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Descriptions of Two New Species of the Genus *Trichodina* Ehrenberg, 1838 (Protozoa: Ciliophora: Peritrichida) from Indian Fresh Water Fishes

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Summary. Two new species of the genus *Trichodina* Ehrenberg, 1838 were obtained during surveys of trichodinid ciliophorans in the Churni River system and adjacent water bodies in West Bengal, India. These are *T. giurusi* sp. n. from *Glossogobius giuris* (Hamilton-Buchanan) and *T. molae* sp. n. from *Amblypharyngodon mola* (Hamilton-Buchanan). This papers deals with taxonomic descriptions of the two new species based on wet silver nitrate impregnated specimens along with prevalence and morphometric comparisons with closely related species.

Key words: Ciliophora, fish ectoparasites, India, *Trichodina giurusi* sp. n., *T. molae* sp. n., Trichodinidae**.**

INTRODUCTION

Since the work of Annandale (1912) who first reported the occurrence of *Trichodina pediculus* Ehrenberg, 1838 from the lymnocnidid medusa, *Lymnocnida indica* in Bombay Presidency of British India no serious work on this complex group had been carried out in India for a long time. Information on the taxonomy of trichodinids and other groups of ciliophorans is available from early 80's. As a result, more than twenty species of trichodinids and one species of chilodonellid ciliophorans representing the genera *Trichodina* Ehrenberg, 1838; *Paratrichodina* Lom, 1963; *Tripartiella* Lom, 1959; *Trichodinella* Šramek-Hušek, 1953 and *Chilodonella* Strand, 1926 were identified from different estuarine and freshwater fishes (Hagargi and Amoji 1979; Mukherjee and Haldar 1982; Das and Haldar 1987; Das *et.al*. 1987; Mishra and Das 1993; Saha *et*. *al*. 1995 a, b; Saha and Haldar 1996, 1997; Asmat and Haldar 1998; Basu and Haldar 1998; Asmat 2000a, b; 2001a, b, c, d; 2002a, b, c; Basu *et al*. 2003; Mitra and Haldar 2003, 2004 a, b). An expedition in search of trichodinid ciliophorans in freshwater fishes of the river Churni revealed the occurrence of two new species of trichodinid ectoparasites belonging to the genus *Trichodina* Ehrenberg, 1838. These are *T. giurusi* sp. n. and *T. molae* sp. n. The present communication reports on the taxonomy, systematics and prevalence of

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these two new species based on silver nitrate impregnated preparations.

MATERIALS AND METHODS

River Churni is one of the many tributaries of the river Ganges and flows through the district of Nadia in West Bengal (23°E, 88.5°W). It is a small and docile river and provides a complete fresh water environment. Samplings were carried out to collect host fishes from the River Churni and adjacent water bodies. Host fishes were brought to the laboratory in living conditions and gill and skin smears were made on grease free slides. Slides containing trichodinid ciliophorans were impregnated using Klein's dry silver impregnation technique (Klein 1958). Examinations of preparations were made under an Olympus phase contrast microscope at ×100 magnifications with an oil immersion lens and photographs were taken with an Olympus camera. All measurements are in micrometers and follow the uniform specific characteristics as proposed by Lom (1958), Wellborn (1967) and Arthur and Lom (1984). In each case minimum and maximum values are given, followed in parentheses by arithmetic mean and standard deviation. In the case of denticles and radial pins, the mode is given instead of the arithmetic mean. The span of the denticle is measured from the tip of the blade to the tip of the ray. Body diameter is measured as the adhesive disc plus border membrane. The description of denticle elements follows the guidelines of Van As and Basson (1989). Sequence and method of the description of denticle elements follows the recommendations of Van As and Basson (1992).

RESULTS AND DISCUSSION

Two new species of trichodinid ciliophorans belonging to the genus *Trichodina* Ehrenberg, 1838 were obtained from the collected fishes. These are *T. giurusi* sp. n. and *T. molae* sp. n. Descriptions of these are provided below.

