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TAXONOMIC STUDY ON TRICHODINIDS PARASITIC ON GILLS OF FRESHWATER FISH, *CARASSIUS AURATUS* FROM CHONGQING, CHINA, WITH THE DESCRIPTION OF *TRICHODINA BREVICIRRA* SP. NOV.

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Abstract: Three trichodinid ectoparasites (Ciliophora: Peritrichida) were isolated from the freshwater fish, *Carassius auratus* collected from Chongqing, China. *Trichodina brevicirra* sp. nov. is described as a new one characterized by the short and slim ray. The other two trichodinids are *Trichodina ngoma* Van As & Basson, 1992 and *Trichodina reticulata* Hirschman & Partsch, 1955. *T. ngoma* Van As & Basson, 1992 is redescribed as a first record in Asia. Three populations of *T. reticulata* Hirschman & Partsch, 1955 fall within the range of morphometry and agree closely in the overall appearance of the adhesive disc. Our observations suggest that denticle and central granules morphology may be more or less variable between and within populations, and that such minor differences should not be overestimated. It should be emphasized that there are 8 to 16 spherical or oval granules in central zone of adhesive disc.

Key words: Trichodinid; *Trichodina brevicirra*; *Carassius auratus*; New species; China
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Trichodinids include those ciliophorans with complex denticles in the adhesive disc. They often occur as parasites on maricultured and freshwater animals, and they can cause serious diseases [1—5]. Among the ten genera within the family Trichodinidae Claus, 1874, *Trichodina* Ehrenberg, 1830 is probably one of the largest and most widely distributed of the ciliate genera. The present paper deals with three trichodinid species belonging to the genus *Trichodina* collected from Chongqing, China. This paper is one of a series of reports on trichodinids of freshwater fishes from Chongqing area[6—15], which aims to extend our knowledge on the diversity and distribution of these ciliophorans.

1 Materials and Methods

Specimens of host fish, *Carassius auratus*, were collected from culture pools in Chongqing, China, during the period from February 2003 to April 2007. Fresh gill smears of hosts were made and those smears with trichodinids were taken back to the laboratory for further silver nitrate staining in order to reveal details of the adhesive disc.

The nuclear apparatus was shown using the methyl green-pyronin stain[16]. 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 ($-Y^1$). Examinations of prepared slides were made under Nikon E600 phase-contrast microscope. All photomicrographs and illustration drawings were made with the help of Nikon-DXM1200 (at × 1000) magnification and computer software CorelDRAW 11.0.

All measurements are presented in micrometers (μm) and follow the uniform specific characteristic system proposed by Lom [17]. The minimum and maximum values are given, followed in parentheses by the arithmetic mean standard deviation. In the case of the denticles and

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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 & Basson [18].

2 Results and Discussion

Phylum: Ciliophora Doflein, 1901.
Class: Oligohymenophorea de Puytorac et al., 1974.
Order: Peritrichida Stein, 1859.
Family: Trichodinidae Claus, 1874.
Genus: *Trichodina* Ehrenberg, 1830.

***Trichodina brevicirra* sp. nov.**

Type-host: *Carassius auratus.*

Location: Gills.

Prevalence: 5/27 (18.5%).

Type-locality: Chongqing (29º5' N, 106º5' E), China.

Date of sampling: February, 2003.

Type-specimens: Syntypes on slide CQB-0302-01 and CQB-0302-02 deposited in the Collection Center of the Key Laboratory of Animal Biology of Chongqing, Chongqing Normal University.

Etymology: The name "*brevicirra*" refers to the typically morphological feature (short and slim ray) of the trichodinid.

