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Four new species of *Trichodina* Ehrenberg, 1830 (Ciliophora: Trichodinidae) from Bangladeshi fishes

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Abstract: During surveys of the trichodinid parasites in freshwater and estuarine fishes in Chittagong Division, Bangladesh four new species of the genus *Trichodina* were investigated and morphologically studied. These are: *Trichodina aplocheilusi* sp. n. from *Aplocheilus panchax*, *Trichodina chittagongensis* sp.n. from *Labeo bata*, *Trichodina kaptaiensis* sp. n. from *Chanda nama* and *Trichodina siddiquae* sp. n. from *Heteropneustes fossilis*. Taxonomic and morphometric data for these trichodinids based on wet silver nitrate impregnated specimens are presented.

Key words: *Trichodina aplocheilusi*, *Trichodina chittagongensis*, *Trichodina kaptaiensis*, *Trichodina siddiquae*, Ciliophora, Trichodinidae, Bangladesh

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 silver impregnation technique. In Bangladesh, very scanty and infrequent information are available on the taxonomy of this particular group of parasites. The existing data on this matter can only be found in Asmat *et al*.[4-9] and Bhouyain *et al*.[10]. During the present survey on the species diversity of the trichodinid ciliates from some fishes of Chittagong Division between January, 2000 and December, 2001, four new species of *Trichodina*, infecting the gills of various fish was found and is described here.

MATERIALS AND METHODS

The host fishes were collected by seine nets and gill nets from the Kaptai Lake in Rangamati Hill District and from the Karnaphuli River at Sadarghat area of Chittagong District between January, 2000 to December 2001. Gill scrapings were made at the collection site. Air-dried gill scrapings were transported to the laboratory. The slides with trichodinid ciliates were impregnated with Klein's silver impregnation technique[11]. Examinations of prepared slides were made under research microscope at 10 x 100 magnification. Measurements were done following the recommendations of Lom[12], Wellborn[13], Arthur and Lom[14] and Van As and Basson[15-16]. For comprehensive morphological details of the ciliates photomicrographs were made. 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}). Measurements are given in µm. The results and photographs were compared to results from other studies.

RESULTS AND DISCUSSION

***Trichodina aplocheilusi* sp. n. (Fig. 1-2, 9, Table 1):** Host. *Aplocheilus panchax*[17]. Locality. Kaptai Lake, Rangamati Hill District. Location. Gills. Prevalence. $^{07}/_{35}$ (20.0%); July-September, 2001. Infection. Low.

Description (n=20): Large sized trichodinid, 58.6-70.7 (64.2±3.1) in diameter. Adhesive disc concave, 50.5-60.6 (54.8±2.7) in diameter (Table 1), surrounded by a finely striated border membrane, 4.0-5.0 (4.6±0.5) wide. Centre of adhesive disc large, 14.1-21.2 (18.8±1.9) in diameter, slightly light stained when treated with silver. Diameter of denticulate ring 28.3-34.3 (31.9±1.5). Denticulate ring consists of 26-30 (27.8±1.4) denticles with 5-7 (6.2±0.5) radial pins per denticle. Adoral ciliary spiral turns about 390-400°.