Trichodina giurusi sp. n. (Figs 1, 2, 5; Table 1)

Small-sized trichodinid. Denticle consisting of broad blade, occupies most area between y-axes. Distal surface of blade slightly rounded, parallel to border membrane. Tangent point flat, like small line rather than point and situated lower than distal surface. Differentiation between distal surface and anterior surface not significant. Anterior surface sloping down backward to form distinct apex, reaches or even extends beyond y+1-axis. Blade apophysis extremely prominent, consisting of 1-2 notches. Anterior and posterior surfaces of blade almost parallel. Posterior margin of blade takes slightly angular curve to form shallow semilunar curve, deepest point of which remains at same level as apex. Blade connection robust. Differentiation between blade and central part not distinct. Central part delicate in comparison to blade, and spine-shaped in most specimens, fits tightly into preceding denticle and extends almost halfway between y-axes. Sections of central part above and below ×-axis similar. Indentation in lower half of central part opposite to ray apophysis of preceding denticle not present. Ray connection broad. Ray apophysis prominent and anteriorly directed. Ray apophysis situated at middle of y-axes. Well-developed ray gradually tapers to sharply pointed end. Rays directed towards y-1 axis. Ratio between the length of the blade and ray is greater than 1. Macronucleus horseshoe shaped but micronucleus could not be detected.

Taxonomic summary

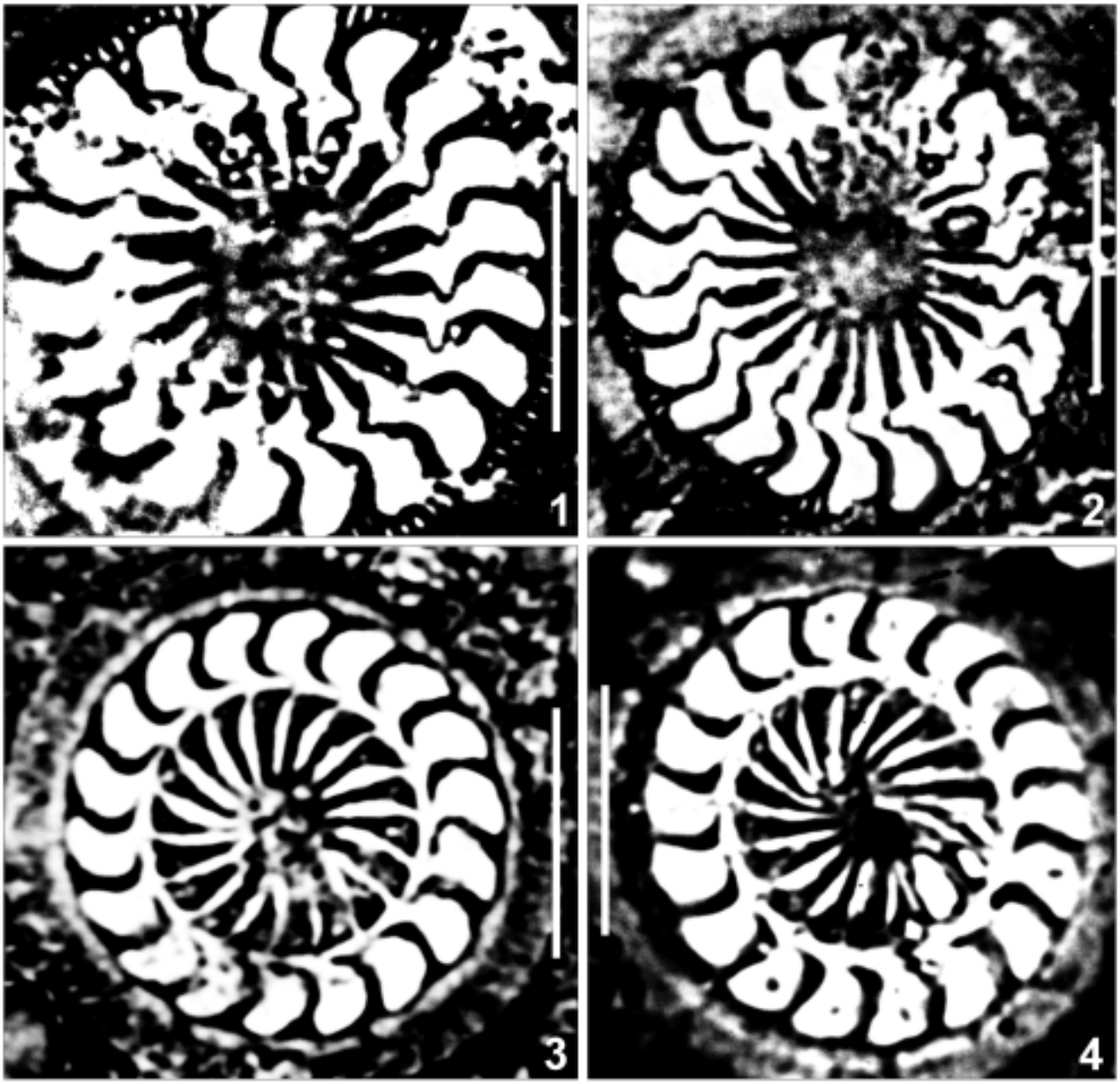
Type host: *Glossogobius giuris* (Hamilton-Buchanan)
Host family: Gobiidae
Type locality: Ranaghat, W. Bengal, India
Location: Gills
Prevalence: 3/56 (5.3 %)

