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Full Length Research Paper

Study of trichodinids (Protozoa, Ciliophora) parasitic on gills of freshwater fishes from Chongqing, China, and identification of a new species *Trichodina cyprinocola* sp. nov.

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This study surveyed freshwater fishes *Cyprinus carpio* and *Carassius auratus* from Chongqing, China, for infection with ectoparasitic trichodinids. Fish specimens were obtained from culture pools, smears of gills were prepared and used for silver nitrate impregnation. Microbiologic studies identified two species of trichodinids belonging to *Trichodina* Ehrenberg, 1838 including one new species from *C. carpio* that was named *Trichodina cyprinocola* sp. nov. Comparisons with closely related species revealed that this new species *T. cyprinocola* has unique sturdy denticles including the sickle blade with bluntly round tangent point, well developed central part, robust, straight but short ray. The other known species *Trichodina borokensis* Arthur and Lom (1984) from *C. auratus* was described here as the first record in China. Our survey also yielded morphometric data and prevalence of infestation for these two species.

Key words: *Trichodina*, new species, freshwater fish, *Cyprinus carpio*, *Carassius auratus*.

INTRODUCTION

As well-known ectoparasites of fishes and mollusks, trichodinid ciliates often parasitize on maricultured and freshwater animals. About 300 nominal *Trichodina* species have been reported from different environments (Lom, 1970, 1973; Lom and Laird, 1969; Lom and Dyková, 1992; Song et al., 2003; Tang and Zhao, 2007, 2010), but their geographic distribution and diversity in Chongqing, China are still largely unknown. As part of a series of ongoing studies on trichodinids of freshwater fishes in the area (Tang et al., 2005a, 2005b, 2007; Tang and Zhao, 2007, 2010**;** Tao and Zhao, 2006; Tao et al., 2008; Zhao et al., 2007; Zhao and Tang, 2007; Zhou et al., 2008), we surveyed the presence of trichodinid species on cultured freshwater Cyprinidae fishes *Cyprinus carpio* and *Carassius auratus,* and are now reporting the first description of a known species in China and identification of a new species.

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MATERIALS AND METHODS

Two freshwater fish species *C. carpio* (approximately 1 year old and ranging from about 8 - 30 cm in total length) and *C. auratus* (approximately 1 year old and ranging from about 15 - 35 cm in total length) were collected from culture pools with water supply mainly from river water in Chongqing, China in 2009, including 25 and 28 fish specimens from culture pools in Dianjiang and Wulong counties, respectively. Wet smears of gills were made fresh and screened for the presence of trichodinids under the Nikon E600 phase-contrast microscope. The nuclear apparatus was shown using the methyl green-pyronin stain (Foissner, 1991). Smears positive for trichodinid were taken back to the laboratory, air-dried, and the staining technique was employed the Klein's dry method (Lom, 1958) for further to reveal the structure of adhesive disc. Prepared slides were examined under the Nikon E600 phase-contrast microscope, photographs were taken using Nikon-DXM1200 camera at 1000x magnification, and illustrations were drawn with computer software CorelDRAW 11.0.

