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Record of three new *Trichodina* species (Protozoa, Ciliophora) parasitic on gills of freshwater fishes from Chongqing, China

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The morphology of three ectoparasitic ciliates, *Trichodina paraheterodentata* sp. nov., *Trichodina pseudominuta* sp. nov. and *Trichodina jialingensis* sp. nov., parasitising the freshwater fishes from Chongqing, China, were investigated following dry silver nitrate and the methyl green-pyronin stain. *T. paraheterodentata* was found on the skin of *Siniperca chuatsi*, and is distinguished mainly by the acute triangle-like blade, the well-developed central part, a little curved and robust ray. *T. pseudominuta* was isolated from the gills of *Carassius auratus*, and is characterized by the slanting parallelogram, relatively developed central part, long and slim ray arranged in helical form with the tip of ray extending past the Y+1 axis in the whole view of the parasite. *T. jialingensis* was collected from the gills of *Ctenopharyngodon idellus*, and is displaying irregular rectangular blade, undeveloped central part and the straight ray. Morphometric data and comparative descriptions of these trichodinids are provided along with details of their prevalence.

Key words: *Trichodina*, new species, freshwater fish, *Siniperca chuatsi*, *Carassius auratus*, *Ctenopharyngodon idellus*, China.

INTRODUCTION

Trichodinid ciliates are well-known ectoparasites of maricultured and freshwater animals, typically fishes, molluscs, as well as amphibians. To date, about 300 nominal trichodind species have been reported from different environments in the world (Lom, 1970, 1973; Lom and Laird, 1969; Lom and Dyková, 1992; Van As and Basson, 1989; Song et al., 2003; Zhao and Tang, 2007; Tang and Zhao, 2011, 2012). In China, the trichodinid ciliates of freshwater fishes have received considerable attention in recent years (Liu and Zhao, 2010; Tang and Zhao, 2011, 2012; Yu et al., 2011; Zhao and Tang, 2007, 2011).

In order to go on with the further studies on trichodinids of freshwater fishes in China, we surveyed the diversity of fishes. Three important freshwater fishes, *Siniperca chuatsi*, *Ctenopharyngodon idellus* and *Carassius auratus* were the targets of our investigation for the trichodinid ectoparasites in the present work. And, three new *Trichodina* species were found from these hosts and described below.

MATERIALS AND METHODS

Specimens of freshwater fishes were collected from Chongqing, China between 2007 and 2009. The two host fishes, *Siniperca chuatsi* (approximately 1 yr old and ranging from 15 - 25 cm in total length) and *Ctenopharyngodon idellus* (approximately 1 yr old and ranging from 18 - 35 cm in total length) were respectively collected

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in Shapingba district and Hechuan district along Jiajing River, respectively. Another host fish, *Carassius auratus* (approximately 0.5 to 1 yr old and ranging from 5 - 15 cm in total length) were obtained from the culture pools in Dazu County. Wet smears of gills were made fresh and screened for the presence of trichodinids under the Nikon E600 phase-contrast microscope. The nuclear apparatuses of the trichodinids were visualized using the methyl green-pyronin stain (Foissner, 1991). Smears positive for trichodinid were taken back to the laboratory, air-dried, and further stained with Klein's dry method (Klein, 1958) to reveal the structure of the adhesive disc. Prepared slides were examined under the Nikon E600 phase-contrast microscope. Photographs were taken using the Nikon-DXM1200 camera at 1000x magnification. Illustrations were drawn with computer software CorelDRAW 11.0.

The position of the micronucleus was given relative to the macronucleus, according to the format described by Lom (1958). In this system, the micronucleus is situated near the terminations of the arms of the macronucleus: (i) externally, near the right termination (+Y); (ii) externally, between the two terminations (-Y); (iii) internally, near the right termination ($-Y^1$).

All measurements were presented in micrometers (µm) following the uniform specific characteristic system proposed by Lom (1958). Minimum and maximum values are given, followed in parentheses by the arithmetic mean and the standard deviation. In the case of the denticles and radial pins, the mode is given rather than the arithmetic mean with the number of specimens examined given in parentheses. The description of denticle elements follows the format recommended by Van As and Basson (1989).

RESULTS

Order Peritrichida Stein, 1879; Family Trichodinidae Claus, 1874; Genus *Trichodina* Ehrenberg, 1838

Trichodina paraheterodentata sp. nov.

