### **Original papers**

## The first record of two trichodinid ectoparasites, *Trichodina* pseudoheterodentata Tang et al. 2017 and *Trichodina* hafizuddini Asmat, 2005 (Ciliophora: Peritricha) from the freshwater fishes in the Baikka Beel of Moulvibazar district in Sylhet division, Bangladesh

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**ABSTRACT.** A parasitological investigation on trichodinid ciliates from several freshwater fishes in the Baikka Beel of Moulvibazar district of Sylhet was carried out between January and December 2015. During the investigation period, two parasitic trichodinid ciliates (Ciliophora: Peritrichia), *Trichodina pseudoheterodentata* Tang et al. 2017 and *Trichodina hafizuddini* Asmat, 2005 were morphologically studied and described with using the silver nitrate impregnation technique. *Trichodina pseudoheterodentata* was isolated from the gills of *Mystus bleekeri*, while *Trichodina hafizuddini* from the gills of *Amblypharyngodon mola*. This investigation has been revealed that these two *Trichodina* species fall within the range of morphometry and agree closely in the overall appearance of the adhesive disc with the original populations. Geographical variation was also observed by considering the body size and number of the denticles. Depending on country's available taxonomical survey on trichodinid ciliates, this study is the first formal report on these trichodinids from any part of Bangladesh.

Key words: Ciliophora, trichodinid, Trichodina pseudoheterodentata, Trichodina hafizuddini, freshwater fish, Mystus bleekeri, Amblypharyngodon mola

#### Introduction

Trichodinids are peritrichous ciliates found worldwide on a variety of hosts [1,2]. The majority of trichodinids are described for freshwater environments [3–7]. The genus *Trichodina* [8] is the largest within the family Trichodinidae [9]. Representatives of the family parasitize or are symbionts of a broad spectrum of aquatic invertebrate and vertebrate hosts [10]. They infect fish skin, fins and gills [11]. The presence of these organisms often become evident after massive development, causing clinical signs in or leading to mortality of infested hosts [8]. To date, more than 300 nominal trichodinid ciliates have been reported from different environments in the world [12,13]. Despite this the *Trichodina* species found on freshwater fishes of Bangladesh have been got little attention.

In Bangladesh, Asmat et al. [14] made the first report on trichodinid ciliates. Since then scanty and infrequent information is available on the taxonomy of the particular group. However, most recent attempt brings a great change in trichodinid ciliates investigation by establishing of 36 species of trichodinid ciliates, representing the four genera *Trichodina* [8]; *Paratrichodina* [15]; *Tripartiella* [16]; and *Trichodinella* [17,18] from various species of freshwater and estuarine ichthyofauna [14,19–33].

The aim of the present survey was to examine which trichodinid species occur on collected wild freshwater fishes from the Baikka Beel, Moulvibazar, Sylhet to follow the progress of these infections and to estimate the rate of prevalence, and

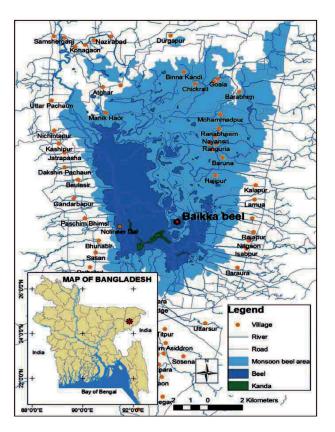


Fig. 1. Map of sampling localities at the Baikka Beel (Source: IPAC [36], www. nishorgo.org)

emphasizing species diversity, morphological variability with reference to existing taxonomic information.