Etymology: The species is named after the specific epithet of the type host *Glossogobius giuris* (Hamilton-Buchanan).

Reference material: Holotype, slide GG-3/2001, and paratype slide GG-2/2002 in the collection of the Protozoology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India and slide GG-1/2002 bearing some paratype materials in the collection of the Harold W. Manter Laboratory of Parasitology, Lincoln, Nebraska, USA (Accession No. HWML 45700).

Remarks: The denticle shape of *Trichodina giurusi* sp. n. obtained from the freshwater fish *Glossogobius giuris* resembles to some extent only two marine species, i.e., *Trichodina jarmilae* Lom *et* Laird, 1969 and *Trichoaina parvula* Lom, 1970.

Trichodina giurusi is comparatively smaller in size than *T. jarmilae* reported by Lom and Laird (1969) from gills of sea raven *Hemitripterus americanus* in Canada. The blade apophysis is extremely prominent in the new species, which is not significant in *T. jarmilae*. In the new species under discussion the distal surface of the blade and anterior blade surface can easily be differentiated. But in *T. jarmilae* this differentiation is not well visible. Extremely prominent apex of the blade of the new species touches or even extends beyond y+1 axis, but in *T. jarmilae* it never touches y+1 axis. The tangent point of the blade is situated almost at the same level as distal point of distal surface in the specimens obtained from *Glossogobius giuris*. In *T. jarmilae* tangent point



Figs 1-4. Photomicrographs of silver nitrate impregnated adhesive discs of trichodinid ciliophorans. **1, 2** - *Trichodina giurusi* sp. n. obtained from the gills of *Glossogobius giuris* (Hamilton-Buchanan); **3, 4** - *Trichodina molae* sp. n. obtained from the gills of *Amblypharyngodon mola* (Hamilton- Buchanan). Scale bars 20 µm.

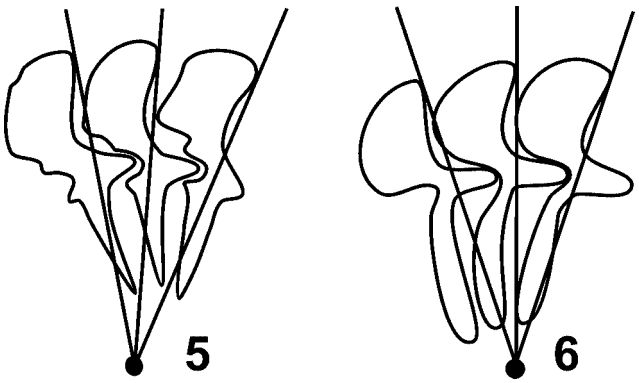
of the denticle is situated enough below the distal point of distal surface of the blade. The posterior surface of the blade of *T. jarmilae* is comparatively deeper than that of *T. giurusi*. In *T. jarmilae* the deepest point of the semilunar curve formed by the posterior blade surface remains slightly upper in position than the apex, but in the new species, deepest point of the semilunar curve remains at the same level as apex. The central part of the denticle of *T. giurusi* is delicate, but that of *T. jarmilae* is elongated and conical. The ray of the new species contains prominent and anteriorly directed ray apophysis. The ray apophysis is completely absent in

Table 1. Morphometric comparison of *Trichodina giurusi* sp. n. with *T. jarmilae* Lom *et* Laird, 1969 and *T. parvula* Lom, 1970. Measurements in µm.