Host and location: *Siniperca chuatsi*; Skin Prevalence: Out of 28 fishes examined, five were infected (18 %); Type locality: Shapingba, Chongqing (29°5' N, 106°5' E), China; Date of sampling: August, 2008. Type-specimens: One holotype (No. CQB-0808-01) and one paratype slide (No. CQB-0808-02) were deposited in the Collection Center of Chongqing Key Laboratory of Animal Biology, Chongqing Normal University. Etymology: The name "*paraheterodentata*" recalls the similarity of this organism to *Trichodina heterodentata* Duncan, 1977. The name "*paraheterodentata*" is a combination of "*para-*" (similar) and "*heterodentata"* (the specific name of *Trichodina heterodentata*).

Morphological description (n = 28) (Figures 1 A - B; 2 A)

Large freshwater *Trichodina* body is 45.0 - 64.0 µm (mean 56.9 ± 6.6) in diameter. The adhesive disc is 37.0 - 55.0 µm (mean 48.3 ± 5.8) in diameter with clear central zone. The border membrane is 3.0 - 6.0 µm (mean 4.4 ± 0.8) wide. The diameter of the denticular ring is 22.0 - 34.0 µm (mean 29.2 ± 4.1). There are about 20 - 22 (n = 28) denticles. The number of radial pins (mode) per denticle is 10 - 12 (n = 28). The denticle spans 12.0 - 19.0 µm (mean 16.1 ± 2.4) and the length of the denticle is 5.5 - 12.0 µm (mean 8.9 ± 1.8). The acute, triangle-like blades are broad and are 4.5 - 7.0 µm (mean 5.5 ± 0.7) in length. The distal blade surface is slightly curved and smooth, but is not parallel to the border membrane. The tangent point is round and is nearly at same level as distal blade surface. The anterior blade surface is straight and smooth and is always extending past the Y+1 axis, but is not parallel to the posterior surface. Apophysis of blade is present and its posterior projection is conspicuous in some specimens. The section connecting the blade and the central part is well developed. The rounded point in the central part of the denticle fits tightly into preceding denticle, extending about half way to the Y-1 axis. The shape of the central part above the X-axis is similar to the part below. The width of the central part is 2.5 - 5.0 µm (mean 3.4 ± 0.7). The ray connection is inconspicuous and is barely distinguishable from ray. The ray is 6.0 - 9.0 µm (mean 8.3 ± 0.9) long and is well developed, tapering gradually to the sharp point and is directed backwards. Ray apophysis is not very prominent in some denticles. The ratio between the denticle above and below the X-axis is less than one. The adoral ciliary spiral turns about 400°- 420° around the peristomial disc. The macronucleus is C-shaped and the micronucleus is oviform and is situated in +Y position.

Remarks

Trichodina Ehrenberg, 1830 is one of the most species and widely distributed of ciliate genera. To date about 300 *Trichodina* species have been described from a range of different hosts, especially maricultured and freshwater animals (Song et al., 2003). Most *Trichodina* species can be divided into five groups based on the denticle blade morphology that is triangular-trichodinid, arc-shaped-trichodinid, round-trichodinid, quadrangular-trichodinid, and club-shaped-trichodinid. The common sickle-shaped and fan-shaped belong to the arc-shaped-trichodinid group. A few remaining species exhibit different blade shapes and cannot be allocated to any of these groups (Zhao and Tang, 2011). *Trichodina paraheterodentata* is one of over 30 species that have a sickle-shaped blade. Among these, *T. paraheterodentata* most closely resembles *Trichodina diaptorni (*Basson and Van As, 1991*), Trichodina kalimbezea (*Van As and Basson, 1992) and *Trichodina magna* (Van As and Bason, 1989) based on blade shape alone. However, the new species can be separated from the latter three by its body diameter, which is 45.0 - 64.0 µm, vs. 26.5 - 36.5 µm for *T. kalimbezea,* 71.2-111.8 µm for *T. magna* and 33.8 - 48.7 µm for *T. diaptorni* (Basson and Van As, 1991; Van As and Basson, 1989; Van As and Basson, 1992). Although *T. paraheterodentata* and *T. diaptorni* overlap in body size, the former has more denticles (20 - 22 vs.15 - 20) than the latter (Basson and Van As, 1991).

