

First Record of *Chilodonella hexasticha* from *Botia rostrata* from Arunachal Pradesh, India with a SEM Study

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Abstract

The present study embodies description of *Chilodonella hexasticha*^{1,10} isolated from edible as well as ornamental fish *Botia rostrata* from Dikrong river Arunachal Pradesh, India. This ciliate is 48.25-56.49(50.38±1.57) µm in length and 40.44-48.82(43.88±2.08)µm in width. Taxonomic description of this species have been depicted as per the norms of Lom 1958.² This communication also holds scanning electron microscopic picture of *C. hexasticha* for a detailed and clear illustration of its morphology along with seasonal prevalence, morphometric details. In conjunction with a detailed morphometric comparison of the present species with previously reported species has been done. This data plays a crucial role in the fishery industry as it causes severe disease chilodonellosis, causing mass mortality of fishes. Also it contains the first record of chilodonellid infestation of ornamental fish in Arunachal Pradesh, India.

Context: Though a lot of studies have been done on fish parasites in India but there is relative paucity in case of infestation of ornamental fish parasites on fish fauna of North East India. So keeping this in mind a survey had been done from March 2019 to march 2020 to examine parasitic infection on *Botia* fish along with a morphometric data.

Aim: The objective of the study is to disseminate adequate knowledge regarding the genus *Chilodonella*. This study has also been compiled some published accounts regarding this genera in order to prepare a little but significant informations that is a vital point in the world of taxonomy.

Setting and Design: All authors had participated in the study design. Sample collection and total work has been done by first author and manuscript writing was done by second author. Corresponding author participated planning and supervision of the work. All authors had critically read and approved of the final manuscript

Materials and Methods:

Sampling: A survey has been accomplished in the middle of March 2019-March 2020. Collection of host fishes had been done from Dikrong River of Arunachal Pradesh. For primary study live fishes were lugged to the laboratory of the Department of Zoology, Rajib Gandhi University, Arunachal Pradesh, India. In Parasitology Laboratory, Department of Zoology, Kalyani University, West Bengal identification has been done.

Parasitological Examination: A total of 537 number fishes have been minutely observed for detection For ectoparasitic infestation. Body of host fishes mainly Skin, gill and fins were intensively examined.

Isolation of Pathogens: Months of the calendar year the host fishes were collocated extensively. Affected fishes were isolated on the verge of signs of parasitic infestation. Thin smears of gill, fin and skin of host fish had been made on grease free slides. After that slides were dried in air and impregnated for 10 min in 2% aqueous AgNO₃ solution (Klein 1958)¹⁵, washed (distilled water), and placed under ultraviolet ray for 25- 30 min. Using Olympus BX 43F



model (100 X) magnification with an oil immersion lens examinations of these slides were made. With the help of Olympus BX 43F model photographs were taken. In the case of measurements the norms of Lom, 1958² have been followed. Minimum and maximum values have been furnished.

Scanning electron microscopic study:

Thin smears gills were made on grease free slides and fixed it in 0.5 % saline solution and subsequently air dried. These slides then treated with 4% gluteraldehyde in 0.1 M phosphate buffer (pH 7.2) and fixed for 4 h in it. After primary fixation, the parasites were again fixed in 2% osmium tetroxide for 3 h in the same buffer. Dehydration of these have been done using ascending grades of alcohol and passed on it in a mixture of absolute alcohol and amyl acetate at the ratio of 3:1 for 30 min. Afterwards these specimens treated with the same mixture of 2:2 and 1:3 ratios for 30 min and finally treated with amyl acetate solution. For drying these specimens in CO₂ Hitachi Hep-2 critical point drier was used and, mounted them on aluminium stubs and lastly coated with gold in an IB-2 coater. Observations of those specimens have been done using Hitachi S-530 (Japan) scanning electron microscope at an acceleration voltage of 10 kv.

Keywords: *Botia rostrata*, Arunachal Pradesh, Chilodonellosis, Dikrong river.

Key message: This communication is a key to address future questions in the areas of infestation of chilodinid group of parasites in Indian (specially North East India) ornamental fishes and gives a broad field of knowledge to the future researchers of this line.

