Full Length Research Paper

Two trichodinids of *Paratrichodina* (Ciliophora, Peritrichida, Trichodinidae) infecting gills of *letalurus punetaus* from Chongqing, China

Fahui Tang, Yuanjun Zhao* and Chunning Liu

Chongqing Key Laboratory of Animal Biology, Chongqing Normal University, Chongqing 400047, P. R. China.

Accepted 13 January, 2012

During a parasitological survey in China, two species of trichodinid ectoparasite (Ciliophora, Peritrichida, Trichodinidae) belonging to genus *Paratrichodina*, were isolated from *letalurus punetaus* (Siluridae). One species was identified as a new species and named as *Paratrichodina rotundiformis* sp. nov. and this new species has round and undeveloped blade with round tangent point, undeveloped triangular central part, slim ray with inclining backward, and whole fitting loose denticle. The other species, *Paratrichodina corlissi* is described for the first time in Asia in the present work. The morphometric data were obtained from specimens prepared using dry silver nitrate and methyl greenpyronin stain techniques. Results of comparison between the new species and closely related species are provided.

Key words: Ciliophora, oligohymenophorea, Peritrichida, Paratrichodina, new species, Ietalurus punetaus.

INTRODUCTION

Trichodinid ciliates are well-known ectoparasites of fishes, amphibians and molluscs, and often occur as parasites on maricultured and freshwater animals. Among the ten genera within family Trichodinidae, four genera, that is, Trichodina, Dipartiella, Trichodinella and Paratrichodina, have been found to occur on marine fishes or molluscs in China (Xu et al., 2001). However, little is known about the species of Paratrichodina under freshwater environment in China. In order to extend our knowledge on the geographic distribution and diversity of these ciliates, we investigate two trichodinids of Paratrichodina on the channel catfish, letalurus punetaus collected from culture pools in Chongqing, China as part of a series of ongoing studies on trichodinids of freshwater fishes in the area (Liu and Zhao, 2010; Tang and Zhao, 2007, 2010, 2011; Tang et al., 2005, 2007; Tao et al., 2008; Yu et al., 2011; Zhao and Tang, 2007, 2011; Zhao et al., 2007).

MATERIALS AND METHODS

Specimens of the channel catfish, *letalurus punetaus* were collected from culture pools in Chongqing, China in 2009. Gill smears of the host were made freshly, and smears with trichodinid were taken back to the laboratory for silver nitrate staining in order to reveal details of the adhesive disc.

The nuclear apparatus was shown using methyl green-pyronin stain (Foissner, 1991). The position of the micronucleus was given relative to that of the macronucleus, according to the procedure described by Lom (1958). In this system, the micronucleus were situated near the terminations of the arms of the macronucleus (i) externally near the right termination (+Y), (ii) externally between the two terminations (-Y) or (iii) internally near the right termination (- Y^1). Examinations of prepared slides were made under a Nikon E600 phase-contrast microscope. All photomicrographs (Figure 1a-e) and illustration drawings were made with Nikon-DXM1200 at a magnification of 1000 × and with the computer software CoreIDRAW 11.0 (Figures 2-5).

All measurements were presented in micrometers (μ m) and the uniform specific characteristic system proposed by Lom (1958) was used. Minimum and maximum values were given, followed by arithmetic mean and standard deviation in the parentheses. For denticles and radial pins, instead of arithmetic mean, the mode was described with the number of specimens examined given in the parentheses. The description of denticle elements was given following the format recommended by Van as and Basson (1989).

^{*}Corresponding author. E-mail: zhaoyuanjuncqnu@126.com. Tel: +86 (0) 23 6536 3077.



Figures 1, 2, 3, 4 and 5. Photomicrographs of silver impregnated specimens of trichodinids. Figures 1, 2 and 3. *Paratrichodina rotundiformis* sp. nov.; Figure 4 and 5, *Paratrichodina corlissi.* (Scale bar = $20 \ \mu m$).