Species	*T. giurusi* sp. n.	*T. jarmilae*	*T. parvula*
Host fish	*Glossogobius giuris*	*Hemitripterus americanus*	*Dasycottus setiger*
Locality	India	Canada	USA
Location	gills	gills	gills
Reference	present study	Lom and Laird (1969)	Lom (1970)
Diameter of			
body	24.4-34.8 (29.7 ± 2.7, 20)	42 (37-45)	35 (30-39)
adhesive disc	20.4-28.6 (24.6 ± 2.2, 20)	26 (23-30)	27 (23-30)
Dimension of body			
denticulate ring	11.7-17.3 (14.9 ± 1.7, 20)	13 (12-16)	14 (13-15)
central area	4.1-7.1 (5.2 ± 0.9, 20)	-	-
Width of border membrane	2.0-3.6 (2.5 ± 0.5, 20)	5	3
Number of			
denticles	20-23 (21, 20)	21 (19-23)	21 (20-23)
radial pins/denticle	5-8 (6, 20)	7 (6)	6-7
Dimension of denticle			
span	7.2-10.8 (8.9 ± 0.9, 20)	-	-
length	3.1-6.6 (4.2 ± 0.9, 20)	5	5
Dimension of denticle components			
length of ray	2.6-4.6 (3.5 ± 0.6, 20)	2-3	4
length of blade	3.6-5.1 (3.8 ± 0.5, 20)	3-4	2.5
width of central part	1.2-2.0 (1.5 ± 0.2, 20)	1-1.5	1.5
Adoral ciliary spiral	390-400°	-	-

Table 2. Morphometric comparison of *Trichodina molae* sp. n. with *T. tenuidens* Fauré-Frémiet, 1943 and *T. puytoraci* Lom, 1962. Measurements in µm.

Species	*T. molae* sp. n.	*T. tenuidens*	*T. puytoraci*
Host fish	*Amblypharyngodon mola*	*Gasterosteus aculeatus*	*Alburnus alburnus*
Locality	India	Poland	Bulgaria
Location	gills	gills	gills
Reference	present study	Lom and Stein (1966)	Grupcheva (1993)
Diameter of			
body	35.7-42.8 (38.2 ± 3.0, 20)	45-69	45 (37-62)
adhesive disc	28.6-34.7 (31.1 ± 2.2, 20)	50 (40-62)	32 (27-38)
Dimension of body			
denticulate ring	12.2-24.5 (19.0 ± 4.6, 20)	31 (25-40)	20 (17-25)
central area	2.6-4.2 (3.3 ± 0.6, 20)	-	-
Width of border membrane	2.9–4.6 (3.6 ± 0.6, 20)	-	3.5 (2.9-4.1)
Number of			
denticles	18-20 (20, 20)	28 (25-33)	24 (20-27)
radial pins/denticle	6-7 (6.3 ± 0.5, 20)	8 (9)	7 (6-8)
Dimension of denticle			
span	8.7-10.1 (9.8 ± 0.8, 20)	-	8.8 (7.6-10.4)
length	3.3-5.1 (4.2 ± 0.6, 20)	7-9	4.5 (2.9-5.3)
Dimension of denticle components			
length of ray	4.1-5.1 (4.4 ± 0.3, 20)	5-7	3.4 (2.9-4.6)
length of blade	3.2-4.6 (3.7 ± 0.5, 20)	4.5-7	3.9 (3.4-4.8)
width of central part	1.1-3.5 (1.7 ± 0.7, 20)	2-2.5	1.4 (1-2)
Adoral ciliary spiral	400-410°	-	-



Figs 5, 6. Diagrammatic drawings of the denticles of trichodinid ciliophorans. **5** - *Trichodina giurusi* sp. n. obtained from the gills of *Glossogobius giuris* (Hamilton-Buchanan); **6** - *Trichodina molae* sp. n. obtained from the gills of *Amblypharyngodon mola* (Hamilton-Buchanan).

T. jarmilae. In *T. giurusi* the ray gradually tapers to a sharp end, but in *T. jarmilae* the width of the ray is almost equal along the entire length and ends with a bluntly rounded tip.

