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Trichodinid Ectoparasites (Ciliophora: Trichodinidae) of Fishes in India

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Abstract: During surveys of the trichodinid parasites in freshwater and estuarine fishes in the state of West Bengal, India five species of the genus *Trichodina* from the gills of host fishes were investigated and morphologically studied. Of these, four are described as new: *Trichodina ahmedi* sp. n. from *Chanda nama*, *Trichodina hafizuddini* sp. n. from *Amblypharyngodon mola*, *Trichodina mossambicusi* sp. n. from *Oreochromis mossambicus* and *Trichodina heterospina* sp. n. from *Sardinella fimbriata*. During the study period *Trichodina martinkae* from *Clarias batrachus* was also recorded for the first time in India. Taxonomic and morphometric data for these trichodinids based on wet silver nitrate impregnated specimens are presented. For each of the new species, comparisons with closely related species are provided.

Key words: *Trichodina ahmedi*, *Trichodina hafizuddini*, *Trichodina heterospina*, *Trichodina martinkae*, *Trichodina mossambicusi*, Trichodinidae, Ciliophora

INTRODUCTION

Members of the family Trichodinidae are best known as ectoparasites of fish with most of the species reported from freshwater environments[1]. The presence of these organisms often becomes evident after a massive development, causing clinical signs in or leading to mortality of infested hosts[2]. More than 250 species of the trichodinid ciliates are recognized as parasite or symbiont on freshwater and marine fish or other organisms. The genus *Trichodina*[3] is the largest of this family. About 200 species of *Trichodina* have been described from fish by Klein's silver impregnation. Since Annandale[4] very scanty and infrequent information are available on the taxonomy of this particular group in this region[5]. This is due to lack of knowledge about proper identification system of this group and also on taxonomy in general. Hence, the existing data[4-28] need a proper and comprehensive analysis. However, the primary objectives of the present study were to identify the trichodinid ciliates inhabiting the freshwater and estuarine fishes and to study on the morphology and taxonomic status of these trichodinids. During this period, a well known species of *Trichodina*, *T. martinkae*, infecting fishes of the genus *Clarias* was identified for the first time in India along with four other new species and is described here.

MATERIALS AND METHODS

Investigations on the occurrence of trichodinid ciliates from fish caught in the ponds and rivers of Nadia, Hooghly and South 24 Parganas districts of West Bengal were carried out between September 1995 to December 1997. Smears of the gills, fins, and skin of host fishes caught by fishing nets revealed the presence of trichodinid ciliates. Gill scrapings were made at the collection site. Air-dried scrapings were transported to the laboratory. The slides with trichodinid ciliates were impregnated with Klein's silver impregnation technique[29] and were examined under the Olympus Phase-contrast microscope at 10x100 magnification. Measurements were made according to the recommendations of Lom[30], Wellborn[31], Arthur and Lom[32] and Van As and Basson[33-34]. Measurements are given in µm. The level of infection was presented as low (1-5 ciliate slide^{-1}), medium (6-10 ciliate slide^{-1}) and high (more than 10 ciliates slide^{-1}). The results and photographs were compared to results from other studies.

RESULTS AND DISCUSSION

Trichodina martinkae **[35] (Fig. 1, 9; Table 1):** Host. *Clarias batrachus*[36]. Locality. Kalyani of Nadia District. Location. Gills. Prevalence. $^{16}/_{120}$ (13.3%). Infection. Low to medium. Reference materials. Lectotypes, Slide CB 1 and CB 2 (07/07/1996) are in the collection of the Department of Zoology, University of Chittagong, Chittagong 4331, Bangladesh.

Trichodina martinkae was described by Basson and Van As[35] from *Clarias gariepinus* from a fish farm in Orange River System of South Africa. Later, Van As and Basson[34] recorded this ciliate from the gills of *Clarias*

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stappersii and *C. theodorae* from Zambesi River System in South Africa. Basson and Van As[36] reported the presence of *T. martinkae* on the gills of *C. fuscus* in Taiwan and opined that this is a parasite of the genus *Clarias* where it is found exclusively on the gills. During the present study *T. martinkae* (Fig. 1, 9) was collected from *Clarias batrachus*, thus confirms the host specificity of this trichodinid as commented by Basson and Van As[36]. The result of the present study extends the known geographic range of *T. martinkae* and *C. batrachus* appears to be a new host.