Two other species with sickle-shaped blades, namely

Trichodina compacta (Basson and Van As, 1989) and *Trichodina acuta* (Lom, 1961), also have body sizes that overlap with *T. paraheterodentata.* Both these species, however, can be separated from *T. paraheterodentata* by the extent to which the rows of oral cilia encircle the peristomial disc (380° - 390° in *T. compacta* and *T. acuta* vs. 400° - 420° in *T. paraheterodentata*) and by the presence (vs. absence) of the circle in the center of the adhesive disc (Basson and Van As, 1989; Lom, 1961). Moreover, the point of the ray is blunt, rounded and almost reaches the central circle in *T. compacta*, whereas the point of the ray tapers gradually to a sharp point and is directed backward in *T. paraheterodentata*.

In terms of its general morphology, the appearance of the adhesive disc, the host, and the site of infestation, *T. paraheterodentata* most closely resembles *T. heterodentata.* So far, there are so many studies on the morphology of *T. heterodentata*. However, a considerable variation exist in those different populations of *T. heterodentata*, and different scholars gave some morphological comparative research to a certain extent (Duncan, 1977; Van As and Basson, 1989; Van As and Basson, 1992; Kruger et al., 1993; Basson & Van As, 1994; Al-Rasheid et al., 2000; Asmat, 2004; Dove and O'Donoghue, 2005; Dias et al., 2009; Martins et al., 2010; Miranda et al., 2012). Those previous reports nearly regarded *T. heterodentata* as a widely distributed trichodinid with its unique morphological characteristic. However, when compared with original populations of *T. heterodentata* described by Duncan (1977), the present new species can be separated by the following combination of characters with the latter: (1) the body diameter of *T. paraheterodentata* is distinctly smaller than any of the three known populations of *T. heterodentata* (45.0 - 64.0 µm in *T. paraheterodentata vs.* 71 - 106 µm in *T. heterodentata* population A, 58 - 108 µm in *T. heterodentata* population B and 70 - 122 µm in *T. heterodentata* population C); (2) *T. paraheterodentata* has a C-shaped (vs. U-shaped) macronucleus; (3) *T. paraheterodenta* has a more robust denticle including a broad blade with round tangent point (*vs*. narrow blade with sharp tangent point); (4) the ray in *T. paraheterodentata* is broad and the tip of the ray is usually directed backward and the three known populations of *T. heterodentata* are featured by narrow ray with the tip of the ray directed forward or parallel with Y axis (Figure 2A - B). It is noteworthy that the population of *T. heterodentata* isolated from *O. mossambicus* in Taiwan is very similar to *T. paraheterodentata* and its identification as *T. heterodentata* may be questionable (Van As and Basson, 1989). Furthermore, it should be noted that there is considerable variations between different populations of *T. heterodentata*, depending on their host and location (Duncan, 1977).

In addition, according to the analysis of 18S rDNA sequence, the molecular phylogenetic research indicated that *T. paraheterodentata* was only 95% similarity to *T. heterodentata*, and first clustered with *Trichodina nobilis* Chen, 1963, rather than with *T. heterodentata* in the phylogenetic tree (Tang et al., 2013), which has further supported the morphological difference between the new species with *T. heterodentata*.

Based on the discussion outlined above, it is concluded that *T. paraheterodentata* is sufficiently distinct for its recognition as a valid species.

Trichodina pseudominuta sp. nov.

Host and location: *Carassius auratus*, gills; Prevalence: Out of 22 *Carassius auratus* examined, four was infected (18.2 %); Type locality: Dazu county, Chongqing, China. Date of sampling: October, 2007. Type-specimens: One holotype (No. CQDL-07-1001) and one paratype slide (No. CQDL-07-1002) were deposited in the Collection Center of Chongqing Key Laboratory of Animal Biology, Chongqing Normal University. Etymology: The name "*pseudominuta*" recalls the similarity of this organism to *Trichodina minuta* Basson (Van As and Paperna, 1983). The name "*pseudominuta*" is a combination of "*pseudo-*" (similar) and "*minuta"* (the specific name of *Trichodina minuta*).