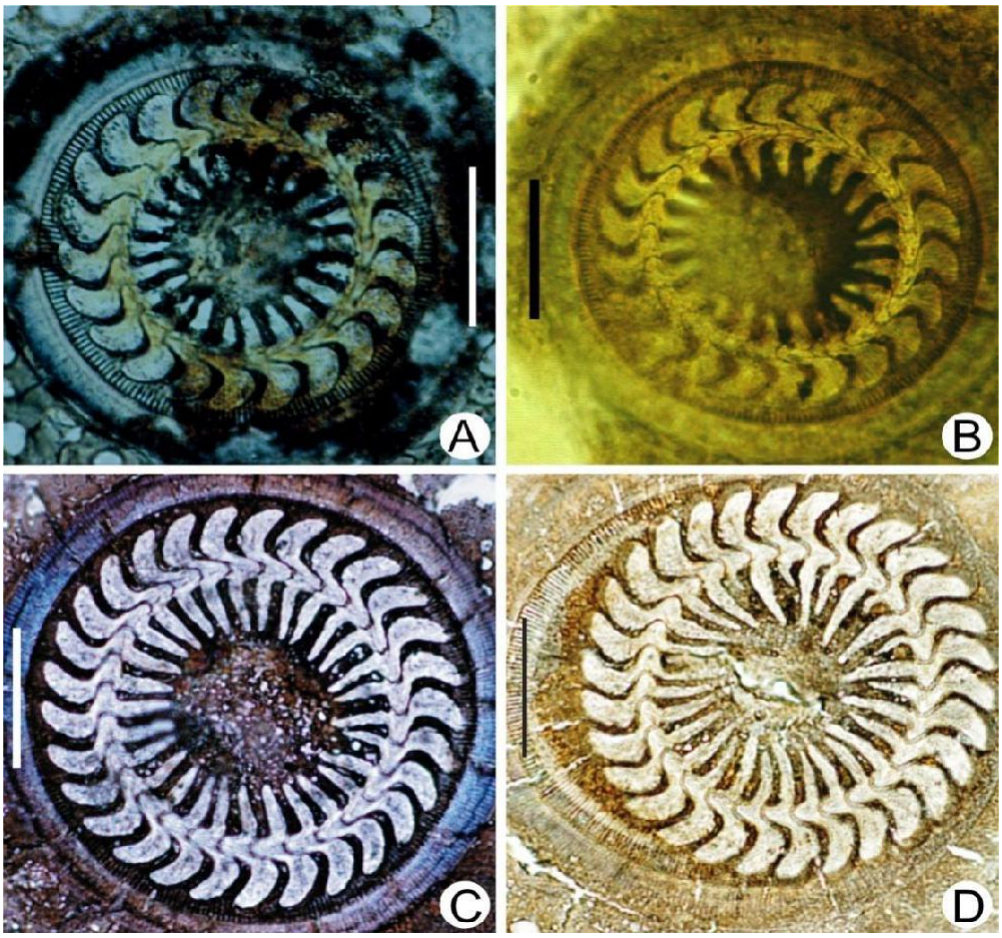

Figure 1. Photomicrographs of silver impregnated adhesive disc of two trichodinids. A-B. *Trichodina cyprinocola* sp. nov.; C-D. *Trichodina. borokensis* Arthur and Lom (Scale bar = 20 µm).

The position of the micronucleus is 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 are 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, 1859; Family: Trichodinidae Claus, 1874; Genus: *Trichodina* Ehrenberg, 1838.

T. cyprinocola sp. nov.

(Figures 1A, 1B and 2A). Type-host and site: *C. carpio*, gills; Type-locality: Dianjiang county, Chongqing (29°5' N, 106° 5' E), China; Date of sampling: May, 2009; Prevalence: Out of 25 *C. carpio* examined, three was infected (12.0%); Type-specimens: Syntypes on slides (NO. CQD-09-0501, CQD-09-0502).

Etymology

The Latin name "*cyprinocola*" refers to the host type for the trichodinid, and it is a composite word composed of a Latin prefix "*cyprino*-" (= *Cyprinus*) and a suffix "*cola*" (= live, reside).

Description (n=16)

Large, freshwater *Trichodina* body diameter is 53.0 to 66.0 µm (58.2 ± 5.3) in silver-impregnated specimens. Adhesive disc is 45.5 to 54.5 µm (49.0 ± 3.7) in diameter, and is surrounded by a finely striated border membrane that is 4.5 to 6.5 µm (5.1 ± 0.6) wide. The adhesive disc is found with clear central zone in adult cells. The diameter of the denticulated ring is 27.5 to 35.5 µm (31.1