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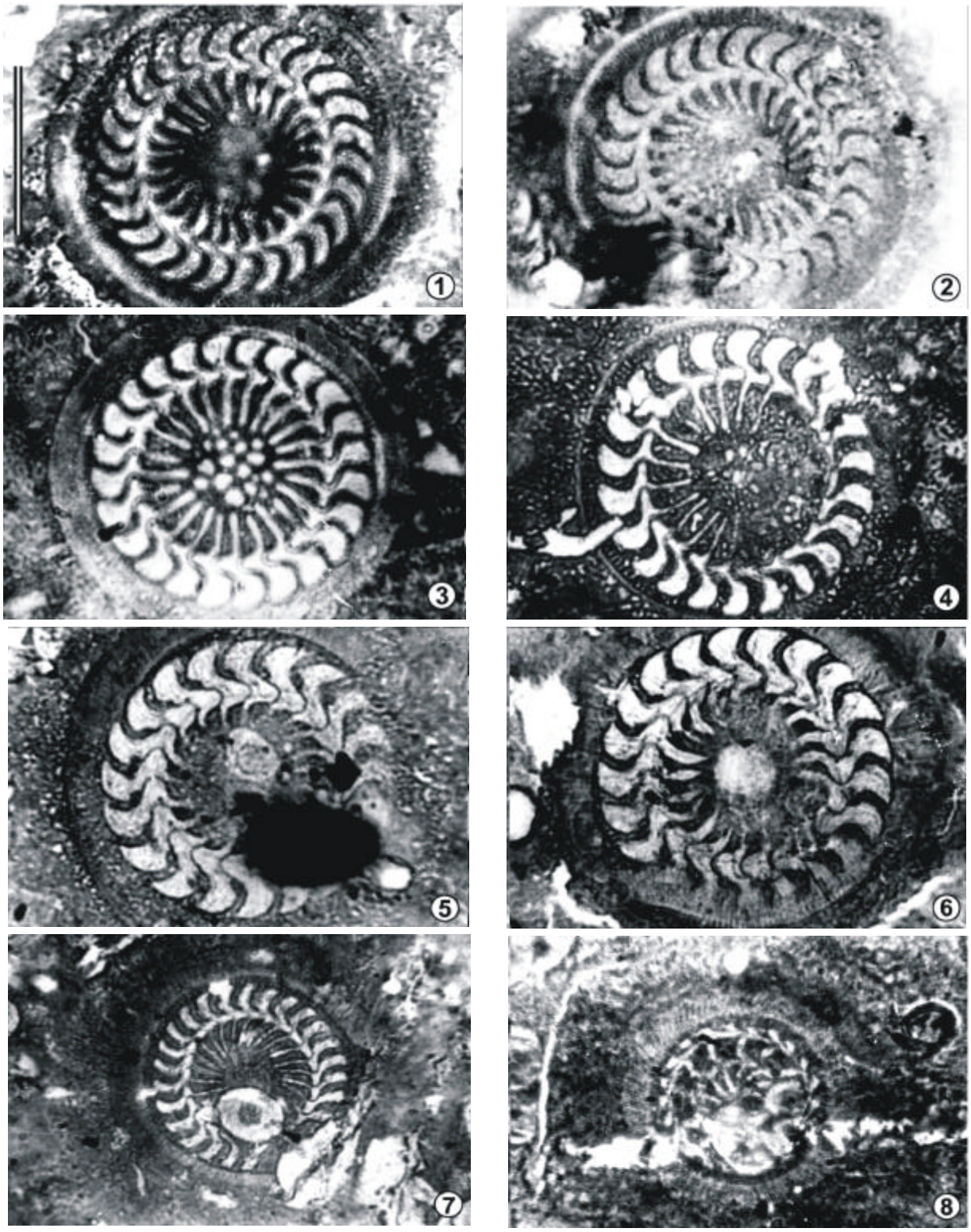

Fig 1-8: Silver impregnated adhesive discs of trichodinids: 1-2 of *Trichodina aplocheilusi* sp. n., 3-4 of *Trichodina chittagongensis* sp. n., 5-6 of *Trichodina kaptaiensis* sp. n., and 7-8 of *Trichodina siddiquae* sp. n. (8 of developing individual). Scale bar 30 µm.

Table 1: Morphometric data of various *Trichodina* sp. obtained from the gills of Bangladeshi fish