#### **Materials and Methods**

Freshwater wild fishes (1.5–7.6 cm and 1.5–30.0 g) were collected from local fish markets and adjacent areas of the Baikka Beel with the help of local people and fishermen during the study period. Gill scrapings were made at the sampling site. Airdried gills scrapings were transported to the laboratory and observing under a binocular dissecting research microscope for sorting out slides with trichodinid ciliates. Fresh gill smears containing slides infected by trichodinids were impregnated with Klein's [34] silver impregnation technique for observing impregnated adhesive disc of trichodinid ciliates as recommended by Lom [35]. All impregnated trichodinid ciliates were observed with the help of a compound microscope, OSK 9712 T-2 at 1000× magnification. Numerous photomicrographs were made using a SONY cyber shot camera in order to have comprehensive morphological data analysis and measurement of these ciliates. All measurements were done with aid of an ocular micrometer and followed the uniform specific recommendation proposed by Lom [35], Wellborn [7], Arthur and Lom [3] and Van As and Basson [10,37]. In case of morphometric and meristic measurements, maximum and minimum values are given, followed in parentheses by the arithmetic mean and standard deviation. For statistical analysis, morphometric data of 20 specimens for the species were considered. Descriptions and measurements of denticles are following the method proposed by Van As and Basson [10].

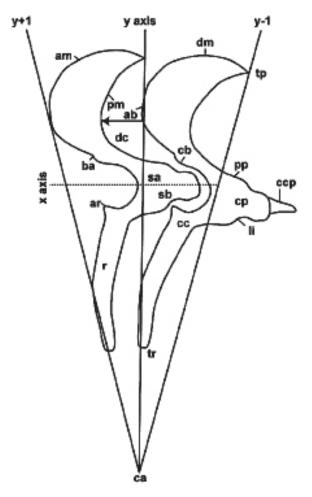


Fig. 2. Denticle structure and construction of X and Y axes as fixed references for description denticles, after Van As and Basson [10]. Explanations: AB, apex of blade; AM, anterior margin of blade; AR, apophysis of ray; B, blade; BA, apophysis of blade; CA, central area of adhesive disc; CB, section connecting blade and central art; CC, section connecting part and ray; CCP, central conical part; CP, central part of blade; DC, deepest point of semi-lunar curve relative to apex; DM, distal margin of blade; PM, posterior margin of blade; PP, posterior projection; R, ray; SA, section of central part above x axis; SB, section of central part below X axis; TP, tangent point; TR, tip of ray.

Species	T. pseudoheterodentata (n=18) [39]	T. pseudoheterodentata (n=20)
Host	Ictalurus punctaus	Mystus bleekeri
Locality	Chongqing, China	Baikka Beel, Moulvibazar, Sylhet
Location	Gills	Gills
Diameter of		
body	73.0-82.5 (76.3±3.2)	48.0-60.2 (55.8±3.8)
adhesive disc	61.5-74.0 (67.0±5.0)	39.9-51.3 (45.4±4.2)
denticulate ring	39.0-47.5 (42.4±3.1)	23.8-29.6 (26.5±1.5)
central area	_	11.2-15.4 (14.1±1.4)
Width of border membrane	4.0-6.0 (4.9±0.7)	4.4-5.4 (5.0±0.2)
Number of		
denticles	23-25	21-25 (23.1±1.0)
radial pins/denticle	10-12	6-8 (7.1±0.9)
Span of denticle	19.0-23.0 (21.0±1.6)	12.1-15.0 (13.8±0.8)
Length of		
denticle	9.5-11.5 (9.9±0.7)	3.4-5.6 (4.6±0.7)
ray	8.5-10.5 (9.4±0.7)	5.8-8.2 (6.9±0.7)
blade	5.5-9.0 (7.0±1.1)	3.7-5.6 (4.6±0.5)
Width of central part	4.5-5.5 (4.8±0.4)	2.0-3.3 (2.6±0.4)
Degree of adoral ciliature	390-410°	400-410°

Table 1. Morphometric comparison of *Trichodina pseudoheterodentata* obtained in the present study with that of Tang et al. [39]. Measurement in micrometer ( $\mu$ m).

