006), trichodinids generally have a low degree of specificity to a single host. Most species are commensals, which cause little or no damage to their hosts, while many are important ecto-parasites, which, if present in large numbers, can cause serious epithelial damage to the skin and gills in fish.

According to Lom and Dykova (1992), the rapid development and direct life cycle of *Trichodina* sp. can be problematic in both recirculation systems and low quality water systems. Trichodinids never occur in large numbers in fish with good condition. The most sensitive are the young categories of fish aged 4 days to 1 year, compared to the older categories which do not get sick, but can be parasite carriers. Despite the natural components of the ecosystem, the presence of these ectoparasites in debilitated fish may be an indicator of deteriorating environmental conditions.

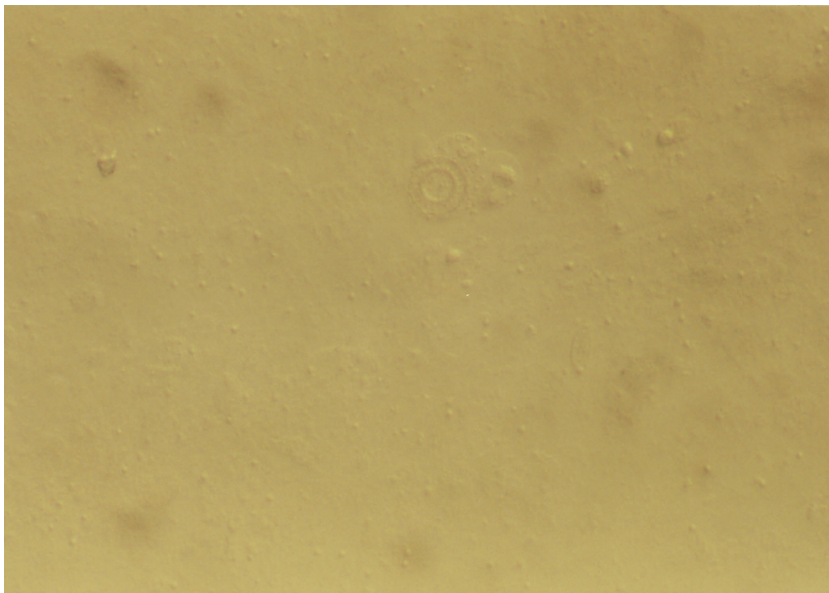


Figure 1. *Trichodina* sp. on gills of *Ctenopharyngodon idella* from fish farm Bukri

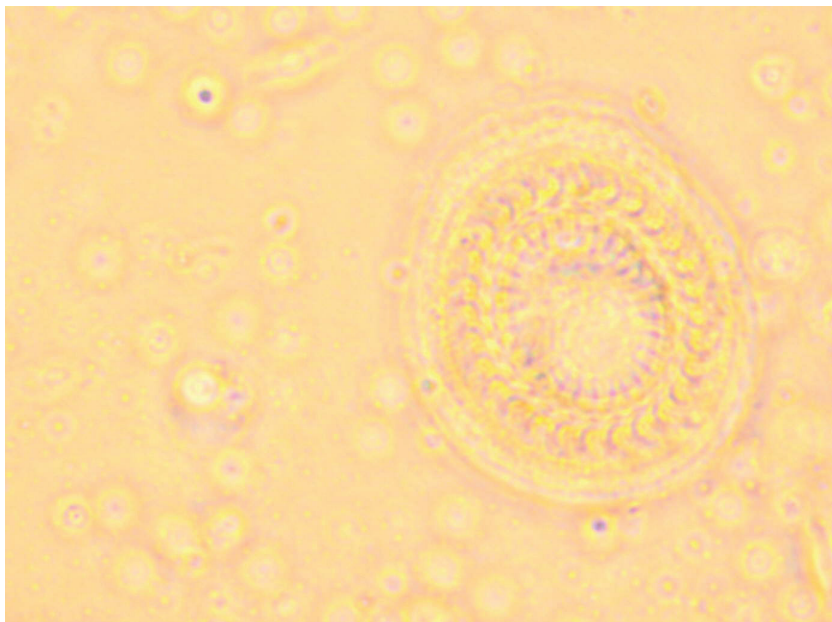


Figure 2. *Trichodina* sp. on liver of *Cyprinus carpio* from fish cage system on reservoir Gradche

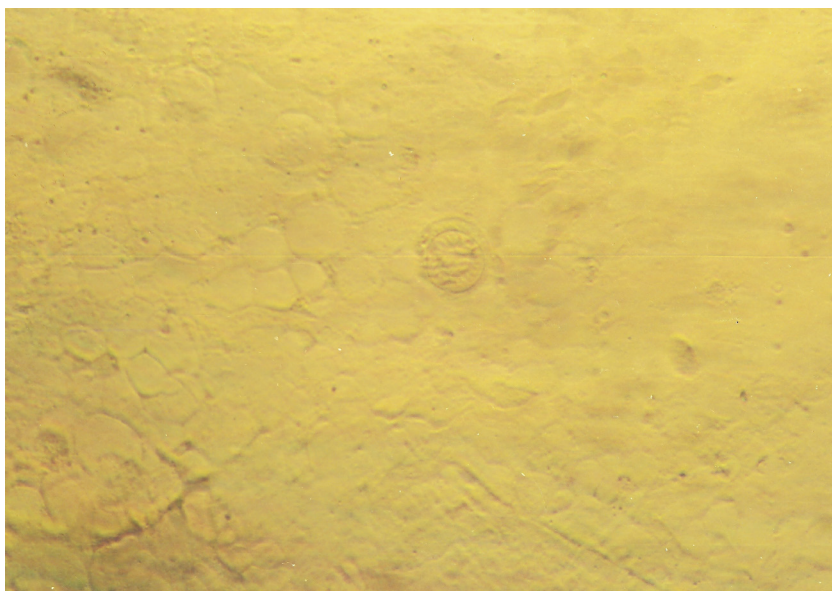


Figure 3. *Trichodina* sp. on fins of *Ctenopharyngodon idella* from fish farm Zhabeni