Species	*Trichodina aplocheilusi* sp. n. (n=20)	*Trichodina chittagongensis* sp. n.(n=20)	*Trichondina kaptaiensis* sp. n. (n=10)	*Trichodina siddiquae* sp. n. (n=11)
Host	*Aplocheilus Panchax*	*Labeo bata*	*Chanda nama*	*Heteropneustes fossilis*
Locality	Kaptai Lake	Karnaphuli River	Kaptai Lake	Kaptai Lake
Diameter of body	58.6-70.7 (64.2±3.1)	36.7-45.9 (40.6±2.7)	45.4-49.6 (47.5±2.2)	40.7-58.1 (50.1±5.3)
Diameter of adhesive disc	50.5-60.6 (54.8±2.7)	29.6-37.7 (33.0±2.5)	35.2-42.8 (37.6±2.3)	30.7-47.9 (40.1±4.7)
Diameter of denticulate ring	28.3-34.3 (31.9±1.5)	19.4-24.5 (21.7±1.3)	21.4-28.6 (24.0±2.2)	19.6-28.6 (25.5±3.7)
Diameter of central area	14.1-21.2 (18.8±1.9)	6.6-12.2 (9.2±1.3)	8.2-15.3(10.1±2.5)	9.2-13.7 (10.4±1.3)
Diameter of clear area	-	-	5.1-10.2 (7.1±1.8)	-
Width of border membrane	4.0-5.0 (4.6±0.5)	2.5-4.1 (3.8±0.5)	4.6-5.1 (4.9 ± 0.2)	4.1-6.0 (5.0±0.8)
Number of denticles	26-30 (27.8±1.4)	22-24 (22.9±0.8)	20-22 (21.0 ± 0.8)	24-29 (26.5±1.4)
Diameter of radial pins/denticle	5-7 (6.2±0.5)	5-8 (6.6±0.8)	8-10 (8.9 ± 0.9)	9-11 (9.8±0.7)
Span of denticle	13.1-19.2 (15.4±1.3)	9.7-13.3 (11.2±1.0)	11.2-13.2 (12.5±0.6)	11.2-15.8 (13.9±1.8)
Length of denticle	7.1-9.1 (7.5±1.6)	5.1-6.1 (5.2±0.2)	4.1-6.1 (4.9±0.7)	5.6-7.7 (6.6±0.7)
Length of ray	4.0-6.1 (5.5±0.7)	3.1-5.2 (4.0±0.5)	4.6-5.6 (5.3±0.3)	4.5-8.2 (6.6±1.4)
Diameter of blade	5.0-7.1 (5.8±1.1)	4.1-6.1 (5.1±0.7)	3.6-4.6 (4.3±0.3)	4.1-6.5 (4.9±0.8)
Width of central part	1.0-3.0 (1.6±0.5)	2.0-2.5 (2.1±0.2)	2.5-3.6 (3.1±0.4)	1.0-3.1 (2.1±0.6)
°Adoral ciliature	390-400	390-400	-	400

Shape of blade slim, sickle-shaped with angularly flattened distal margin. Tangent point sharp, slightly lower than distal margin. Anterior margin angularly curves down, crosses y+1 axis, forms distinct apex near base of blade, adjoins central part of preceding denticle. Anterior blade apophysis not clearly visible. Apical depression well developed but never impregnates. Posterior margin slightly curves down forming sickle-shaped shallow crescent with deepest point at same level as apex (Fig. 9). Posterior blade apophysis absent. Blade connection well developed. Interblade space moderate. Central part strong, widely triangular with bluntly rounded point, often extends halfway past to y-1 axis, fitting firmly with preceding denticle. Indentation in lower central part not visible. Section above and below x axis similar. Ray connection short but broader than blade connection. Ray apophysis not visible. Ray straight, gradually pointed towards end. In some cases, central groove distinct. Ray slanted posteriorly, so that tip touches or often crosses y-1 axis.

Trichodina aplocheilusi sp. n. may be characterized by having slim, sickle-shaped blade with angularly flattened blade; moderate interblade space; widely triangular central part with bluntly rounded point; posteriorly slanted and gradually pointed ray; and light stained, large central area. Based on the overall shape of denticle, especially the general shape of blade and posteriorly slanted ray, only *Trichodina dampanula*[18] is similar to *T. aplocheilusi*.