Introduction

India is a developing country and progressive advancement of fishery sector plays a major role in its economy. Ornamental fish culture or aquaculture acts the prime role in fish industry. And North East India is leading contributor of this field. But this cost effective business having trouble due to parasitic infestation that leads to huge economic losses in this field. *Chilodonella* is one of the potent ciliate ectoparasite of fishes and found mainly in the freshwater and euryhaline environment. This ciliate is the causative agent of severe fish disease 'chilodonellosis'. This parasite mainly affects the epithelium specially gills. The affected fishes secrete excessive mucus and displays signs of irritation, skin lesions. Several reports of fish death caused by *Chilodonella* have reported in Australia³ and South Africa.⁴ It counts immense economic losses in commercial tropical fish stores.⁵ Economic losses due to *Chilodonella* sp. have been reported by Leibovitz (1980)⁵ and Shariff (1984).⁶ There is a lack of information about chilodonellid infestation of ornamental fishes in India. Though in India some sort of work have been done of chilodinid infection on edible fishes by Mitra and Haldar, 2004;⁷ Mitra and Bandyopadhyay, 2012⁸. So, in this regard a survey has been conducted from march 2018 to march 2019. From this pilot study we have isolated and identified *Chilodonella hexasticha* from ornamental fish *Botia rostrata* from Dikrong river, Arunachal Pradesh, India, for the first time.

This ciliophoran species sometime misinterpret with *C. piscicola*. For the first time both the species had been identified by Andre in 1912⁹ from ornamental fish *Carassius auratus*. In 1926 Wenyon¹⁰,

later in 1931 Kahl¹¹ and Ten Kate agreed with the distinctness of these two species. While in 1935 Schaperclaus¹² claimed that both the species are same and Krascheninnikow (1952)¹³ accepted the view. Later Kazbuski and Migala (1974)¹⁴ break the confusion of the taxonomy of this species and clearly conceded on the licitness of both species, viz., *C. piscicola* and *C. hexasticha*.

Both the species have distinct morphological characteristics by which one can differentiate them. The shape of *C. piscicola* is less elliptical with a clear notch on its posterior part of body, whereas *C. hexasticha* has oval body and absence of notch on its posterior end. Number of kineties are also different. Presence of more kineties in case of *C. piscicola* both right (range 7-15) and left (range 12-13) kineties in comparison to *C. hexasticha* which bears five to seven kineties in the right and seven to nine in the left band.

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Result

Table 1 : Morphometry of *Chilodonella hexasticha* (Kiernik, 1909) Kahl 1931, obtained in the present study (n=20).

Characters	Range	Mean	SD	SE	CV%
Length of the body	48.25-56.49	50.38	1.57	0.35	3.1
Width of the body	40.44-48.82	43.88	2.08	0.46	4.74
No. of right kinety	5-7	5.95	0.57	0.12	9.57
No. of left kinety	7-9	7.5	0.59	0.13	7.8

Table 1 contains morphometric details of chilodonellid species. All measurements are in μm .

Morphometric comparison of *C. hexasticha* obtained in the present study with those of Mitra and Haldar, 2004 and Mitra and Bandyopadhyay, 2012

Chilodonella hexasticha (Kiernik, 1909) Kahl, 1931

Taxonomic Summary

Type host: *Botia rostrata*

Host family: Botiidae

Type locality: River Dikrong (27°2'30"N, 93°54'57"E)

Location: Gill filaments

Prevalence: 22.34%(120/537)

Type specimen: *Chilodonella hexasticha*

Lectotype: Slide no-AP/PARA/KLY/CH/45 has been deposited in the Parasitology Laboratory, Department of Zoology, University of Kalyani.

Description

On the basis of twenty specimens this description has been made (table 1) and morphometric comparison among the previous reported species have been also included here (table 2). Body of this ciliophoran is less elliptical with flatten dorsoventral side. Length measuring 48.25-56.49 (50.38 \pm 1.57) μm and width measuring 40.44-48.82 (43.88 \pm 2.08) μm . The dorsal side is devoid of any cilia except a tiny ciliary row on its tips. There are two ciliary system in on the ventral surface of the body. One is right ciliary kinetics and another one is left ciliary kinetics. Both the ciliary systems are loosely aligned and differentiated by a non-ciliary zone (Figs. 1). The right ciliary system is slightly curved and consists of 5-7(5.95 \pm 0.57) kineties. On the other side the straight left ciliary system consisting 7-9(7.5 \pm 0.59) kineties. Absence of notch in posterior side of the body. Macronucleus is oval in shape. Granular cytoplasm along with a cytostome. Presence of a cytopharynx (Fig. 1).

