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Surveillance of Protozoan Infestation in Gill and Skin of *Channa punctatus* Collected from Local Market of Kolkata

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Authors' contributions

This work was carried out in collaboration between both authors. Author RD designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SM managed the analyses of the study. Authors SM and RD managed the literature searches. Both authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aims: The present study is aimed at examining the protozoan parasitic population on the gill and skin of fresh water murrel with detailed histopathological study along with measurement of index of infection of parasite infected fish.
Study Design: Fish samples were collected from local market of Kolkata and examined for parasitological and histopathological study in controlled laboratory condition.
Place and Duration of Study: Post Graduate Department of Zoology for a time period of six months.
Methodology: *Channa punctatus* (size 16-20 cm, weighing 15-17 gm) were collected from local market of Kolkata. Skin and gill filaments were analysed in controlled laboratory condition for examining the presence of protozoan parasites along with the routine histopathological study.

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Photo-documentation was carried out under microscope fitted with a digital camera.
Results: The freshwater fish were found to be infected with protozoan species with marked lesions in skin and gill.
Conclusion: In this experimental analysis, protozoan species were identified in the skin and gill of *Channa punctatus* with the highest index of infection during the time span of September and October. The study is indicative of the harmful influence of parasite on fish health.

Keywords: Parasite; skin; gill; fish.

1. INTRODUCTION

Fish health management is the concept of proactively regulating the host, pathogen and environment to maximize the optimal condition for sustained growth and health. In order to get better nutrition from fish, they must be free from diseases and mishandling. Parasitic diseases of fish are very common all over the world. Fish are one of such hosts that act as either definitive, paratenic (transport) or intermediate host in the life cycle of many protozoan parasites. Parasitic diseases, either alone or in conjugation with other environmental stresses, may influence weight or reproduction of the host, alter its population characteristics, or affect its economic importance [1]. Parasitic infestation has harmful influence on fish health that inhibits the normal growth of the fish and leads to high mortality rates. The snake-headed fish; itis the representative of the Channiform family Channidae which is the most important species of inland fisheries of India*. C. punctatus* is one of the most popular fish with delicious taste. This fish has very good commercial value in tropical countries as well as in the Indian subcontinent [2]. The present study is indicative of extensive distribution of parasites in gill and skin of edible freshwater fish. The effects of parasites are one of the factors hindering high production of *C. punctatus*. The parasitic infection of this experimental fish results in economic losses due to not only mortality, but also higher treatment costs and decreasing growth that reduces the expansion of aquaculture.

2. MATERIALS AND METHODS

2.1 Study Location and Sampling

Channa punctatus (size 16-20 cm, weighing 15-17 gm) were collected from local market of Kolkata, West Bengal, India during September to March and freshwater fish were transported to the laboratory in large containers. The fish were separately maintained at temperature ranging between 14°C-30°C in aquariums of 20 liter capacity with continuously aerated and dechlorinated tap water (pH 7.2-7.4; hardness 185-200 mg/l as CaCO3; alkalinity 170-175 mg/l as CaCO3) for four days before being taken for experimentation. The animals were fed with boiled eggs and earthworms [2].Water was renewed periodically so as to maintain the dissolved oxygen.The specimens were devoid of feeding prior to the test period to reduce the quantum of excretory products in the aquarium to avoid vomiting by the fish.

2.2 Parasitological Studies

Fish samples (50) were taken in live condition during each time interval for experimentation, killed by hand and examined immediately for parasitological study using microscope fitted with digital camera. A clean spatula was held to the body of each individual and it was drawn backwards towards the tail in a smooth movement, lifting off a small amount of mucous from the different sites of the body for investigating parasites from skin. Later on, for each sites, mucous scrapings were placed on a clean glass slides and examined under microscope for observing the presence of parasites. In gill biopsy, fine pair of scissors were used to cut open the operculum from both sides to reveal the opercular cavity. Gill filaments were taken out by cutting off the two ends of the gill arches and kept on Petri dishes with the saline solution of NaCl (0.67%). The body smear and gill were prepared on grease free, clean slides with a drop of 0.67% NaCl solution and air dried for the presence of spores. For permanent preparations, smears were fixed in Schaudinn's fixative and stained with borax carmine for thirty minutes and then after dehydrating in alcohol graded series of 50%, 70%, 90% and 100%, the parasites were cleaned with xylene and mounted in Canada Balsam.