Morphological description (n = 26) (Figures 1 C - D, 2C)

Trichodina pseudominuta is a small to medium sized freshwater *Trichodina*. Its body diameter is 41.0 - 51.5 µm (44.6 ± 3.9). The adhesive disc is 32.5 - 41.5 µm (35.9 ± 3.6) in diameter, and is surrounded by a finely striated border membrane that is 3.5 - 5.5 µm (4.5 ± 0.6) wide, The adhesive disc has a clear central zone in adult cells. The diameter of the denticulated ring is 18.0 - 23.0 µm (20.3 ± 1.9) and the number of denticles is about 20 - 23. The number of radial pins per denticle is 7 - 8. The denticle spans 10.5- 16.5 µm (112.9 ± 1.8) and the length of the denticle is 5.0 - 7.5 µm (6.0 ± 0.8). The blade is 4.5 - 7.5 µm (5.8 ± 0.7) in length. The distal blade surface is smooth and round, parallel to border membrane, and is higher than the bluntly round tangent point. The anterior and posterior surfaces are smooth and are parallel to each other. The anterior surface extends past the Y+1 axis and the posterior surface forms an arc-shape with deep point. Apophysis of blade and posterior projection is absent. The central part is relatively developed with rounded point fitting into the preceding denticle, but not extending about half way to the Y-1 axis. The shape of the central part above and below the X-axis is nearly the same. The width of central part is 2.0 - 4.0 µm (2.8 ± 0.5). The ray connection is inconspicuous and barely distinguishable from the ray. The ray is relatively thin and is obliquely attached and slanted a little forward with a sharp point. The length of the ray is 4.0 - 6.5 µm (5.0 ± 0.7) and the ray apophysis is not obvious or absent in some specimens. Adoral ciliary spiral turns about 380º-400º around the peristomial disc. The macronucleus is C-

shaped, while the micronucleus is ellipse shaped and is situated in +Y position.

Remarks

Considering the overall morphology of the adhesive disc, *Trichodina pseudominuta* sp. nov. is similar to two known species, *Trichodina minuta* (Basson et al., 1983) and *Trichodina kazubski* (Van As and Basson, 1989).

When compared with *T. minuta*, the blade morphology of *T. minuta* shows some resemblance to some extent. As a result, the name of new species is mainly derived from this aspect. Nevertheless, the obvious difference existing between these two species can be observed from the following comparisons. The new species possesses a larger body-size (41.0 - 51.5 µm vs. 28.2 - 38.0 µm) but a smaller adhesive disc (18.0 - 23.0 µm vs. 22.4 - 33.7 µm). Furthermore, the most significant difference between these two species can be observed from the morphology of the denticle: 1) The morphology of blade in the two trichodinid species is different. The blade of *T. pseudominuta* presents a slanting parallelogram as a result of the anterior and posterior surfaces being parallel to each other. And the distal blade surface is very smooth and a bit curved. In contrast, the quadrangular blade is truncated with a very straight distal blade surface in *T. minuta*. Furthermore, the apophysis of the blade in *T. minuta* is obvious, whereas this structure in *T. pseudominuta* is absent. 2) Although the rays in both species are slender, the ratio between the denticle above and below the X-axis in these two trichodinids is different. The ratio is more than one in *T. minuta* because of its short ray, while the ratio is about one in the new species because of its long ray. 3) Moreover, *T. minuta* is found on the skin, fins, and gills of the freshwater fishes *Oreochromis mossambicus*, *Pseudocrenilabrus philander*, *Tilapia sparrmanii,* and *Barbus trimaculatu*s from South Africa, while the new species was isolated from the gills of another host *Carassius auratus*.

According to cell size and the morphology of the adhesive disc, *T. kazubski* does exhibit some resemblance to *T. pseudominuta*. Nevertheless the new species can still be distinguished from *T. kazubski* by certain aspects of the adhesive disc morphology: 1). *T. kazubski* possesses broad blades with clear angularity and sharp tangent point, as well as obvious blade apophysis and ray apophysis. In contrast, the new species has smooth blade with bluntly round tangent point. In addition, the connection between the blade and the central part in *T. pseudominuta* is much thicker than that in *T. kazubski*. 2). The ray of *T. kazubski* is relatively more developed than that of the new species. And in the new species, the direction of the ray inclines forward and presents helical form with the tip of ray extending past the Y+1 axis in the whole view. However, the same case doesn't exist in *T. kazubski* 3). The new species possesses a smaller denticle number than that in *T. kazubski* (20 - 23 in *T. pseudominuta* and 22 - 26 in *T. kazubski*).