#### **Results and Discussion**

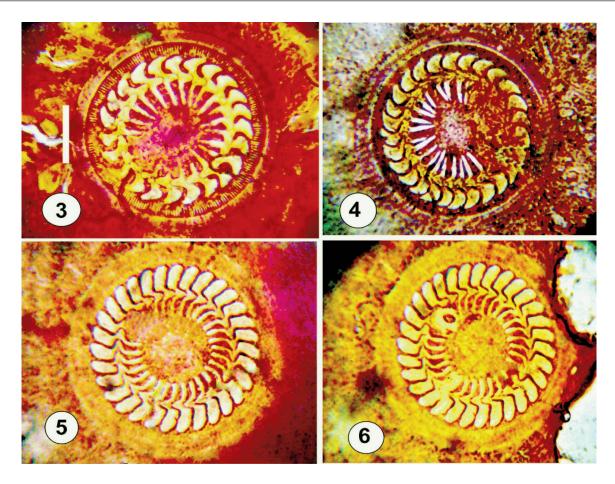
*Trichodina pseudoheterodentata* Tang et al. **2017** (Figs 3-4, 7; Table 1)

Host: Mystus bleekeri [38], Locality: The Baikka Beel (24.3514°N, 91.6979°E) of Moulvibazar district in Sylhet division, Bangladesh. Location on host: Gills. Prevalence: Thirty eight out of fifty specimens examined (76%). Intensity of infection: High. Voucher specimens: Two slides (CUZM-MB-1-BB and CUZM-MB-2-BB) with sliver impregnated specimens prepared on 29 April 2015 have been deposited in the Museum of Department of Zoology, University of Chittagong 4331, Bangladesh.

**Description (n=20):** Large trichodinid ciliate, cup shaped body with 48.0-60.2 (55.8±3.8) diameter; concave adhesive disc 39.9-51.3(45.4±4.2); surrounded by finely striated, wide border membrane 4.4-5.4 (5.0±0.2); central area large, impregnates slightly less than rest of the disc, diameter 11.2–15.4 (14.1±1.4), without any silver nitrate impregnated granules; diameter of denticulate ring 23.8–29.6 (26.5±1.5), consisting of 21-25 (23.1±1.0) denticles with radial pins per denticle, 6-8 (7.1±0.9); span and length of denticle 12.1-15.0 (13.8±0.8) and 3.4-5.6 (4.6±0.7) diameter respectively; blade 3.7-5.6 ( $4.6\pm0.5$ ); length of ray 5.8-8.2 ( $6.9\pm0.7$ ) and central part 2.0-3.3 ( $2.6\pm0.4$ ) in width. Adoral ciliary spiral about 400-410°.

Denticle morphology: Blade wide, quadrilateral obliquely and sickle-shaped with sharp tangent point (Fig. 3). Distal margin of blade smooth and little curved not parallel to border membrane, lying somewhat away from border membrane and all but same level of tangent point. Anterior margin angulary curved but not parallel with posterior margin. Anterior margin extending beyond y+1 axis (Fig. 7), prominent anterior blade apophysis always present. Posterior margin of blade distinct to mark semilunar curve with deepest point of curve at below apex. Blade connection thick and posterior blade apophysis present. Central part strong, straight or slightly curved downwards with bluntly rounded, robust point of tip extending just past halfway to y-1 axis and strongly interlinked into preceding denticle. Shape of central part above and below x axis similar. Indentation on lower central part indistinct. Ray connection well developed and markedly distinguishable from entire ray. Ray comparatively stout, long and tip of ray directed parallel with y axis. Ray apophysis not developed.

Trichodina pseudoheterodentata was first



Figs 3-6. Photomicrographs of silver impregnated adhesive discs of *Trichodina pseudoheterodentata* (3-4) and *Trichodina hafizuddini* (5-6). Scale bar 20 µm.

described by Tang et al. [39] from the gills of channel catfish Ictalurus punctatus from the Changshou Lake in Chongqing, China. The present study confirms the existence of T. pseudoheterodentata for the first time in Bangladesh from freshwater catfish Mystus bleekeri. Based on the appearance of adhesive disc T. pseudoheterodentata is almost resembles to Trichodina heterodentata [40]. Besides morphological features of T. pseudoheterodentata reveal that it is freshwater large sized Trichodina species, cell diameter 73.0-82.5 µm, possess robust denticles with broad, sickle-shaped blade, width central part, horse-shoe shaped macronucleus and spherical shaped micronucleus situated in +Y position, adoral ciliary spiral turns about 390-410° around peristomal disc.