RESULTS AND DISCUSSION

Order, Peritrichida; Family, Trichodinidae; Genus, *Paratrichodina*.

Paratrichodina rotundiformis sp. nov.

Type-host and site: *letalurus punetaus*, gills; Typelocality: Shapingba District, Chongqing Municipality (29°5' N, 106°5' E), China; Date of sampling: October, 2009; Prevalence: Out of the 16 *letalurus punetaus* examined, only one was infected (6.25%); Type-specimens: Syntypes on slide No. CQH-09010-01, CQB-09010-02 was deposited in the collection center of Chongqing Key Laboratory of Animal Biology, Chongqing Normal University, China.

Etymology

The special Latin name "*rotundiformis*" refers to the blade shape (round blade) of the trichodinid, and it is a composite of the Latin stem "*rotundi* -" (= rotundity) and

the Latin suffix "formis" (= form).

Description (Figure 1a-e)

The following morphological description of the new species were based on 18 specimens measured (n = 18). Small freshwater Paratrichodina with compressed body, diameter 25.0-35.0 µm (31 ± 2.7); adhesive disc 21.0-29.0 μ m (26 ± 2.2); width of border membrane 2.0-3.0 μ m (2.5 ± 0.2) ; diameter of denticle ring 12.0-18.0 µm (16 ± 1.7); number of denticles 24-27; number of radial pins per denticle usually 4-5; span of denticle 6.0-8.0 µm (7.5 ± 0.6); length of denticle 3.0-4.0 μ m (3.5 ± 0.5); blade generally developed and round, length 3.0-4.5 µm (4 ±0.4); smooth distal blade surface and a little curved and higher than tangent point; anterior and posterior surfaces straight and smooth and not in parallel with each other with anterior surface just touching the Y+1 axis; blade apophysis and posterior projection present but not distinct; central part barely developed with sharp point fitting very loosely into preceding denticle and not far extending half way beyond Y-1 axis; shapes of the central part above and below the X-axis were almost similar and were 1.0-1.5 μ m (1.2 ± 0.4) in width; ray



Figure 2. Diagramatic drawing of the denticles of *Paratrichodina rotundiformis* sp. nov. (drawn from the present work).



Figure 3. Diagramatic drawing of the denticles of *Paratrichodina incise.* (redrawn from Lom, 1963).

connection hardly conspicuous and barely distinguishable from ray; ray was very slender and smooth but obliquely attached and slanted a little posteriorly with a sharp point of ray; ray apophysis absent and length of ray was 2.0- $3.0 \mu m$ (2.5 ± 0.4); ratio between denticle above and below X axis about one. Adoral ciliary spiral turns about 150-220° around peristomial disc.

Remarks

In Family Trichodinidae, *Paratrichodina* (Lom, 1963) is a relatively small genus. The character of the genus is that denticles have well-developed rays, and are wedged together only by central parts. Anterior projection may be present or absent. If present, projection is situated near the base of the blade and is not in contact with the notch in the blade of the preceding denticle, and adoral spiral makes a turn of 150-280° (Lom, 1959; Basson and Van As, 1989). So far, only about 10 species of this genus have been reported from the gills of freshwater and marine fishes, and some species have been reported from the urinary tract of fishes and tadpoles in Eurasia, USA and Africa (Li and Desser, 1985; Lom, 1963; Kazubski and El-Tantawy, 1986; Lom and Haldar, 1976, 1977; Lom and Dykova, 1992).

In China, there have been only two reported *Paratrichodina* species, *P. globonuclea* and *P. obliqua*, from marine fishes (Xu et al., 2001). The differences among those recorded *Paratrichodina* reported in the present study, were discussed below.