± 2.7) and the number of denticles is about 22 to 24 (n=16). However, the number of radial pins (mode) per denticle is 9 to 10 (n=16). The span of denticle is 12.0 to 14.5 µm (13.5 ± 0.8), whereas the length of denticle is 7.5 to 9.5 µm (8.3 ± 0.7). The *Trichodina* blade is very broad and sickle-shaped, filling most of the space between the Y-axes, and is 5.0 to 6.5 µm (5.6 ± 0.5) in length. The distal blade surface is smooth and curved, parallel to the border membrane, and obviously higher than the bluntly round tangent point. The anterior and posterior surface are straight and smooth. The anterior surface always touches the Y+1 axis or it just extends to it; whereas the posterior surface forms a narrow curve with the deepest point. The apophysis of the blade and posterior projection are present but not distinct, though the blade connection is very thick. The central part is very developed with rounded point fitting tightly into the preceding denticle, and not just extending about half way to Y-1 axis. The shape of the central part that is above the X axis is similar to that below it; although the width of the central part is 2.5 to 4.0 µm (3.4 ± 0.5). The ray connection is inconspicuous and barely distinguishable from the ray, which is robust, straight but relatively short, tapering to its sharp tip, attached to the Y axis, not inclining forward or backward. The ray apophysis is prominent, and the length of the ray is 4.0 to 6.0 µm (5.0 ± 0.6). The ratio between the denticle above and below the X axis is about one. The adoral ciliary spiral turns at about 380 to 400° in the peristomial disc. Nonetheless, the macronucleus which is horseshoe-shaped, and the micronucleus which is oval, are situated in +Y position.

Remarks

In regard to the overall morphology of the adhesive disc, *T. cyprinocola* sp. nov. is similar to two known species, *Trichodina gulshae (*Asmat et al., 2003) and *Trihcodina heterodentata* Duncan, 1977 (Asmat et al., 2003; Duncan, 1977).

Similar to *T. gulshae*, *T. cyprinocola* also possesses close body size and similar sickle-shaped blade, but it displays several unique characteristics distinguishable from *T. gulshae* (Figures 2A and 2B). First, with respect to the denticle morphology, in *T. cyprinocola*, apophysis of blade is present but not distinct, blade connection is strong and ray is very robust, short, and not inclining forward or backward. However, in *T. gulshae*, apophysis of blade is more prominent, blade connection is relatively thinner, and the ray is much more slender and slanted in anterior direction. Second, *T. cyprinocola* is a parasite from the cultured Cyprinidae fish *C. carpio* , whereas *T. gulshae* was isolated from the Siluriformes fish *Mystus cavasisus* at Sadarghat area of the Karnaphuli river in Chittagong.

Based on the overall shape of the denticles, the present species resembles more closely to *Trichodina heterodentata* Duncan, 1977, especially the original population A of *T. heterodentata* (Duncan, 1977). However, *T. cyprinocola* is also different from *T. heterodentata* in several aspects (Figures 2A and 2C). First, the adhesive disc of *T. heterodentata* is significantly larger than that of *T. cyprinocola* (71 - 106 vs. 53 - 66 µm) with a greater number of radial pins per denticle (11 in *T. heterodentata vs.* 9 - 10 in *T. cyprinocola).* With reference to the morphology of denticles, the new species possesses much more broader sickle-blades with round tangent point (*vs.* sharp tangent point); its distal blade surface is significantly higher than the round tangent point, unlike that of *T. heterodentata* that is lower than or at the same level as the tangent point. Besides, in the new species, the anterior surface always touches or just extends to Y+1 axis (*vs.* extends beyond Y+1 axis in *T. heterodentata*); its ray is very robust, straight and short, and the tapering tip does not incline forward or backward, attaching to Y axis. Nevertheless, the ray in *T. heterodentata* is less developed and a little curved, morphologically different from the new species.

Based on the significant differences in a combination of morphologic features, especially the denticle morphology, *T. cyprinocola* is considered a new trichodinid species.

T. borokensis

Host and Site: *Carassius auratus*, gills; Locality: Wulong county, Chongqing, China; Date of sampling: March, 2009 (Figures 1C, 1D and 2D); *Prevalence*: Out of 28 *C. auratus* examined, three were infected (10.7%).