Trichodina dampanula is an endoparasite infecting the urinary bladder and ureters of an amphibian, *Bufo guttaralis*. The species was described by Bank *et al*.[18] from South Africa. However, the two species differ in several other respects. For example, in *T. dampanula*: i) the blade is more angular and of almost equal breadth throughout with truncated distal margin (*vs* sickle-shaped blade with angularly flattened blade), ii) the anterior blade apophysis is distinct (*vs* absent in the present species), iii) the blade connection is weakly developed (*vs* well developed), iv) the central part is slender and tubular (*vs* strong and conical in shape), v) although slightly narrow at the point of attachment to the central part, rest of the ray is of equal width ending with rounded point (*vs* broad with distinct central groove and gradually ends in a fine point), vi) the centre of the adhesive disc in silver stained specimen is similar to the rest of the adhesive disc (*vs* lightly stained); and vii) *T. dampanula* is isolated from the urinary bladder and ureters of *Bufo guttaralis* as an endoparasitic ciliate (vs the presently described species was collected from the gills of a freshwater fish, *Aplocheilus panchax*). Bank *et al*.[18] commented that the urinary bladder of toads and frogs requires a unique and highly specific adaptation for parasites to survive. Their laboratory observations also showed that *T. dampanula* can survive for only a few minutes in pond water because they rapidly absorb water, resulting in the cells bursting. Thus, *T. aplocheilusi* can easily be distinguished from all other trichodinid ciliates obtained from the gills of fish as well as endoparasites of fish and other animals.

Type Host	*Aplocheilus panchax*[17] (Cyprinodontiformes: Aplocheilidae)
Type locality	Kaptai Lake (22°29'N 92°17'E), Hill District of Rangamati
Type location	Gills
Type specimens	Holotype, slide AP 1 (09/07/2001); paratypes, slide AP 2 (09/07/2001) are in the collection of the Department of Zoology, University of Chittagong, Chittagong 4331, Bangladesh
Etymology	Named after the generic name of the type host

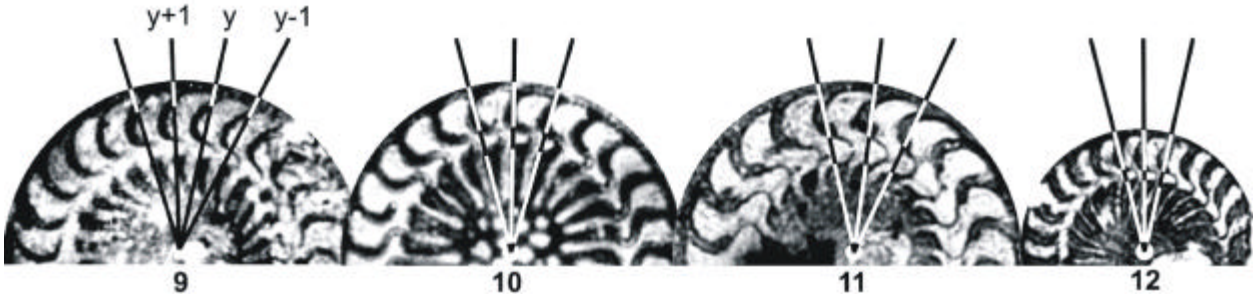

Fig. 9-12: Denticles of trichodinids in relation to y axis: 9 of *Trichodina aplocheilusi* sp. n., 10 of *Trichodina chittagongensis* sp. n., 11 of *Trichodina kaptaiensis* sp. n., and 12 of *Trichodina siddiquae* sp. n.

***Trichodina chittagongensis* sp. n. (Fig. 3-4, 10, Table 1):** Host. *Labeo bata*[17]. Locality. Karnaphuli River, Chittagong. Location. Gills. Prevalence. $^{8}/_{60}$ (13.3%). Infection. Low to medium.

Description (n=20): Medium-sized trichodinid, 36.7-45.9 (40.6±2.7) in diameter. Centre of adhesive disc contains one to many (upto 26) white irregularly rounded non-impregnable particles (Fig.3-4). Particles loosely arranged, slightly elevated from adhesive disc and clustered round central area. Denticulate ring, 19.4-24.5 (21.7±1.3) in diameter (Table 1), consists of 22-24 (22.9±0.8) denticles with 5-8 (6.5±0.8) radial pins to one denticle. Adoral ciliary spiral turns about 390-400°.