Table 2 : Contains morphometric comparison of *C. hexasticha* found in present study with previously reported ones

Characters	<i>C. hexasticha</i>	<i>C. hexasticha</i>	<i>C. hexasticha</i>
Host	<i>Nandus nandus</i>	<i>Labeo rohita</i> ; <i>Cyprinus carpio</i>	<i>Botia rostrata</i>
Locality	Churni river, West Bengal, India	Vidyadhari river, Sunderban, West Bengal, India	Dikrong river, Arunachal Pradesh, India
Site of infection	Gills	Gills	Gills
Reference	Mitra and Haldar,2004	Mitra and Bandyopadhyay, 2012	Present study
Length of the body	38.8-59.2 (48.3 ± 6.3)	26.7-54.0 (38.3±1.1)	48.25-56.49(50.38±1.57)
Width of the body	35.7-53.0 (43.2±5.0)	22.1- 49.3 (32.1±0.2)	40.44-48.82(43.88±2.08)
No. of right kinety	5-7 (6.7 ± 0.6)	5-7	5-7(5.95±0.57)
No. of left kinety	6-8 (6.9 ± 0.4)	6-8	7-9(7.5±0.59)

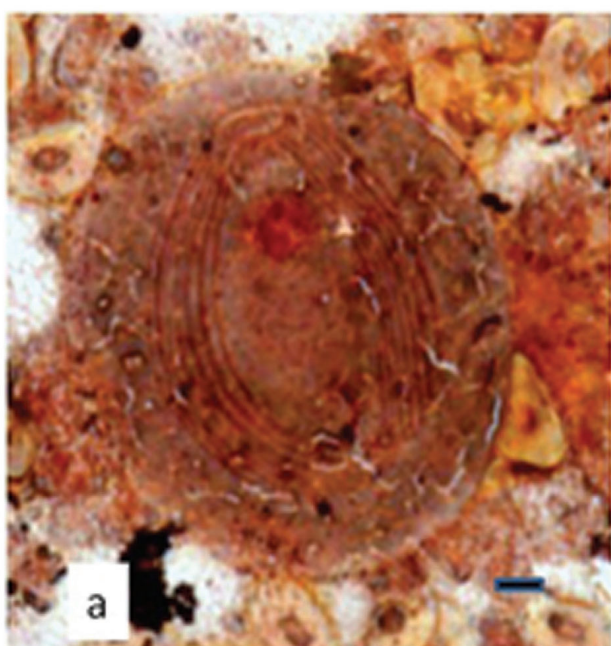


Fig. 1: (a) Photomicrograph of *Chilodonella hexasticha*, stained with 2% aqueous Silver nitrate solution, magnification 100X



Fig. 1: (b) Line diagram of *Chilodonella hexasticha*

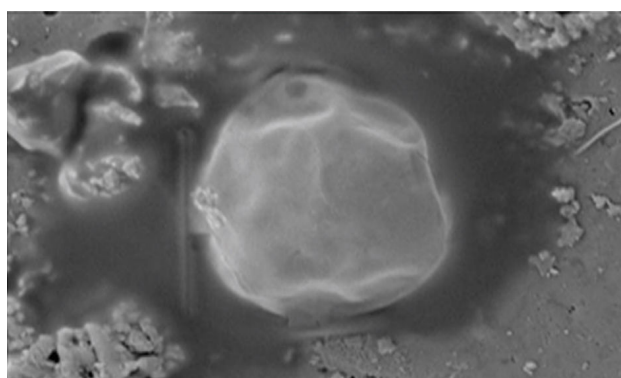


Fig. 1: (c) Scanning electron microscopic image of *C. hexasticha*

Intraspecific variability

During the present study, the specimen shows no significant variability in shape and structure of the denticles

Discussion

The present study reveals first record of *C. hexasticha* infection procured from ornamental as well as edible fish *Botia rostrata* collected from Arunachal Pradesh, India for the first time. Identification of this has been made following the view of Lom and Dykova (1992).¹⁶ In India this species was first recorded by Mitra and Haldar, 2004⁷ from freshwater wild fish, *Nandus nandus*. Later, in 2012 Mitra and Bandyopadhyay⁸ reported this species from fingerlings of *Labeo rohita* and *Cyprinus carpio*.

Hence based on the above mentioned data it can be concluded that the present species seems to be new host and new geographical records that has a great significance in the field of parasitology.

Conclusion

In conclusion it can be stated that there is a urge for furthermore investigation on the harmfulness of *Chilodonella* sp. on ornamental fishes in India and a need of appliance of molecular taxonomic techniques to furnish a clear species identification within this genus.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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