Trichodina giurusi is also smaller than *T. parvula* in its body dimensions reported by Lom (1970) from the gills of marine fish *Dasycottus setiger* and *Radulinus asprellus* in the Pacific Coast of USA. The main differences between these two species lie in the structures of the blade and the ratio of blade**:** central part. The blade of *T. parvula* is angular in orientation to the y-axes, while that of the new species is straight. Both prominent apex and blade apophysis are present in *T. giurusi.* However, these are not present in *T. parvula*. The anterior and posterior margins of the blade of *T. giurusi* are parallel but these are not so in *T. parvula*. The anterior margin touches, or even extends beyond the y+1 axis, but in case of *T. parvula* the anterior margin never crosses the y+1 axis. The ratio between the blade and ray is greater than 1 in the species under discussion, but in both the populations of *T. parvula* reported from *Dasycottus setiger* and *Radulinus asprellus* this is much lesser than 1.

There are also habitat differences between *T. giurusi* and these trichodinids since *T. giurusi* is a freshwater species whereas *T. jarmilae* and *T. parvula* are marine. The superficial resemblance in the blade, central part and ray structures notwithstanding, we propose to designate the trichodinid ciliophoran as a new species. Morphometric comparison between *Trichodina giurusi* sp. n. and the other two closely related species is presented in Table 1.

***Trichodina molae* sp. n. (Figs 3, 4, 6; Table 2)**

Medium-sized trichodinid. Concave adhesive disc bears dark central area. The denticulate ring consists of uniquely shaped denticles. Blade broad, almost semilunar, occupies most area between y-axes. Distal margin of blade flat; slightly rounded and remains in close proximity to border membrane. Tangent point rounded, situated lower than distal point of distal surface. Anterior margin slopes down gradually to form inconspicuous apex that almost touches and rarely extends beyond y+1 axis. Blade apophysis not visible. Posterior margin of blade forms shallow curve, deepest point of which remains at same level or slightly lower than apex. Blade connection thick. Posterior blade projection not observed. Central part slender, conical, extends halfway past y-1 axis in most cases, and fits tightly into preceding denticle. Sections above and below x-axis similar in shape. Ray apophysis absent. Ray connection narrow. After origin, ray gradually flattens laterally and then terminates with bluntly rounded end to take a spatula shape. Rays directed slightly anteriorly towards y+1 direction. Macronucleus horseshoe shaped. Micronucleus could not be detected.

Taxonomic summary

Type host: *Amblypharyngodon mola* (Hamilton-Buchanan).

Host family: Cyprinidae.

Type locality: Ranaghat, W. Bengal, India

Location: Gills.

Prevalence: 17/285 (5.9 %).

Etymology: The species is named after the specific epithet of the type host *Amblypharyngodon mola* (Hamilton-Buchanan).

Reference material: Holotype, slide AM-14/2001, and paratype slide AM-12/2001 in the collection of the Protozoology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India and slide AM-2/2001 bearing some paratype materials in the collection of the Harold W. Manter Laboratory of Parasitology, Lincoln, Nebraska, USA (Accession No. HWML 16742).

Remarks: The present trichodinid species is characterized by a broad blade, elongated conical central part, a narrow junction between the central part and ray and especially the depressed spatulate ray with bluntly rounded tip and, as such, differs significantly from other known trichodinid species. It shows some resemblance with *Trichodina tenuidens* Fauré-Frémiet, 1943 (Lom and

Stein 1966) and *Trichodina puytoraci* Lom, 1962 (Grupcheva 1993) only if the blade and ray structures are considered.

Trichodina tenuidens and *T. puytoraci* resemble our specimen in the blade and ray structures, but differ mainly in having a central subdivided clear area in the form of non-impregnable granulation, which is totally absent in the specimen under discussion. Instead, the central area of the adhesive disc is totally dark in *T. molae*. Both *T. molae* and *T. tenuidens* have spatulate rays, but their blade structure is completely different. The blade of *T. tenuidens* is elongated, while this is broad and slightly rectangular in the specimen under discussion. The number of denticles as reported by Lom and Stein (1966) in *T. tenuidens* is also higher (25-33 in Polish and 23-32 in Russian populations (*vs.* 18-20 in *T. molae*). *T. puytoraci* resembles the trichodinid specimen obtained from *Amblypharyngodon mola* when blade, central part and ray structures are compared. The blade is comparatively broader in *T. molae*. The tip of the ray is swollen in *T. puytoraci* (Grupcheva 1993), but rather spatulate in the new species. Considering all these differences we propose the trichodinid ciliophoran obtained from gills of the freshwater fish *Amblyphryngodon mola* a distinct one and designate in this paper as *Trichodina molae* sp. n. Morphometric comparison of *Trichodina molae* sp. n. obtained in the present study with closely related species is presented in Table 2.

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