Blade broad and slightly falcate, filling most of space between y +1 axis (Fig. 10). Distal margin truncated or flat and parallel to border membrane when situated close to it. Tangent point blunt and situated slightly lower than distal margin. Anterior margin curves down smoothly and sometimes touches y+1 axis, forming a shallow apex. Apical region never impregnates. Anterior and posterior blade apophysis absent. Posterior margin forms elongated, shallow, semilunar curve with deepest point lying lower than apex. Blade connection long and narrow and as thick as ray. Central part rather slender, with bluntly pointed end that never touches y-1 axis but fits tightly with preceding denticle. Section above and below x-axis similar. No indentation in lower central part. Ray straight or slightly curved and slender, of same thickness throughout, but thicker at end with rounded tip. Ray runs parallel to y-axes. Axis passes along posterior margin of ray. Ray apophysis present, but not distinctly visible.

The centre of the adhesive disc of *Trichodina chittagongensis* sp. n. has one to many non-impregnable, irregularly-shaped and loosely arranged particles and can be compared to *Trichodina tenuidens*[19], *T. reticulata*[20] and *T. puytoraci*[21].

Trichodina tenuidens has been reported mainly from the gills and fins of the marine fish. Only the centre of the adhesive disc containing some argentophobic granules resembles the present trichodinid, but the size and shape of these clear structures and that of the denticle and also the morphological data are different in the two species. Lom and Stein[22] gave a detailed account of *T. tenuidens* and presented many photomicrographs to show its highly variable central area of the adhesive disc which resemble *T. reticulata, T. partidisci, T. domerguei* or *T. puytoraci.* Not only the central area, but the shape of the blade and ray also vary in different specimens. Lom and Stein[22] commented that the differences suggest the possibility that they might be the product of an extremely broad variation of *T. tenuidens.* Unlike *T. tenuidens*, the denticle of *T. chittagongensis* possesses a broad and falcate blade with a flat distal margin. Furthermore, the ray in *T. chittagongensis* is straight and of the same thickness with a thickened tip.

T. reticulata has been described from different hosts from various localities, mostly cyprinids and in the majority of cases from the genus *Carassius*[23]. The centre of the adhesive disc of *T. reticulata* always bears a compact mass of comparatively large sized circular cell-like structure. In case of *T. chittagongensis* these structures are mostly irregular-shaped, loosely arranged and varying in number from one to 26. Basson and Van As[24] redescribed *T. reticulata* by the method proposed by Van As and Basson[15]. A longitudinal ridge extending from the ray, across the central part to the blade is a distinct feature of the denticle in *T. reticulata*. This ridge is absent in the trichodinid described in this paper. In addition, the shape of blade is truncated and angular in *T. reticulata*, but falcate in *T. chittagongensis.* The morphological data are also different in the two species.
Lom[21] established *T. puytoraci* from the gills of *Mugil auratus*, *M. saliens* and *M. cephalus* of the Rumanian Black Sea coast. *T. chittagongensis* differs from *T. puytoraci* in several aspects, such as in *T. puytoraci*: I) the distal margin of the blade (*vs* truncated and lying in close contact with the border membrane), ii) the tip of ray is widened or swollen (*vs* thicker to a lesser extent), iii) the ray apophysis is distinct

(*vs* rarely distinguishable), iv) the clear structures in the centre of the adhesive disc resemble grains of coffee or wheat, i.e., rounded or oval with a prominent black indentation mark (*vs* irregular-shaped, smooth and smaller in size); and v) the zone of adoral cilia spirals about 370-380° (*vs* 390-400°). Compared with the Lom's[20] original description of the species from *Mugil cephalus*, the measurements indicate the relatively small dimensions of *T. chittagongensis*.

Type Host	*Labeo bata*[17](Cypriniformes: Cyprinidae)
Type locality	Karnaphuli River at Sadarghat (22°18´N 91°53´E) in Chittagong District, Bangladesh
Type location	Gills
Type specimens	Holotype, slide LB 1 (10/08/2000); paratypes LB 2 (10/08/2000) are in the collection of the Department of Zoology, University of Chittagong, Chittagong 4331, Bangladesh
Etymology	Named after the district of the type locality of the type host.

***Trichodina kaptaiensis* sp. n. (Fig. 5-6, 11; Table 1):** Host. *Chanda nama*[17]. Locality. Kaptai Lake, Rangamati Hill District. Location. Gills. Prevalence. $^{7}/_{250}$ (2.8%). Infection. Low.