***Trichodina ahmedi* sp. n. (Fig. 3-4, 11; Table 1):** Host. *Chanda nama*[38]. Locality. Kalyani of Nadia District. Location. Gills. Prevalence. $^{105}/_{498}$ (21.1%). Infection. Low to medium.

Denticle morphology: Blade of denticle moderately spaced, broad, angular, almost sickle-shaped, filling more than half space between y axes (Fig. 11). Shape of blade triangular in developing individuals (Fig. 4). Distal margin not clearly distinguishable from anterior margin lying close to border membrane. Anterior margin slopes down angularly to y+1 axis, forming sharply rounded or conical apex which touches y+1 axis. Apical depression well developed. Anterior blade apophysis distinctly visible. Posterior margin forms shallow crescent with deepest part at same level as apex, sometimes slightly lower than apex. Tangent blunt, sometimes forming small line rather than point. Blade connection strong, nearly as thick as ray connection. Central part stout, wide triangular with bluntly rounded point, extending halfway to, rarely touching, y-1 axis, interlocking firmly with preceding denticle. Shape of central part above and below x-axis similar. Indentation in lower central part sometimes present. Ray connection short, well developed (but very delicate in developing individuals), with indistinct ray apophysis. Ray strong, dagger-shaped with narrow base and broad middle containing a prominent central groove, ending in sharply rounded tip (Fig. 3). Ray slightly bent backwards, orienting anteriorly, so tip of ray touches or crosses y+1 axis.

The variability in the structure of denticles, concerning mainly the thickness of the blade and the shape of ray is evident in different populations. Typically, the blade is moderately spaced, broad, angular, almost sickle-shaped, but in many cases appear as spoon-shaped or almost rounded filling most of the interblade space. The shape of ray also varies considerably in different individuals. In most cases, ray is strong, dagger-shaped with narrow base and broad middle containing a prominent central groove and ends in sharply rounded tip. In some cases, ray is straight, slanting anteriorly or in other cases, the part after the ray apophysis becomes broadened like a soupspoon. These differences seem to exhibit the individual variation because any subpopulation could include many of these variants.

The described trichodinid may be characterized by having lightly stained central area of the adhesive disc and the denticle consists of almost sickle-shaped blade with conically rounded apex, well-developed central part and dagger-shaped ray with central groove and pointed tip. The species shows resemblance to *Trichodina ranae* da Cunha[37] as demonstrated by Kazubski[38]. *T. ranae*, an inhabitant of urinary bladder in various aquatic frogs in Europe was recorded from Portugal[37], Italy[39], France[40], Germany[41] and Poland[38]. General appearance of the adhesive disc of the present trichodinid appears to be similar to *T. ranae* by having sickle-shaped blade, backwardly bent ray and same kind of variations in denticles. But in close examination striking differences reveal the described trichodinid as a distinct form. The main differences concern the structure of the denticle and the central area of the adhesive disc. In *T. ranae*, i) the central part of denticle is slender and tubular (*vs* stout and wide triangular in shape); ii) the ray is slender, straight, finger-like with variable thickness but having no ray apophysis and central groove (*vs* more backwardly bent and dagger-shaped ray, sometimes having distinct ray apophysis and central groove); and iii) the central area of the adhesive disc contains whitish granules (*vs* lightly stained central area). Morphometric data on body dimensions are close to each other, but the number and length of denticle is higher than the described species. However, on account of the photomicrographs and morphometric data given by Kazubski[38], closeness of both trichodinids may be ascertained.

Type Host	*Chanda nama*[38] (Perciformes: Ambassidae)
Type Locality	Kalyani of Nadia District (23°3'N 88°4'E), West Bengal, India
Type Location	Gills
Type Specimens	Holotype, slide CN 1 (06/12/1995); paratypes, slide CN 2 (06/12/1995) are in the collection of the Department of Zoology, University of Chittagong, Chittagong 4331, Bangladesh
Etymology	Named in honour of the great Bangladeshi parasitologist, Abu Tweb Abu Ahmed

***Trichodina hafizuddini* sp. n. (Fig. 5-6, 12; Table 1):** Host. *Amblypharyngodon mola*[38]. Locality. Hooghly River of Hooghly district. Location. Gills. Infection. Low. Prevalence. $^{34}/_{700}$ (4.8%).