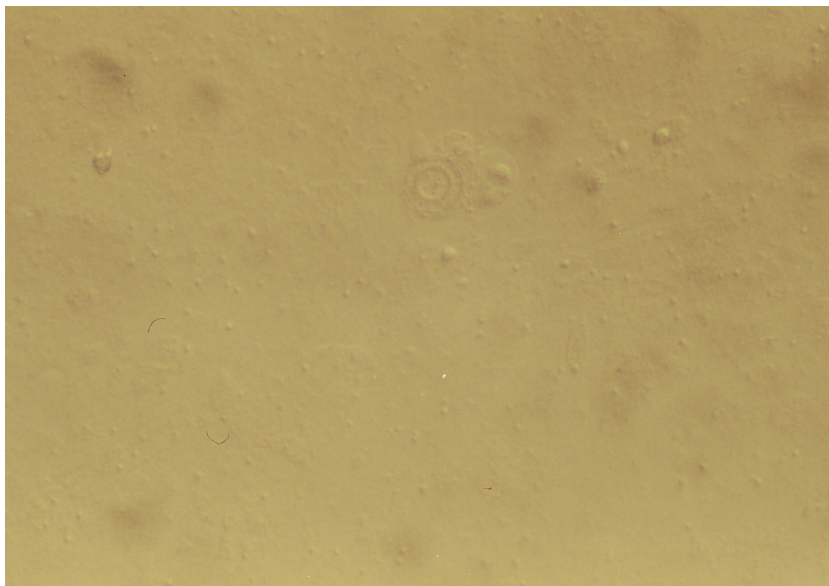


Figure 4. *Trichodina* sp. on gills of *Ctenopharyngodon idella* from fish farm Bukri

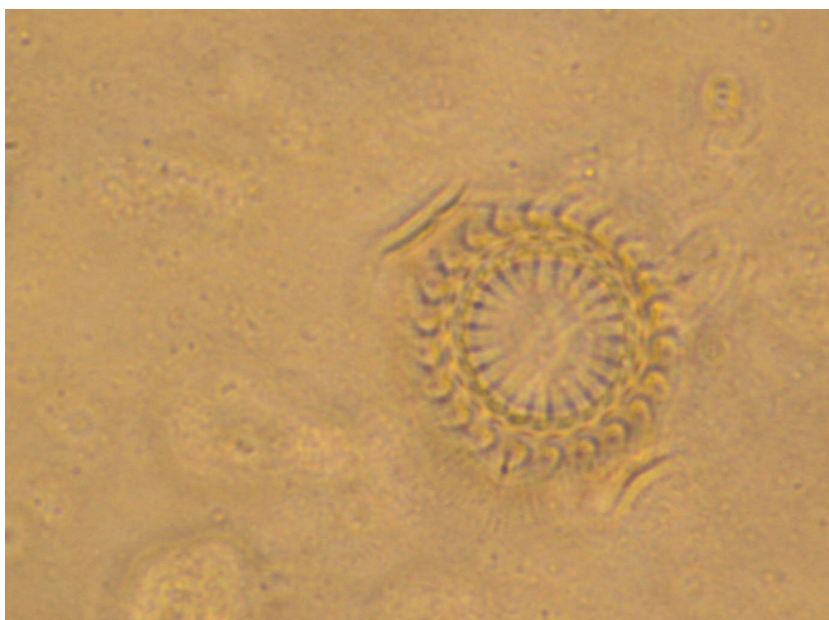


Figure 5. *Trichodina* sp. on gills of *Cyprinus carpio* from fish cage system on Globochica reservoir

Most trichodinids are ectocommensal and they use the fish only as a substrate for attachment, while they feed on suspended bacteria. High bacterial loads provide abundant food for trichodinids, which subsequently proliferate on hosts and then cause attachment-related pathologies. According Khan (2004) and Huh et al. (2005), most trichodinids are not pathogens, but when the relationship host/parasite/environment is misbalanced by nutritional deficiency, poor water quality, infectious and/or parasitic diseases, trichodinids may proliferate, being responsible for severe epidermal lesions and disease outbreaks.

## Conclusions

The presence of this protozoan in common carp are determined throughout all seasons, but trichodins usually never occur in large numbers to seriously damage the health of the host, if other, non-specific factors do not act at the same time.

Our findings of *Trichodina* sp. in grass carp in the present study is considered as the first record in Macedonia. Also, this fish species is regarded as new host for *Trichodina* sp. in Macedonian waters.

Total, the prevalence with *Trichodina* sp. in common carp was 35.67%, while the mean intensity 4.56. The prevalence with *Trichodina* sp. in common carp by season was as following: spring – 11.82%, summer – 3.44%, autumn – 7.85%, winter – 7.05%, while the mean intensity was: spring – 4.55; summer – 4.67; autumn – 4.40; winter – 4.40.

The prevalence with *Trichodina* sp. in grass carp was 11.84%, while the mean intensity 7.56.

Clinical signs of Trichodiniasis in fish are slow movement, loss of appetite, black coloration, necrosis and ulcer on different parts of the body and excessive accumulation of mucous in gill filaments. If the trichodinids occur in aquaculture, it is usually indicates eutrophication or poor water quality.

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