Description (n = 14)

Large, freshwater *Trichodina* body diameter is 67.5 to 76.0 µm (71.5 ± 2.8). The adhesive disc is 60.0 to 67.0 µm (62.4 ± 2.4) in diameter, and is surrounded by a finely striated border membrane that is 5.5 to 7.0 µm (6.4 ± 0.5) wide. The adhesive disc is found with a clear central zone in adult cells. Diameter of the denticulated ring is 38.0 to 40.5 µm (39.0 ± 1.0) and the number of denticles is about 28 to 31 (n = 14). However, the number of radial pins per denticle is 9 to 10 (n = 14). The span of the denticle is 17.0 to 20.0 µm (18.4 ± 1.2); whereas, the length of the denticle is 7.5 to 8.5 µm (8.2 ± 0.3). Blade spathulate is 7.0 to 8.5 µm (7.6 ± 0.5) in length. The distal blade surface is smooth and round, but not parallel to the border membrane, and is higher than the bluntly round tangent point. It is observed that the anterior and posterior surfaces are smooth, and almost parallel to each other. The anterior surface extends past Y+1 axis, while the posterior surface forms an arc-shaped with the deepest point. The apophysis of the blade and posterior projection is absent. The central part is relatively developed with a rounded point fitting tightly into the

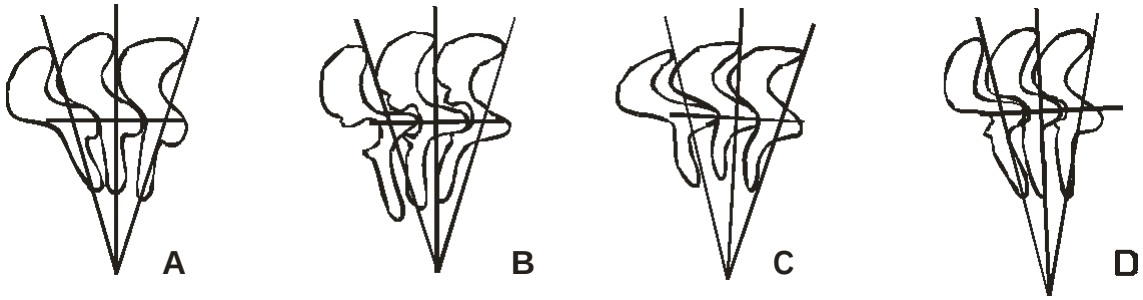

Figure 2. Diagramatic drawing of the denticles in four *Trichodina* species: A. *Trichodina cyprinocola* sp. nov.; B. *T. gulshae* Asmat et al., 2003; C. *T. heterodentata* Duncan, 1977; D. *T. borokensis* Arthur and Lom.

preceding denticle, extending about half way to the Y-1 axis. The shapes of the central part above and below the X-axis are similar; though the width of the central part is 3.5 to 4.5 µm (4.0 ± 0.3). The ray connection is inconspicuous and barely distinguishable from the ray, which is relatively thick, obliquely attached and a little slanted posteriorly with a sharp point. The length of the ray is 6.0 to 9.5 µm (7.8 ± 1.3), and the ray apophysis is prominent in some specimens. Adoral ciliary spiral turns at about 370 to 390° in the peristomial disc. Nonetheless, the macronucleus is U-shaped, and the micronucleus is ellipse, and situated in +Y position.

Remarks

T. borokensis was first identified on gills of *Pelecus cultratus* from northwestern Rybinsk Reservoir, USSR about two decades ago (Arthur and Lom, 1984). Since then, there have not been any reports on this species, particularly in the world. *T. borokensis* exhibits some similarities to *Trichodina jiroveci* Grupcheva and Lom (1980) described from the gills of *Neogobius fluviatilis* from Bulgaria and *Trichodina cobitis* Lom, 1961 described from freshwater fishes of Czechosolvakia (Grupcheva and Lom, 1980; Lom, 1961). However, the distinct differences from those two species were discussed clearly in the original report of *T. borokensis*. This species can be characterized by the morphology of the adhesive disc: the denticles possess curved blades which appear rather spathulate and terminate in a bluntly rounded point; the anterior surface of the blade is smooth, lacking any notch or indentation. In the majority of denticles, the ray is obliquely attached and is thus slanted a little posteriorly. In the present study, we identified a trichodinid species in *C. auratus* from Chongqing, China that has denticle morphology extremely similar to *T. borokensis* originally described by Arthur and Lom (1984) with the only exceptions of a slightly different body size and denticle number (67.5 - 76.0 µm in our population *vs.* 55.6 - 71.4 µm in the original population; 28 - 31 in our population *vs.* 24 - 28 in original population). This finding has established the first record of a new host for *T. borokensis* in the world, and extended the known geographic and host range of *T. borokensis*.

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