With reference to the body size and denticle dimensions, the morphometric data of P. rotundiformis fall well within the range of P. incisa and P. erectispina. However, the new species differs from P. incisa in a combination of taxonomic features especially the host type and denticle shape (Figures 2 and 3). P. incisa is mainly found in Cyprinidae fishes and some tadpoles of frog; whereas the new species is isolated from the Siluridae fish, letalurus punetaus. Furthermore, the morphological difference of the denticles of the two organisms is distinct. For example, the new species possesses remarkably round blade with a little curved distal blade surface, straight anterior surface, undeveloped blade apophysis and oblique ray; whereas in P. incisa the blade is stubby club-shaped with much more curved distal blade surface and anterior surface, and there is well developed blade apophysis and erect ray.

P. rotundiformis also possesses body size similar to that of *P. erectispina*, but displays several unique characteristics that are distinguishable from *P. erectispina* (Figures 2 and 4). First, with respect to the denticle morphology, the blade of *P. rotundiformis* is round, and the apophysis of blade is present but not distinct; ray is short, straight and inclines backward. However, in *P. erectispina*, the blade is club-shaped, and the apophysis of the blade is much more prominent; the ray is longer, thinner and erect very much compared with that of *P. rotundiformis*. In addition, the adoral ciliary spiral turns are also remarkably different (150-220° in *P. rotundiformis versus* 230-280° in *P. erectispina*).

According to the cell size and host type, P.



Figure 4. Diagramatic drawing of the denticles of *Paratrichodina erectispina.* (redrawn from Lom & Haldar, 1977).



Figure 5. Diagramatic drawing of the denticles of *Paratrichodina corlissi.* (drawn from the present work).

rotundiformis exhibits some resemblance to *Paratrichodina corlissi*, but can be distinguished from *P. corlissi* by certain aspects of adhesive disc morphology (Figures 2 and 5), that is, *P. corlissi* has less denticles (22-23 versus 24-27) and more adoral ciliary spiral turns (250-270° versus 150-220°) than *P. rotundiformis*. Furthermore, the blade morphology can further aid in distinguishing these two species easily. In *P. corlissi*, the blade is club-shaped and anterior blade surface does not touch the Y+1 axis; whereas these structures in *P.*

rotundiformis are distinctly different, that is, the blade is round, and in most cases, the anterior blade surface touches or just extends to Y+1 axis.

On the basis of the significant differences in a combination of morphological features, especially the denticle morphology, *P. rotundiformis* is considered as a new species of genus *Paratrichodina*.

Paratrichodina corlissi

Host and Site: *letalurus punetaus*, gills; Locality: Hechuan District, Chongqing Municipality, China; Date of sampling: August, 2009; Prevalence: Out of the 18 *letalurus punetaus* examined, one was infected (5.56%).

Description (Figures 1d, e, and 5)

The following morphological description of P. corlissi was based on 18 specimens measured (n = 18). Small freshwater Paratrichodina; body diameter 26.5-29.0 µm (27.6 ± 0.9) ; adhesive disc 23.0-26.5 µm (24.3 ± 1.1) in diameter surrounded by a finely striated border membrane of 23.0-26.5 μ m (24.3 ± 1.1) in width; adhesive disc with clear central zone in adult cells; diameter of denticulated ring 14.0-17.0 μ m (15.7 ± 1.0) and number of denticles about 22-23; number of radial pins per denticle 5; span of denticle 6.5-8.0 μm (7.1 \pm 0.4); length of denticle3.0-3.5 μ m (3.2 ± 0.2); blade clubshaped and 3.0-4.0 μ m (3.5 ± 0.3) in length; distal blade surface smooth, straight, nearly in parallel with border membrane, and higher than the bluntly tangent point; anterior and posterior surfaces not very smooth and not parallel with each other; anterior surface not extending beyond Y+1 axis; posterior surface L shape with deep point; apophysis of blade present and posterior projection absent; central part not developed and cone-shaped with sharp point fitting loosely into preceding denticle and not extending half way to Y-1 axis; shapes of the central part above and below the X-axis similar; width of central part 1.0-2.0 μ m (1.6 ± 0.3); ray connection very inconspicuous and barely distinguishable from ray; ray relatively thin, needle-shaped, obliquely attached and slanted a little posteriorly with a sharp point of ray; length of ray 2.0-3.5 μ m (2.7 ± 0.5) and ray apophysis absent; adoral ciliary spiral turns 250-270° around peristomial disc (Figu re 1c); macronucleus U-shaped and micronucleus ellipse situated at +Y position.