Presently discussed trichodinid specimens found from the gills of *M. bleekeri* have coincident morphological features of that originally described by Tang et al. [39]. However, morphometric data of presently discussed specimen shows a range of variation. Body dimension of *T. pseudodeterodentata* that described by Tang et al. [39], is larger than presently described specimens (Table 1). Number of denticles, width of border membrane falls with the range. However, dimension of adhesive disc, denticular ring, number of radial pins per denticles, and measurement of denticle component are comparatively smaller than that of population from Tang et al. [39].

# *Trichodina hafizuddini* Asmat, 2005 (Figs 5-6, 9; Table 2)

Host: Amblypharyngodon mola [38]. Locality: The Baikka Beel (24.3514°N, 91.6979°E) of Moulvibazar district in Sylhet division, Bangladesh. Location on host: Gills. Prevalence: Twenty eight out of seventy specimens examined (40%). Intensity of infection: Low to medium. Voucher specimens: Two slides (CUZM-AM-1-BB and CUZM-AM-2-BB) with sliver impregnated specimens prepared on 5th October 2015 have been deposited in the Museum of Department of Zoology, University of Chittagong 4331, Bangladesh.

**Description (n=20):** Large trichodinid ciliate with hemispherical shaped body, 48.0-52.3 (49.8±1.3) in diameter. Diameter of adhesive disc

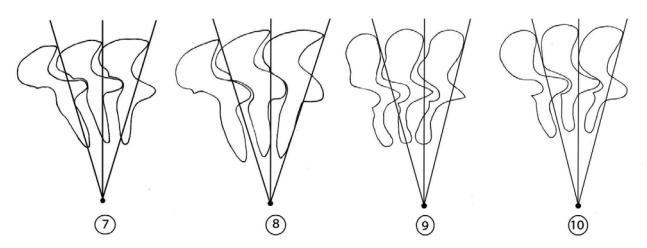
Species	<i>T. hafizuddini</i> (n=20) [25]	T. hafizuddini (n=20)
Host	Amblypharyngodon mola	Amblypharyngodon mola
Locality	Hooghly River, India	Baikka Beel, Moulvibazar, Sylhet Bangladesh
Location	Gills	Gills
Diameter of		
body	45.9-62.3 (51.3±5.2)	48.0-52.3 (49.8±1.3)
adhesive disc	35.7-54.1 (42.2±5.1)	39.7-41.7 (40.3±0.6)
denticulate ring	24.0-38.2 (27.6±3.5)	24.7-27.9 (26.6±0.8)
central area	13.7-24.5 (17.6±2.9)	12.6-16.3 (14.1±0.8)
Width of border membrane	$4.1-5.1 \ (4.4 \pm 0.4)$	4.9-5.8 (5.2±0.3)
Number of		
denticles	28-31 (29.7±0.8)	30-33 (31.3±0.8)
radial pins/denticle	7-11 (8.9±1.3)	4-6 (5.3±0.8)
Span of denticle	_	12.5-15.3 (14.0±0.8)
Length of		
denticle	5.1-8.2 (6.0±0.9)	3.7-5.6 (4.6±0.5)
ray	3.1-6.6 (4.5±1.1)	4.0-6.3 (5.1±0.5)
blade	4.6-6.6 (5.3±0.5)	5.3-8.1 (6.6±0.7)
Width of central part	1.5-2.2 (1.9±0.2)	1.7-3.0 (2.3±0.4)
Degree of adoral ciliature	390-405°	380-390°

Table 2. Morphometric comparison of *Trichodina hafizuddini* obtained in the present study with that of Asmat [25]. Measurement in micrometer (µm).