Denticle morphology: Blade broad, butter spoon-shaped with almost parallel border (Fig. 5-6), filling entire space between y axes. Distal margin conically round and close to border membrane. Inter-blade space generally not very large, contains

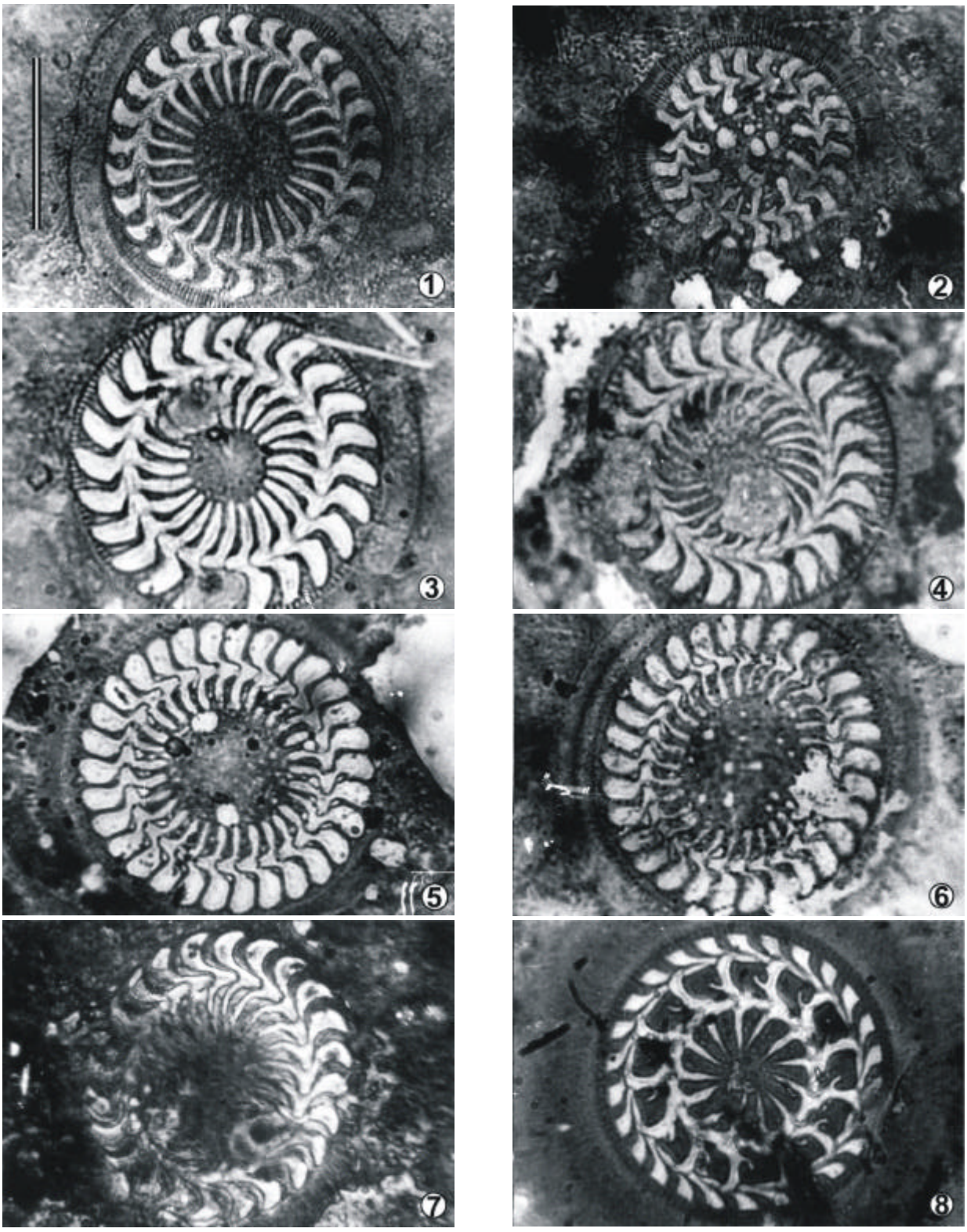

Fig 1-8: Silver impregnated adhesive discs of trichodinids: 1 *Trichodina martinkae*, 2 *Trichodina heterospina* sp. n., 3-4 *Trichodina ahmedi* sp. n. (4 of developing individual), 5-6 *Trichodina hafizuddini* sp. n. and 7-8 *Trichodina mossambicusi* sp. n. (8 of developing individual) from India. Scale bar-30 µm.