Description (n=10): Flattened, disc-shaped, medium-sized trichodinid, 45.4-49.6 (47.5±2.2) in diameter. Adhesive disc concave, 35.2-42.8 (37.6±2.3) in diameter (Table 1), and surrounded by a finely striated border membrane, 4.6-5.1 (4.9±0.2) wide. Central area of adhesive disc bears a clear, sometimes diffused, area which almost devoid of any argentophilic particles and surrounded by a circular perimeter, 8.2-15.3 (10.1±2.5). Denticulate ring consists of 20-22 (21.0±0.8) denticle with 8-10 (8.9±0.9) radial pins per denticle. Adoral ciliary spiral could not be detected.

Blade of denticle broad, filling most of space between y and y+1 axes (Fig. 11). Distal margin of blade flat, running parallel to border membrane. Tangent point slightly lower than distal margin. Anterior margin forms angle with y axes. Posterior margin indentation forms small semilunar arch with y axes. Deepest point of semilunar arch lies on same level as apex. Apex conical, rarely extends beyond y axes. Apical depression well developed, never impregnates with silver. Anterior apophysis of blade prominent, so a notch appears in between apex and anterior apophysis. Blade connection broad, not clearly distinguishable from blade. Posterior blade apophysis not clearly visible. Central part robust, broadly triangular (Fig. 5-6) with rounded tip. Tip extends more than halfway past, sometimes touches y-1 axis. Section of central part above x axis sloping at steep angle. Indentation in lower central part absent. Connection between ray and central part thin. Ray slightly curved in posterior direction with broad mid-region and sharp pointed tip. Tip not touches perimeter of central clear area. Ray apophysis not prominent, but central groove clearly visible.

Like other trichodinids, *Trichodina kaptaiensis* sp. n. is also characterized by its silver impregnated adhesive disc morphology. It shows some resemblance to the denticle shape of *Trichodina acuta*[25] from the skin and gills of different host fishes in Bohemia, and of *T. compacta*[15] from the skin and fins of different host fishes in South Africa and Israel.

T. acuta was originally described by Lom[25] from five species of freshwater fishes and from the skin of tadpoles belonging to several species of frogs in Republic Czech. Since then, it was reported to have a wide geographical distribution. Like *T. acuta*, the centrally located clear area of *T. kaptaiensis* also relatively smaller, the space between the circle and the tips of the rays is distinct and it lacks any darker spots, but the shape of the denticle in the two species is significantly different. In *T. acuta*: i) the distal margin of the blade slopes in an anterior direction towards the apex (*vs* the distal margin has a flat section, closely associated with and running parallel to the border membrane); ii) the blade is narrow and the apex does not extend beyond the y+1 axis (*vs* in some cases the apex extend beyond the y+1 axis); iii) the distinction between the central part and the blade are more pronounced (*vs* less pronounced); and iv) the posterior projection of the blade is prominent and distinct spaces can be observed between them (*vs* not prominent).

Trichodina compacta was described by Van As and Basson[15] from the skin and fins of some fishes in South Africa. Later, Van As and Basson[16] reported *T. compacta* from other parts of the Zambesi River system. The overall dimensions and the shape of denticles of *T. kaptaiemsis* correspond closely with the description of *T. compacta*. Both the species have similar blade shape, denticle fitting, but can easily be distinguished from *T. compacta* by the morphology of the ray, the central part and the central clear area. In *T. compacta*: i) the ray is thick (*vs* slender); ii) the central part is not clearly distinguishable from the blade or the ray (*vs* inseparable from the blade, the ray is quite distinct and the ray connection in thin); iii) the point

of ray is blunt, rounded and almost touching the central clear circle (*vs* the ray ends in a sharp point and always remain far away from the central clear area); iv) the clear area contains dark spots (*vs* absence of dark spots); and v) the margin of blade is not closely associated with the border membrane (*vs* closely associated).