39.7–41.7 (40.3 $\pm$ 0.6), surrounded by a finely striated border membrane 4.9–5.8 (5.2 $\pm$ 0.3). Centre of disc well impregnated, nearly similar to the rest of adhesive disc without any argentophobic granular processes or patches, 12.6–16.3 (14.1 $\pm$ 0.8) in diameter. Denticulate ring diameter 24.7–27.9 (26.6 $\pm$ 0.8), consists of 30–33 (31.3 $\pm$ 0.8) with radial pins 4–6 (5.3 $\pm$ 0.8) per denticle. Span and length of

denticle 12.5–15.3 (14.0  $\pm$ 0.8) and 3.7–5.6 (4.6 $\pm$  0.5) respectively. Length of blade 5.3–8.1 (6.6 $\pm$ 0.7), ray 4.0–6.3 (5.1 $\pm$ 0.5), central part 1.7–3.0 (2.3 $\pm$ 0.4) in width. Inter-blade space small to medium.

**Dencticle morphology:** Blade stout, spoon shaped and slightly curved (Fig. 5). Distal margin of blade conically rounded, almost proximate to border membrane. Tangent point smooth, like small line



Figs 7-10. Diagramatic drawing of the denticles of *Tichodina* species: 7. *Trichodina pseudoheterodentata* from the gills of *Mystus bleekeri* in Bangladesh; 8. from the gills of *Ictalurus punctatus* in China, redrawn from Tang et al. [39]; 9. *Trichodina hafizuddini* from the gills of *Amblypharyngodon mola* in Bangladesh; and 10. from the gills of the *Amblypharyngodon mola* in India, redrawn from Asmat [25].

rather than point and below distal margin. Anterior margin slightly swollen behind slop of distal margin and a shallow concave like depression present above base of apex. Inter-blade space small, having a few number of minute argentophobic glistening particles. Blade connection prominent. Apex extends beyond y+1 axis (Fig. 9). Posterior margin of blade different to mark any shallow semicircular curve remains slightly lower than apex. Posterior projection indistinct. Central part slender, triangular in shaped, directed downwardly, with sharp point of tip, tightly fitting into preceding denticle and extends half way to y axis. Ray shorter than blade, gently slanted in posterior direction, with marked constriction slightly apart to distinct ray apophysis. Post constricted part of ray gradually inflated and ending in bulbous rounded tip. Argentophobic particles present between ray bases. Tip of ray extending almost half way to y-1 axis.

Asmat [25] established *Trichodina hafizuddini* as a new species from the gills of freshwater mud perch *Amblypharyngodon mola* from the Hooghly River of Hooghly in West Bengal, India. In accordance with Asmat [25], *T. hafizuddini* is characterized by moderately spaced, broad, angular, almost sickle-shaped blade, but in many cases appear as spoon – shaped or almost rounded filling most of the inter-blade space. Ray shorter than blade with central groove and sometimes constriction just below ray apophysis. Post constriction part of ray to some extent inflated, ending in rounded tip.

The present paper reports on the occurrence of this species for the first time from the same host A. mola in Bangladesh. We found that the trichodinid ciliate obtained from A. mola shows some significant variability in comparison to the population reported from the River of Hooghly in West Bengal, India when denticle structure is considered. Asmat [25] observed anterior margin of blade slightly curves down and forms shallow apex at the base of blade. In our study, we observed that anterior margin slightly swollen behind slope of distal margin and a shallow concave depression is visible that situates above the base of apex. Another important difference is the central part. Asmat [25] reported that central part of the Trichodina hafizuddini is slender, tubular with bluntly rounded point. Besides present study reveals that central part is triangular in structure with sharp point of tip. These denticle structural variations may be distinguishable as geographical distribution and environmental condition in the locality of the

described trichodinid.

Values for the diameter of body, adhesive disc, deticulate ring and central area are slightly smaller than data given by Asmat [25]. However, width of border membrane, number of denticles, radial pins per denticle, length of denticle, blade and ray length of the present studied specimens fall in the range as reported by Asmat [25] (Table 2).

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