argentophobic granules. Tangent flat, forming small line rather than point and situated lower than distal margin (Fig. 12). Anterior margin slightly curves down and forms shallow apex at base of blade and sometimes extends beyond y+1 axis. Blade apophysis prominent. Posterior margin indentation forms small semilunar curve with deepest point at below apex. Blade connection thin. Posterior blade apophysis absent. Central part slender, tubular with bluntly rounded point extending rarely halfway to y-1 axis and interlinked tightly into preceding denticle. Sections above and below x-axis similar. Indentation in lower central part rarely seen. Ray connection short and thin. Ray apophysis, though not always prominent, directed upward towards central part. Ray shorter than blade, bearing central groove and sometimes constriction just below ray apophysis. Post constriction part of ray to some extent inflated, ending in rounded tip. Ray slightly curved posteriorly, but remains almost parallel to y axes. Argentophobic particles present between ray bases.

The ciliate may be characterized by having denticles which possess spoon-shaped blades with parallel lateral margins, but curved distal surface; slender central parts; rays are shorter than the blades, of uniform thickness and curved in the posterior direction or slanted anteriorly; and the central area contains a few to many non-impregnable round particles. This trichodinid closely resembles *Trichodina caspialosae*. This species was recorded by Lom[42] from the gills of *Alosa braschnikowi meotica* in the Rumanian Black Sea coast. The photomicrograph of the silver impregnated adhesive disc of this species as inserted by Lom[42] is close to the present species in having slender central part; rays of uniform thickness which are smaller than the blades; and a huge central area but the other features of the denticle and adhesive disc in the two species is significantly different. In *T. caspialosae*: i) the blade is triangular in shape with an angular anterior margin (*vs* distinctly butter-spoon in shape with parallel lateral margins and curved distal surface); ii) the interblade space is wide (*vs* narrow); iii) the central conical part is wide and conical (*vs* slender and tubular); iv) the ray is straight (*vs* backwardly curved or slanted anteriorly); v) the ray apophysis is absent (*vs* present and distinct); and vi) there is no argentophobic particles in the central area, in the interblade spaces and the ray bases (*vs* present). The morphometric data of *T. caspialosae* is also significantly smaller than the presently discussed ciliate.

Type host	*Amblypharyngodon mola*[38] (Cypriniformes: Cyprinidae)
Type Locality	Hooghly River (Latitude 22°00'N Longitude 88°07E) at Triveni of Hooghly District, West Bengal, India
Type Location	Gills
Type Specimens	Holotype, slide AM 1 (25/11/1996); paratypes, slide AM 2 (25/11/1996) in the collection of the Department of Zoology, University of Chittagong, Chittagong 4331, Bangladesh
Etymology	Named in honour of the great Bangladeshi parasitologist, A. K. M. Hafizuddin

***Trichodina heterospina* sp. n. (Fig. 2, 10; Table 1):** Host. *Sardinella fimbriata*[45]. Locality. Matla River of South 24 Parganas District. Location. Gills. Infection. Low.

Denticle morphology: Blade of denticle broad, erect, but slightly angular and fills most of space between y axes. Distal margin nearly adjoins border membrane, mostly flat and runs parallel to this membrane (Fig. 2). Tangent flat, forms a line rather than sharp point, lying slightly below distal margin. Anterior margin angular with y+1 axis (Fig. 10), forms an angular apex at base. Apex extends to y+1 axis, but never touches this line. Apical depression well developed and never impregnates. Anterior blade apophysis absent. Blade connection as thick as ray connection. Posterior margin also angular, forms a shallow crescent that lies at same level as apex. Posterior blade apophysis absent. Central part wide triangular with sharply rounded point, extending slightly more than halfway to y-1 axis. Indentation in lower central part absent. Shape of central part above x axis slightly sloped posteriorly, but lower section forms nice triangle. Ray connection short and broad, having no ray apophysis. Rays alternately long and short. Long ray strong, straight, narrowed at base but greatly broadened distally, ending in inflated round tip. Short ray sometimes very difficult to recognize. Ray slightly slanted anteriorly, tip of ray touches y+1 axis.

The described species of *Trichodina* is unique among the trichodinids in having loosely packed denticulate ring consisting of angularly erect blade with truncated or slightly curved distal margin and flat tangent point; strong and straight rays with narrow base and inflated round tip, alternates with reduced rays; and the centre of the adhesive disc contains a few marble-shaped round or rod-like bright particles. Only a few individuals were obtained in the smears made from the gills of *S. fimbriata*, a common estuarine fish of West Bengal. Until more information on the adoral ciliary spiral is available, the present trichodinid is recognized as a species of *Trichodina*, mainly because of agreement in the nature of wedging of the denticles.