Based on the comparison between the related species, *T. pseudominuta* is considered to be a new member of the *Trichodina* genus.

***Trichodina jialingensis* sp. nov.**

Host and location: *Ctenopharyngodon idellus*, gills. Prevalence: Out of 37 *Ctenopharyngodon idellus* examined, five was infected (13.5%). Type locality: Hechuan District, Chongqing, China. Date of sampling: August, 2009. Type - specimens: One holotype (NO. 0908H1) and one paratype slide (No. 0908H2) were deposited in the Collection Center of Chongqing Key Laboratory of Animal Biology, Chongqing Normal University. Etymology: The name "*jialingensis*" mainly references to the sample locality of this parasite, because the present trichodinid species was collected from the Hechuan district along Jialing River in Chongqing.

Morphological description (n = 26) (Figures 1 E-F, 2F)

Trichodina jialingensis is a large freshwater *Trichodina* species. Its body diameter is 41.0 - 52.0 µm (47.6 ± 3.2). The adhesive disc 33.0 - 44.0 µm (39.1 ± 3.2) in diameter and the border membrane is 3.5 - 5.0 µm (4.5 ± 0.5) in width. The adhesive disc shows clear central zone in adult cells. The diameter of the denticulated ring is 16.0 - 27.5 µm (23.3 ± 3.3) and the number of denticles is about 22 - 25. The number of radial pins per denticle is 7 - 8. The denticle spans 10.5 - 13.0 µm (11.7 ± 0.6) and the length of the denticle is 5.0 - 6.5 µm (5.8 ±0.5). The blade is 4.0 - 5.5 µm (5.1 ± 0.5) in length. The distal blade surface is smooth, not parallel to border membrane, and is nearly at the same level as the blunt tangent point. The anterior and posterior surfaces are smooth and are nearly parallel to each other. The anterior surface is just touching the Y+1 axis. The posterior surface forms a wide arc-shape with the deepest point. Apophysis of blade and posterior projection is absent. The central part is not well developed with the rounded point fitting into the preceding denticle, and extending about half way to the Y-1 axis. Shapes of the central part above and below the X-axis are similar. The width of the central part is 1.5 – 3.0 µm (2.3 ± 0.4). Ray connection is inconspicuous and hardly distinguishable from ray. Ray is developed and not oblique, parallel to or attached to the Y-axis. The Length of ray is 3.5 - 5.5 µm (4.6 ± 0.6) and ray apophysis is present but not obvious. Adoral ciliary spiral turns about 370°- 390° around the peristomial disc. Macronucleus is C-shaped, and the micronucleus is spherical and situated in +Y position.

Remarks

Trichodina jialingensis n. sp. differs from its known congeners by a combination of taxonomic features, especially