2.3 Analysis of Parasitic Infestation

The analyses of parasitic infestation for index were carried out by the following formulae of [3] as

$$\text{Index of infection} = \frac{\text{No of host infected x No.of parasites collected}}{\text{Total host examined}}$$

2.4 Histopathology

Tissue samples for histology were obtained from *Channa punctatus* and routine histopathological study was carried out following the method of [4]. Tissues of skin and gill were obtained with the help of a fine, sharp and sterile scalpel. The tissues were cut into small pieces and washed in a physiological saline solution (0.58% NaCl) to remove mucous, blood etc. The tissues were immediately fixed in Bouins solution (Saturated picric acid: 75 parts, 37-40% formaldehyde: 25 parts and glacial acetic acid: 5 parts) and later they were passed through standard alcoholic gradation (70% ethylalcohol: 24 hr → 90% ethylalcohol: 30 min × 4 → 100% ethylalcohol: 30 min× 4 changes). Finally the tissue sections were immersed in cedar wood oil for five days. Before embedding, the excess cedar wood oil was removed by washing with xylol (5 min × 2changes). Embedding was done by placing the tissue in a mixture of xylol and paraffin (1:1) bath for 30 mins at 60ºC and then in a paraffin bath at 60ºC for 90mins. For block making, 'L' shaped moulds of copper were kept on a glass plate and some wax was poured into the square formed by the two 'L' moulds. The tissues were quickly transferred from the molten wax into the molten wax in the 'L' moulds and more molten wax was poured into the 'L' moulds. The wax was allowed to cool down and solidify. The blocks of tissues were trimmed with a sharp knife such that the embedded tissue came close to the surface. The blocks of tissues were mounted on metallic block holders by heating them on flame such that the block tissue gets mounted with its base on the block holder and the tissue at the top. The tissue sectioning was done with a microtome with an average tissue thickness of 5-6 µ. The tissue sections were floated in hot water bath at 55ºC and the sections were lifted on clean grease free slides with Mayer's albumin smeared on the surface. After lifting, the sections were stretched, dried and fixed with gentle heat. Staining was done routinely with Harris haematoxylin and eosin studies. Staining set was made by keeping the following fluids in the couplin jars and transferring the tissue slides in the following sequence: Xylol pure I (2 min) → Xylol pure II (2 min) → Absolute ethylalcohol (50 min) → 70% Ethylalcohol (5 min) → 50% Ethylalcohol (5 min) → Water (1 min) → Haematoxylin (10 min) → Acid alcohol (0.5% HCl in 70% ethylalcohol) (few dips) → Water (1 min) → 50% Ethylalcohol (5min) → 70% Ethylalcohol (5 min) → 90% Ethylalcohol (5 min) → Eosin → 90% Ethylalcohol (5 min) → Absolute ethylalcohol (5 min) → Xylol pure III (2 min) → Xylol pure IV (1 min). After the slides were lifted from xylol IV and excess xylol was drained off, the stained tissue sections were mounted with coverslip on Canada Balsam.

3. RESULTS AND DISCUSSION

The specimens of *Channa punctatus* were found to be infected with protozoan species like *Chilodonella sp., Trichodina sp. and Actinophrys sp.* (Figs. 1, 2, 3 and 4). The area of infection includes skin and gill. In *Channa punctatus*, the highest index of infection (80) was recorded in the time interval of Sep-Oct whereas the lowest index of nfection (28.8) was observed in the time interval of Nov-Dec (Fig. 5). Out of the examined protozoan parasites, the maximum infection of *Chilodonella sp*, in skin and gill was noticed (Table 1).

Several damages were observed to have been caused by the parasites found on the body parts of the sampled fish~~es~~. Erosion of the epithelium of the skin (Fig. 6A and 8A) and thickening of the gills (Fig. 6B and 8B) as well as excess mucus secretions on the gills of the sampled fish~~es~~ was caused by the parasites. The sheer number~~s~~, of *Chilodonella sp.* covering the gills (Fig. 7) also could cause mechanical blockage of oxygen transfer. Excess mucus production and removal of the skin epithelium was caused by *Trichodina sp.* as seen in (Fig. 9). This resulted in sluggish movement, loss of appetite, emaciation, loss of condition with larger head and darker skin than normal. Some of infected fish showed pale skin patches and more slimy skin. Excess mucus formation on the skin was also observed in the sampled fish infected by *Chilodonella sp.* (Fig. 6A). This made the skin to appear slimy and exhibited cloudiness and showed evidence of irritation as it tried to "scratch" off the organisms by rubbing against the walls of the fish pond. Fish is important to the human populace in trade and economy; it is of importance in the diet of different countries especially in the tropics and subtropics where malnutrition is a major problem [5]. Parasites are the most diverse and common pathogens, the aqua-culturist may likely encounter and parasitic diseases are very