Type Host	*Chanda nama*[17] (Perciformes: Ambassidae)
Type locality	Kaptai Lake (22°29'N 92°17'E), Rangamati Hill District, Bangladesh
Type location	Gills
Type specimens	Holotype, slide CN 1 (11/01/2001); paratypes, slide CN 2 (09/02/2001) and CN 3 (11/01/2001) are in the collection of the Department of Zoology, University of Chittagong, Chittagong 4331, Bangladesh
Etymology	Named after the type locality of the type host.

***Trichodina siddiquae* sp. n. (Fig. 7-8, 12; Table 1):** Host. *Heteropneustes fossilis*[26]. Locality. Kaptai Lake, Rangamati Hill District. Location. Gills. prevalence. $^{6}/_{60}$ (10.0%); March-April, 2001. Infection. Low.

Description (n=11): Medium-sized trichodinid with saucer- to disc-shaped body, 40.7-58.1 (50.1±5.3) in diameter (Table 1). Adhesive disc concave, 30.7-47.9 (40.1±4.7) in diameter, surrounded by finely striated border membrane, 4.1-6.0 (5.0±0.8) wide. Adhesive disc stains uniformly in silver impregnated specimens. Denticle ring, 19.6-28.6 (25.5±3.7) in diameter, consist of 24-29 (26.5±1.4) denticles with 9-11 (9.8±0.7) radial pins per denticle. Adoral zone of cilia turns about 400°.

Blade broad and elongated filling major part of y axes (Fig. 12). Distal margin slightly curved or truncated, parallel to border membrane when truncated. Tangent point blunt to y axis, sometimes pointed, and on same level as distal margin of blade. Anterior margin obliquely curves down. Apex of anterior margin sometimes impregnates and rarely extends beyond y+1 line. Posterior margin forms extended curve. Deepest point of curve formed by posterior margin situated at same level as apex. Anterior apophysis of blade sometimes prominent. Section connecting blade and central part moderate. Posterior blade apophysis not visible. Central part comparatively wide, tubular (Fig. 7-8), extending halfway past, sometimes touches, y axis. Shape of central part above x axis sloped while lower part straight. Tip of central part rounded, fitting tightly into preceding denticle. Small indentation on proximal side of central part corresponding to anterior apophysis of ray present. Axis passes through base of central part. Ray thin, tapers slightly to sharp, rounded point. Central groove not present. Ray directed in anterior direction, so that tip extends past y+1 axis.

Trichodina siddiquae sp. n. may be charaterised by having a dark centred adhesive disc with denticle consist of slender, rectangular blade; truncated distal margin; well-developed apex; comparatively wide central part with more sloped upper part and rounded tip which extends the y axis and touches the y+1 axis; the y axis passes through the base of the central part; thin ray of same thickness throughout with bluntly rounded tip; and the tip of ray touches the y-1 axis. Based on these characters *T. siddiquae* show some resemblance to *Trichodina uniforma*[15].

T. uniforma was described by Van As and Basson[15] from the skin, fins and gills of *Carassius auratus* in the Komati River system of South Africa. The species has so far only been found on the goldfish and appears to be restricted to one locality. The general appearance of *T. siddiquae* is quite similar to *Trichodina uniforma*. But in close observation the two species differ in many aspects, viz., in *T. uniforma*, I) the distal margin of the blade is truncated (*vs* slightly curved and not situated parallel to the border membrane), ii) the tangent point to y axis is on the same level as the distal margin of the blade (*vs* flat and lower than the distal margin), iii) the apex is lower than the deepest point of the semilunar

Type Host	*Heteropneustes fossilis*[26] (Siluriformes: Heteropneustidae)
Type locality	Kaptai Lake (22°29'N 92°17'E), Rangamati Hill District, Bangladesh
Type location	Gills
Type specimens	Holotype, slide HF 1 (09/07/2001); paratypes, slide HF 2 (09/07/2001) are in the collection of the Department of Zoology, University of Chittagong, Chittagong 4331, Bangladesh
Etymology	Named after the pioneer researcher on the trichodinid ciliates in Bangladesh, Papia Salina Siddiqua

curve (*vs* at the same level as the deepest point), iv) the blade connection is thick (*vs* thin), v) the anterior blade apophysis is absent (*vs* present); and vi) the body dimension is large (*vs* medium in size).

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