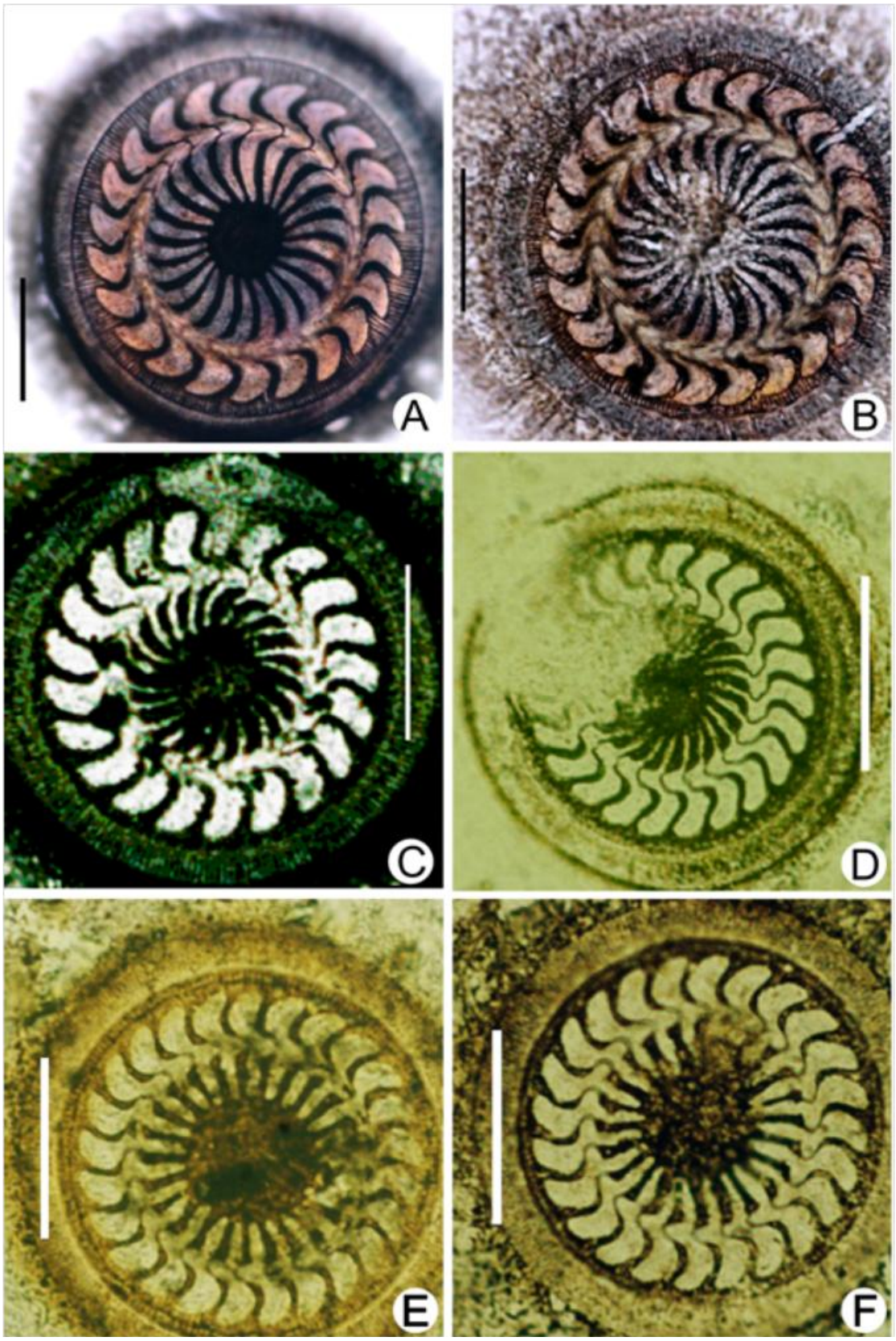

Figure 1. Photomicrographs of silver impregnated adhesive disc of three new trichodinid species. A-B. *Trichodina paraheterodentata* sp. nov.; C-D. *Trichodina pseudominta* sp. nov.; E-F. *Trichodina jialingenesis* sp. nov. (Scale bar = 20 µm).