common in fish all over the world and are of particular importance in the tropics [6]. Fish parasites result in huge economic losses and they increase mortality; increase farm inputs via increased treatment expenses and cause reduction in growth rate and possibly weight loss during and after the period of parasite disease outbreak.In the experimental study, the protozoan species such as *Trichodina sp.* (Fig. 3), *Chilodonella sp.* (Figs. 1 and 2) and *Actinophrys sp*. (Fig. 4) were identified in the skin and gill of *Channa punctatus*. The analysis for the index of infection was carried out in (Fig. 5). The highest index (80) in *Channapunctatus* was observed during the time span of Sep-Oct as compared to the lowest index (28.8) during the time span of Nov-Dec (Fig. 5). The index of infection in the fish varied across different time span in fishes (Fig. 5). This may be due to the influence of a change in atmospheric conditions on the definitive host causing physiological changes which influence the occurrence of parasite population. The index of infection was found to be lowest during Nov-Dec as lower temperature during midwinter causes arrested the development of parasites in the host and the environment. Gills were observed to harbour the highest number of protozoan parasites. This could be due to the fact that the gills are the centre of filter feeding and are sites of gaseous exchange. This observation agrees with the reported works of [7,8]. Infection in the gills caused severe degeneration, necrosis and consequent degeneration of the branchial epithelium and occultation of the capillaries. The epithelium degradation of the gill was counteracted by the extreme process of epithelial hyperplasia. Infection with *Trichodina sp.* caused epidermal necrosis of the skin and excess mucus formation on the skin.