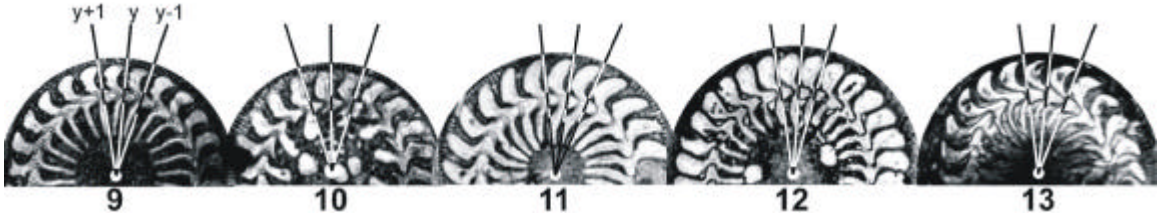

Fig 9-13: Denticles of trichodinids showing relation to y axes: 9 *Trichodina martinkae*; 10 *Trichodina heterospina* sp. n.; 11 *Trichodina ahmedi* sp. n.; 12 *Trichodina hafizuddini* sp. n.; and 13 *Trichodina mossambicusi* sp. n.

Type host	*Sardinella fimbriata*[45] (Clupeiformes: Clupeidae)
Type locality	Matla River (Lattitude21°5'N Longitude 88°5'E) at Canning of South 24 Parganas District, West Bengal, India
Type location	Gills
Type Specimens	Holotype, slide SF 1 (25/05/1997); paratypes, slide SF 2 (25/05/1997) in the collection of the Department of Zoology, University of Chittagong, Chittagong 4331, Bangladesh
Etymology	Named after alternate long and short rays of denticles in the ciliate

***Trichodina mossambicusi* sp. n. (Fig. 7-8, 13; Table 1):** Host. *Oreochromis mossambicus*[46]. Locality. Kalyani of Nadia District. Location. Gills. Prevalence. $^{9}/_{385}$ (2.3%). Infection. Low.

Denticle morphology:. Blade of denticle moderately falciform, filling most of space between y and y+1 axes (Fig. 13). Distal margin slightly curved, lying almost at same level as sharply pointed tangent. Anterior margin curves forming rounded apex that never extends beyond y+1 axis. Apical depression in few blades of each specimen impregnates lightly, strong impregnation found in developing individuals (Fig. 8). Anterior blade apophysis sometimes clearly visible. Deepest point of crescent formed by posterior margin lies at same level as apex. Posterior projection of blade not visible. Blade connection well-developed, of same thickness as ray (Fig. 7). Central part moderate with bluntly rounded point, extends slightly beyond y-1 axis and fitted tightly into preceding denticle. Shape of section above and below x-axis similar. Ray well-developed, tapers to a fine point and slightly curved in posterior direction, but remains parallel to y axes. Ray connection short and well-formed, ray apophysis not prominent.

Only two existing species of *Trichodina* having dark centers resemble to some extent the present one by the morphology of the silver impregnated adhesive disc. The two species are *Trichodina siluri*[43] from the gills of *Silurus glanis* and *Trichodina esocis*[44] from the skin of *Esox lucius* in Czech Republic. The general shape of blade with sharp tangent point of immature specimen of *T. siluri* is to a lesser extent similar to the described specimens, but the shape of ray, both in adult and immature, is quite different, which in *T. siluri* is more or less straight with same width and blunt tip, whilst backwardly curved and gradually pointing in the present species. Moreover, the anterior margin of the blade in the presently described species bears no irregular notches, which is present in *T. siluri*. The falciform blade of *T. esosis* with sharp tangent point, deep posterior margin indentation and also measurements are quite similar to the present species, but the considerably narrower blade and forwardly slanted, straight and blunt-tipped ray of same thickness is distinctly different from the described species in which the reverse features are prominent. These features justify the establishment of this trichodinid as a new species.

Type host	*Oreochromis mossambicus*[46] (Perciformes: Cichlidae)
Type locality	Kalyani of Nadia District (23.3N 88.4E), West Bengal, India
Type location	Gills
Type Specimens	Holotype, slide OM 1 (14/08/1996); paratypes, slide OM 2 (14/08/1996) in the collection of the Department of Zoology, University of Kalyani, Kalyani 741235, Nadia, West Bengal, India
Etymology	Named after the species name of the type host,

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