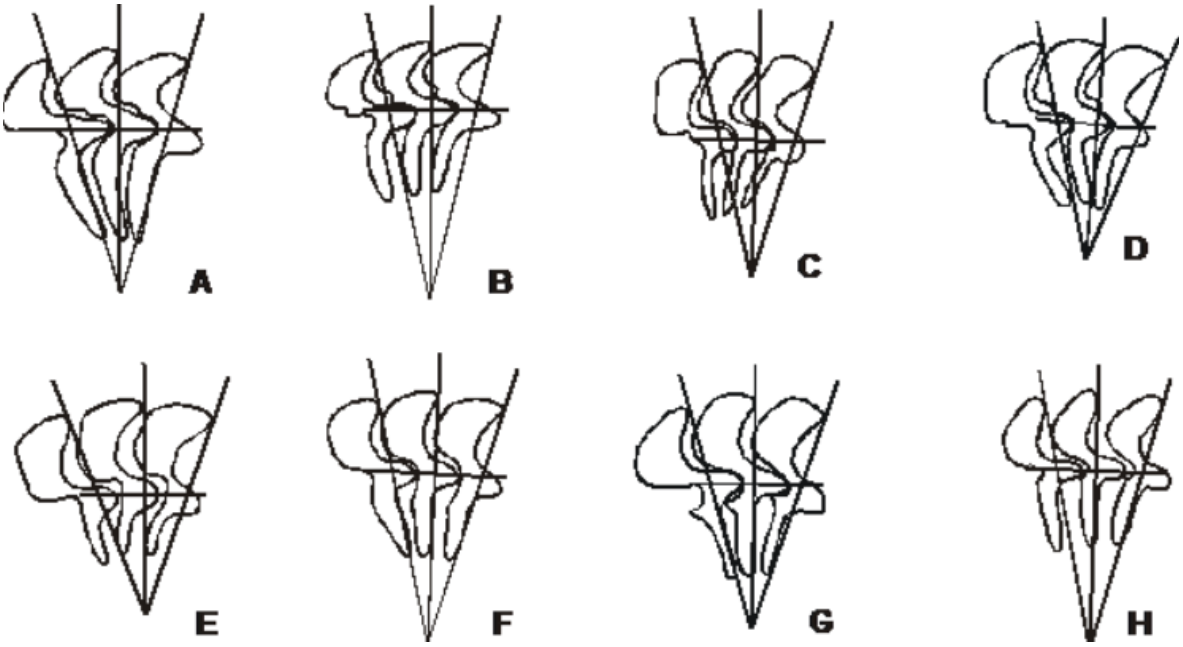

Figure 2. Diagramatic drawing of the denticles of *Trichodina* species: A. *Trichodina paraheterodentata* sp. nov.; B. *Trichodina heterodentata* Duncan, 1977 (drawn from Duncan, 1977); C. *Trichodina pseudominta* sp. nov.;D. *Trichodina kazubski* Van As and Basson, 1989 (drawn from Van As and Basson, 1989); E. *Trichodina minuta* Basson, Van As and Paperna. 1983 (drawn from Basson, Van As and Paperna); F. *Trichodina jialingensis* sp. nov.; G. *Trichodina nkasa* Van As and Basson, 1992 (drawn from Van As and Basson, 1992). H. *Trichodina fahaka* Al-Rasheid et al., 2000 (drawn from Al-Rasheid et al., 2000).

body size and adhesive disc. It resembles, to some extent, *Trichodina nkasa (*Van As and Basson, 1992) found from the Zambesi River at Katima Mulilo in South Africa (Van As and Basson, 1992). *T. jialingensis*, isolated from gills of grass carp, *Ctenopharyngodon idellus* in Chongqing, differs from *T. nkasa*, which parasitizes the gills of catfishes, *Synodontis leopardinus* and *S. macrostigma*, by having a distinctly larger body (41.0 - 52.0 µm vs. 29.5 - 39.5µm) and larger denticle number (22 - 25 vs. 19 - 22). In contrast, the adoral ciliary spirals in the two species represent obviously different (370°- 390° in *T. jialingensis* vs. 400°- 425° in *T. nkasa*). With reference to the morphology of adhesive disc, although the denticle shape of *T. jialingensis* is similar to that of *T. nkasa*, the new species can also be clearly distinguished from that of the latter, especially by the difference in the blade. *T. jialingensis* has relatively smooth and irregular rectangular blade, while the blade in*T. nkasa* is almost rectangular. Moreover, in the new species, the distal blade surface is lower than the tangent point or at the same level as the tangent point, but the tangent point is significantly higher in *T. nkasa*. Notably, *T. nkasa* possesses prominent ray apophysis, while the same structure is present in *T. jialingensis* but is not obvious.

T. jialingensis is also similar to *Trichodina fahaka (*Al-Rasheid et al., 2000) in terms of the general appearance of the denticles, especially the straight ray (Al-Rasheid et al., 2000). However, the known *T. fahaka* has typical acute triangular blade, which fills part of the space between the Y-axes. In contrast, the irregular rectangular blade fills almost the entire space between Y-axes in *T. jialingensis*. In *T. fahaka,* its distal blade surface is remarkably higher than the tangent point, while the distal blade surface is lower than the tangent point or at the same level as the tangent point in *T. jialingensis*. In *T. fahaka*, the denticulated ring comes into a unitary by the fitting tightly central part; At the same time, because of the short ray, there is a very wide space left in the clear central zone, which does not occur in the new species. In the case of host type, *T. fahaka* is a parasite of the feral fish, *Tetradon fahaka,* from Nile River, Egypt, but the present new species was collected from cultured fresh water fish, *Ctenopharyngodon idellus* in China. In addition, *T. fahaka* is distinctly smaller than *T. jialingensis* (31.4 - 34.3 µm vs. 41.0 - 52.0 µm).

In view of these differences discussed above, we believe that *T. jialingensis* should be considered a new species.

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