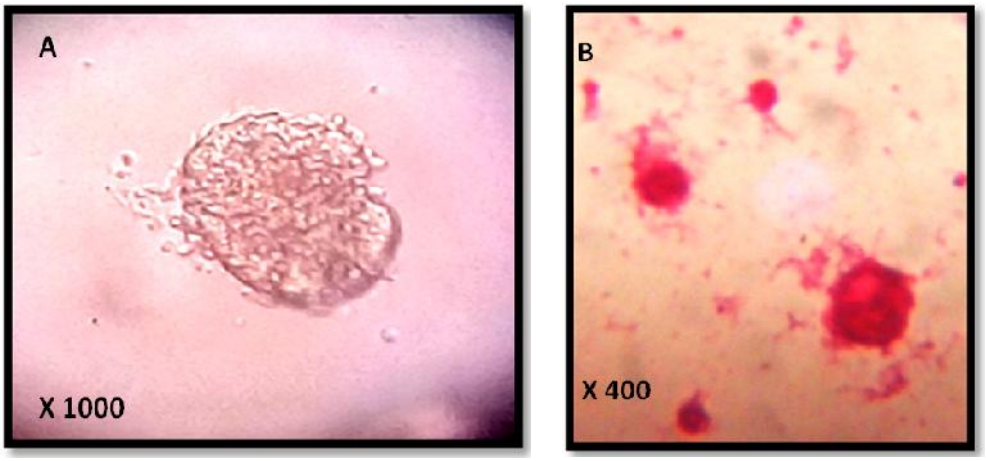

Fig. 1. (A&B). Photomicrograph of *Chilodonella sp*. isolated from skin

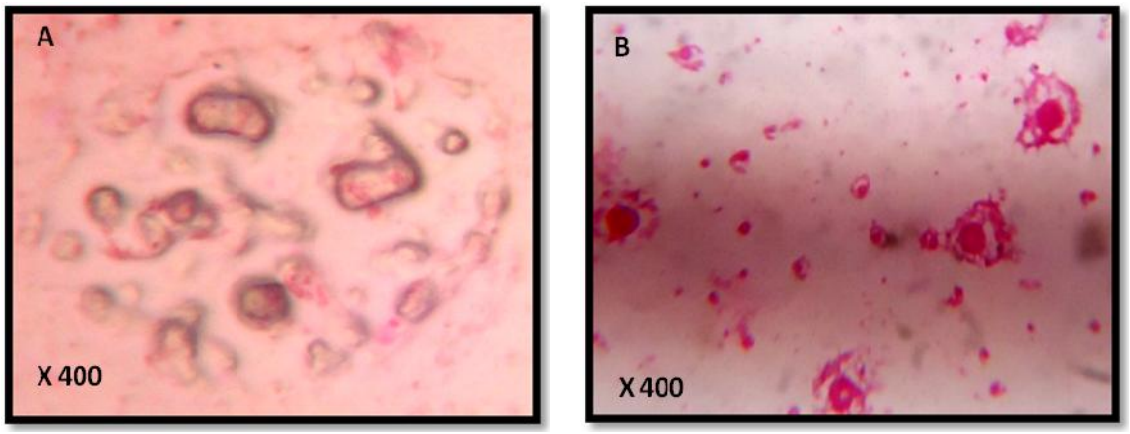

Fig. 2. (A&B). Photomicrograph of *Chilodonella sp*. isolated from gill

Table1. Prevalence of infection of protozoan parasites

Time period	Total no. of host examined	No. of host infected	Parasite groups infected	Parasite species	Site of infestation	No. of parasites
Sep-Oct	50	50	Protozoa	*Chilodonellasp.*	Gills, Skin	74
				Trichodina sp.	Skin	6
				Actinophryssp.	Gills, Skin	0
Nov-Dec	50	48	Protozoa	*Chilodonellasp.*	Gills, skin	21
				Trichodina sp.	Skin	15
				Actinophryssp.	Gills, Skin	0
Feb-Mar	50	50	Protozoa	*Chilodonellasp.*	Gills, Skin	52
				Trichodina sp.	Skin	20
				Actinophryssp.	Gills, Skin	3