Remarks

Paratrichodina corlissi was first identified on gills of *Gobio* gobio in ex-Czechoslovakia. *Gobio kessleri* was identified in Bulgaria and described by Lom and Haldar (1977) about 30 years ago. Since then, there have not been any reports on this species, particularly in Asia. Up to now,

there have been only more than ten *Paratrichodina* species in the world. This *Paratrichodina* species can be characterized by the morphology of the adhesive disc, the denticles possess club-shaped blades, cone-shaped central part and needle-shaped ray. In the present study, we identify a trichodinid species from gills of *letalurus punetaus* from Chongqing, China that has denticle morphology extremely similar to that of *P. corlissi* as originally described by Lom and Haldar with the only exception in adoral ciliary spiral turns (250-270° in our population *versus* 180-240° in the original population). This is the first record of a new host for *P. corlissi* in Asia, and extends the known geographic and host range of *P. corlissi*.

ACKNOWLEDGEMENTS

The present work was supported by grants from the National Natural Science Foundation of China (No. 30970329, No. 31101637 and No. 31172068), project of Chongqing Science and Technology Commission (No. CSTC, 2010CA1010), the Science Research Foundation of the Education Committee of Chongqing (No.KJ090814) and Science Founding of Chongqing Normal University (No. 11XLB025).

REFERENCES

- Basson L, Van As JG (1989). Differential diagnosis of the genera in the family Trichodinidae (Ciliophora: Peritrichida) with the description of a new genus ectoparasitic on freshwater fish from southern Africa. Syst. Parasitol., 13: 153-160.
- Foissner W (1991). Basic light and scanning electron microscopic methods for taxonomic studies of ciliated protozoa. Eur. J. Protistol., 27: 313-330.
- Kazubski SL, El-Tantawy SAM (1986). The ciliate Paratrichodina africana sp. n. (Peritricha, Trichodinidae) from *Tilapia* fish (Cichlidae) from Africa. Acta Protozool., 25: 433-438.
- Li LX, Desser SS (1985). The protozoan parasites of fish from two lakes in Algonquin Park, Ontario. Can. J. Zool., 63: 1846-1858.
- Liu CN, Zhao YJ (2010). Morphological and taxonomic study of four species of trichodinids parasitic on gills of Siluriformes fishes from freshewater. J. Chongqing Normal Univ., 27(1): 16-21.
- Lom J (1958). A contribution to the systematics and morphology of endopasitic trichodinids from amphibians of uniform specific characteristics. J. Protozool. 5: 251-263.
- Lom J (1959). On the systematics of the genus *Trichodinella* Sramek-Husek (=Brachyspira Raabe). Acta Parasitol. Polonica, 7: 573-590.
- Lom J (1963). The ciliates of the family Urceolariidae inhabiting gills of fishes (the trichodinella-group). Vest. Cesk. Spol. Zool., 27: 7-19.
- Lom J, Dykova I (1992). Protozoan parasites of fishes. Develop. Aquacul. Fisher. Sci., 26: 1-315.
- Lom J, Haldar DP (1976). Observations on trichodinids endocommensal in fishes. Trans. Am. Micros. Soc., 95: 527-541.