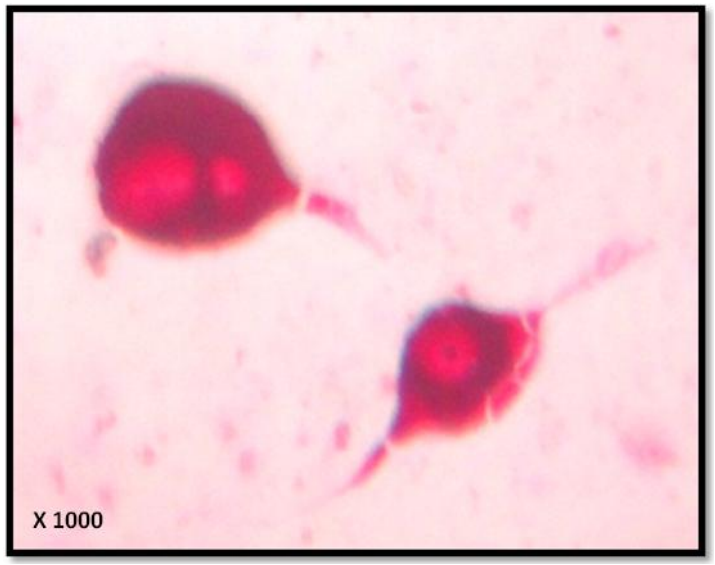

Fig. 3. Photomicrograph of *Trichodina sp*. isolated from skin

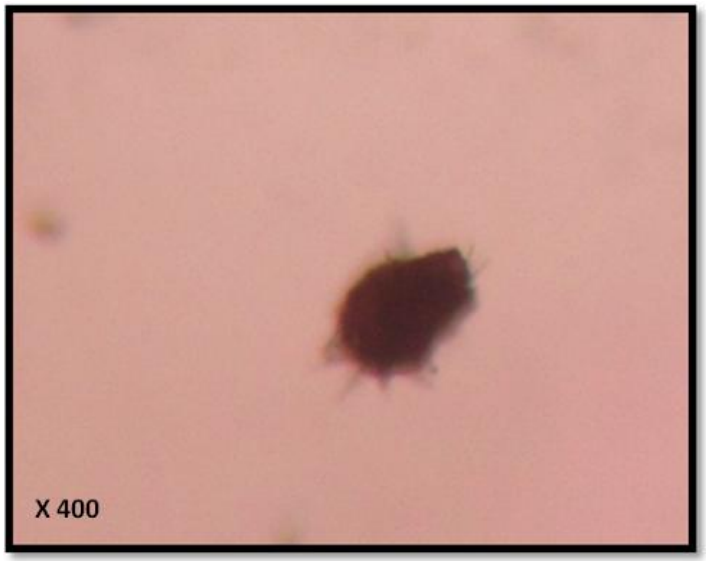

Fig. 4. Photomicrograph of *Actinophrys sp*. isolated from gill

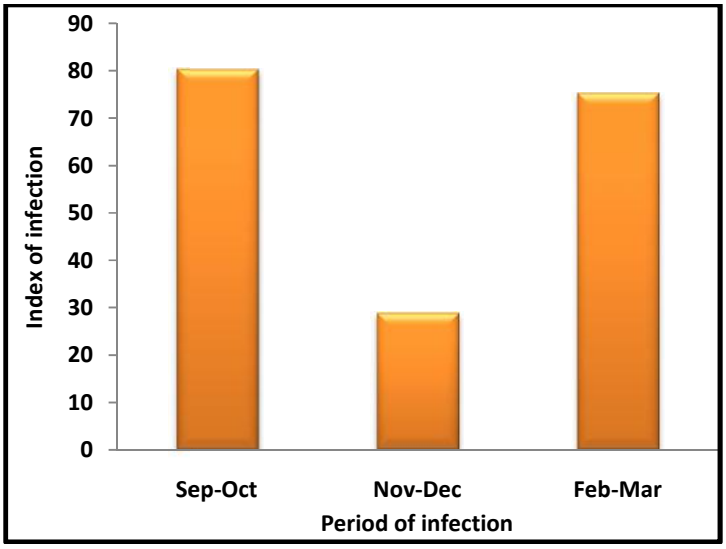

Fig. 5. Index of infection of protozoan parasites at different time span of the year

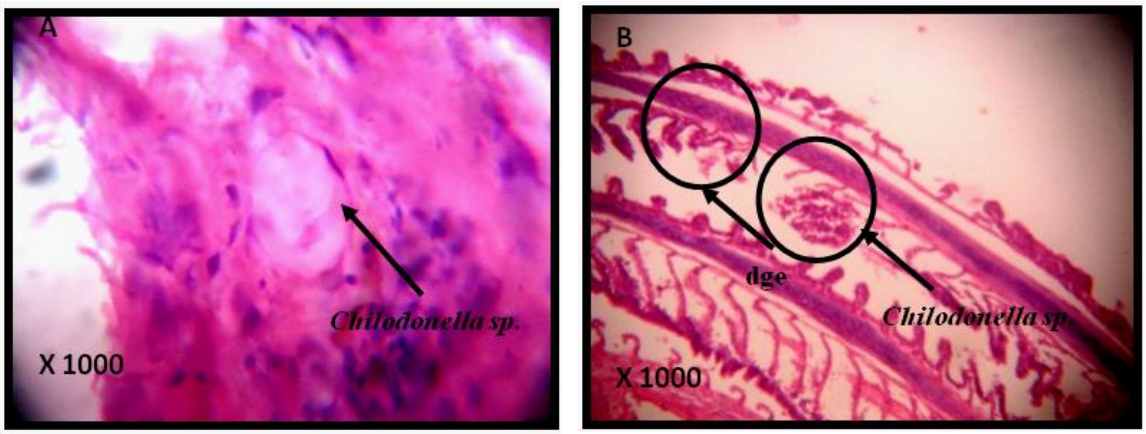

Fig. 6. Photomicrograph of *Chilodonella sp*. on (A). Skin and (B).Gill epithelium exhibiting degeneration (dge)