- Lom J, Haldar DP (1977). Ciliates of the genera *Trichodinella*, *Tripartiella* and *Paratrichodina* (Peritricha, Mobilina) invading fish gills. Folia Parasitol., 24: 193-210.
- Tang FH, Zhao YJ (2007). Taxonomic study on three species of *Trichodina* Ehrenberg, 1838 with pathologic research on gill tissue of *Carassius auratus* caused by *Trichodina heterodentata* Duncan, 1977. A study on trichodinids from freshwater fishes in Chongqing II.
 J. Chongqing Norm. Univ. Nat. Sci. Ed., 24 (3): 8-12.
- Tang FH, Zhao YJ (2010). Taxonomic study on trichodinids parasitic on gills of freshwater fish, *Carassius auratus* from Chongqing, china, with the description of *Trichodina brevicirra* sp. nov. Acta Hydrobio. Sin., 34: 1004-1011.
- Tang FH, Zhao YJ (2011). Study of trichodinids (Protozoa, Ciliophora) parasitic on gills of freshwater fishes from Chongqing, China, and identification of a new species, *Trichodina cyprinocola* sp. nov. Afr. J. Microbiol. Res., 5(26): 5523-5527.
- Tang FH, Zhao YJ, Tang AK (2005). Presence of ectoparasitic trichodinids (Ciliophora, Oligohymenophorea, Peritrichida) on the gills of cultured freshwater fish, *Carassius auratus* in Chongqing, China, with the the description of a new species of the genus *Trichodina*. Acta Zootax. Sin., 30: 35-40.
- Tang FH, Zhao YJ, Tao YF (2007). Trichodinids (Ciliophora: Peritrichida) parasitic on gills of freshwater fishes, *Carassius auratus* and *Aristichthys nobilis* from China, with the description of *Trichodina subtilihamata* sp. nov. Zootaxa, 1582: 39-48.
- Tao YF, Zhao YJ, Tang FH (2008). Seven spwcies of Trichodinid ectoparasites (Ciliophora: Peritrichida) from freshwater fishes, *Hypophthalmichthys molitrix*, Aristichthys nobilis and Ctenopharyngodon idellus, with the description of Trichodina Chongqingensis sp. nov. Acta Hydrobio. Sin., 32: 124-129.
- Van As JG, Basson L (1989). A further contribution to the taxonomy of Trichodinidae (Ciliophora: Peritrichia) and a review of the taxonomic status of some fish ectoparasitic trichodinids. Syst. Parasitol., 14: 157-179.
- Xu KD, Song WB, Warren A, Choi JK (2001). Trichodinid ectoparasites (Ciliophora: Peritrichida) of some marine fishes from coastal regions of the Yellow Sea and Bohai Sea. Syst. Parasitol., 50: 69-79.
- Yu SS, Zhao YJ, Tang FH (2011). Geographical distribution and diversity of trichodinid ectoparasites (Ciliophora, Oligohymenophorea, Mobilia) from the gills of fresh and estuarine fishes in Zhejiang Province, China and coastal regions of the East China Sea. Eur. J. Sci. Res., 64 (1): 61-74.
- Zhao YJ, Tang FH (2007). Trichodinid ectoparasites from the freshwater fish *Misgurnus anguillicaudatus* (Cantor) and mollusc *Anodonta woodiana* (Lea) of Chongqing in China, with descriptions of two new species of *Trichodina* Ehrenberg, 1838. Syst. Parasitol., 67: 65-72.
- Zhao YJ, Tang FH (2011). Taxonomic study on trichodinids (Protozoa, Ciliophora) infecting on gills of Freshwater Fishes, *Cyprinus carpio* and *Mylopharyngodon piceus* from China, with the description of *Trichodina regularis* sp. nov. Eur. J. Sci. Res., 58 (2): 231-237.
- Zhao YJ, Tang FH, Tang AK (2007). A taxonomic study on species of *Trichodinella* (Raabe, 1950) Sramek-Husek, 1953 and *Tripartiella* Lom, 1959 with seasonal population dynamics of *Trichodinella epizootica* (Raabe, 1950) Sramek-Husek, 1953. A study on trichodinids from freshwater fishes in ongqing I. J. Chongqing Norm. Univ. Nat. Sci. Ed., 24: 1-6