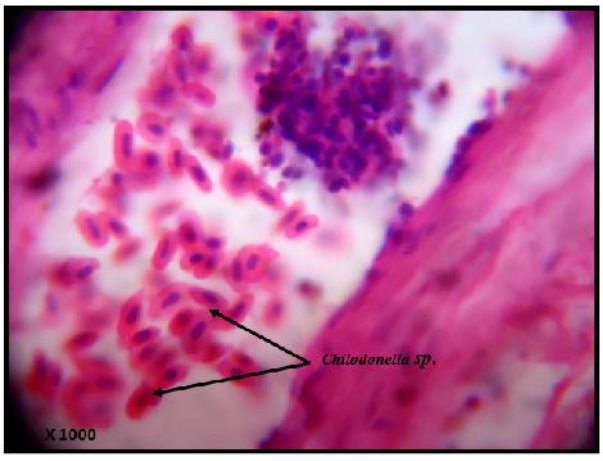

Fig. 7. Photomicrograph of *Chilodonella sp*. on the gill epithelium

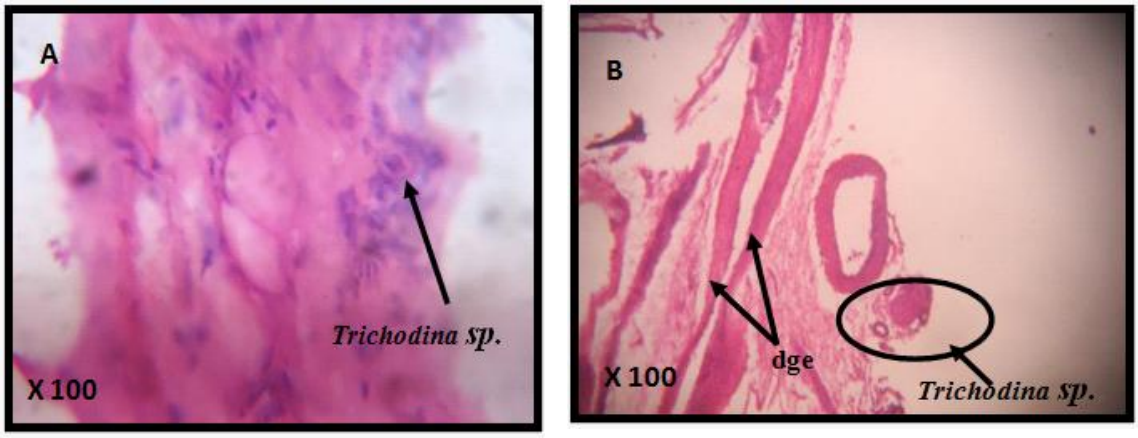

Fig. 8. Photomicrograph of *Trichodina sp*. on (A). Skin and (B).Gill epithelium exhibiting degeneration

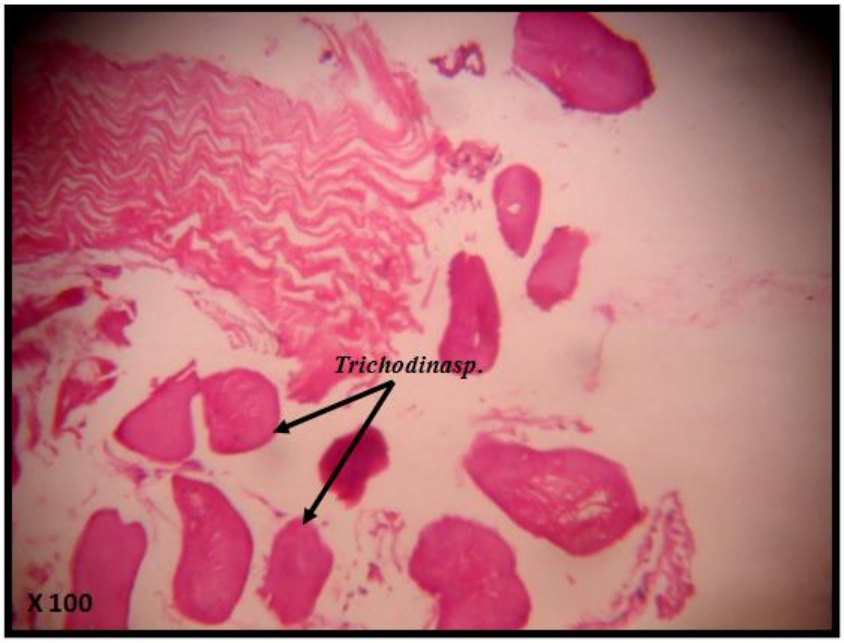

Fig. 9. Photomicrograph of *Trichodina sp*. on the skin

4. CONCLUSION

The present study indicates the presence of *Chilodonella sp*. and *Trichodina sp*. on the gill and skin of freshwater murrel where *Channa punctatus* results in depilatory effect on the host. Emphasis should be given for adopting contro measures to interrupt the steps of parasitic transmission from one host to another. Attention demands controlling the parasite with a view to increase the protein production together with the rapid growth of the fish.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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