Description (Plate I-1, 2; Plate II-5) (*n* = 22): Medium to large-sized, freshwater *Trichodina*. Cell diameter 48.0—56.0μm (52.9 ± 2.7). Diameter of adhesive disc 40.0—47.0μm (43.3 ± 2.3). Adhesive disc with clear central zone. Border membrane 4.5—6.0μm (5.3 ± 0.4) in width. Diameter of denticulated ring 25.0—30.0μm (27.2 ± 2.1). Number of denticles about 25—30 (22). Number of radial pins (mode) per denticle 8—10 (22). Span of denticle 11.0—15.0μm (13.1 ± 1.8). Length of denticle 6.0—7.0μm (6.5 ± 0.3). Blade broad and oblique-quadrangle shaped, with upper part wider than lower part, 5.0—6.0μm (5.4 ± 0.5) in length. Distal blade surface plane and straight, but not parallel to border membrane. Tangent point very sharp and as same level as distal blade surface. Anterior blade surface straight and smooth, always extending past Y+1 axis, not incompletely parallel to posterior surface, but forming a arch-shaped with Y-1 axis. Apophysis of blade present and posterior projection obvious in some denticle. Section connecting blade and central part relatively developed. Central part well developed with rounded point fitting tightly into preceding denticle, just extending about half way to Y-1 axis. Shape of central part above X axis not similar to the part below. Width of central part 1.5—2.0μm (1.8 ± 0.3). Ray connection very inconspicuous and barely distinguishable from ray. Ray relatively short and very slim, tapering gradually to sharp point, parallel to Y axis. Ray apophysis not prominent or absent and length of ray 4.0—6.0μm (5.3 ± 0.6). Ratio between denticle above and below X axis more than one. Adoral ciliary spiral turns about 380°—400° around peristomial disc. Macronucleus U-shaped, and micronucleus oviform, situated in +Y position.

Discussion: Among those known *Trichodina* species, two trichodinids, *Trihodina paranigra* Tang *et al*., 2005 and *Trichodina cobitis* Lom, 1961, are found to be similar to the new species on the basis of the morphology of adhesive disc [6, 19].

When compared to *T. paranigra*, denticle shapes of both species show some resemblance, but the new species can be still easily distinguished from the latter based on the important points followed characteristics. 1) It possesses a larger number of radial pins per denticle (8—10 vs. 7—8); 2) Based on the morphometric data, it is little smaller *T. paranigra* (48—56μm vs. 56—65μm); 3) The most significant difference between these two species can be observed from the morphology of the denticle: firstly, shapes of blade are different, in new species, the blade presents oblique-quadrangle shaped, as its upper part is wider than lower part, which forms a very sharp tangent point, whereas there is no case occurred in *T. paranigra*. Secondly, section connecting blade and central part of *T. brevicirra* is more developed than that of *T. paranigra*. Thirdly, shape of central part above X axis is not similar to the part below (vs. similar in *T. paranigra*) and ratio between denticle above and below X axis is more than one (vs. about one in *T. paranigra*). Finally, ray of new species is shorter and slimmer than that of *T. paranigra*.

In comparison with *T. cobitis*, two species possess the close morphometric data except diameter of denticle ring (25—30μm vs. 20—24μm). However, morphological differences can assist to differentiate them. In the case of denticle morphology, obvious differences can be easily observed. The tangent point of blade is very sharp and it is always as the same level as distal blade surface in the new species whereas tangent point is not so sharp and lower than distal blade surface in the latter. Moreover, tip of ray almost touches or extends past Y+1 axis in *T. cobitis*, but not yet in *T. brevicirra*.

According to the comparison with closely related species, *T. brevicirra* is considered to be a new member of the genus of *Trichodina* Ehrenberg, 1830.

***Trichodina ngoma* Van As & Basson, 1992**

Host and site: Gills of *Carassius auratus*.

Locality: Chongqing, China.

Date of sampling: February, 2004.

Prevalence: 1/29 (3.45%).

Description (Plate I-3, 4; Plate II-8) (*n* = 16): Medium to large size, freshwater *Trichodina*. Cell diameter 56.0—62.0μm (59.6 ± 2.3). Diameter of adhesive disc 44.0—50.0μm (47.8 ± 2.1). Center of disc clear and without any granules in adult and young cells (Plate I-3,4). Border membrane 4.0—6.0μm (5.2 ± 0.5) in width. Diameter of denticulated ring 27.0—32.0μm (30.3 ± 2.4). Number of denticles about 26—29 (16). Number of radial pins per denticle 8—10 (16). Span of denticle 14.0—17.0μm (15.8 ± 2.4). Length of denticle 5.0—7.0μm (6.2 ± 0.9). Blade quadrangular, 5.0—7.0μm (6.1 ± 0.7) in length, not filling most of space between Y-axes. Distal blade surface relatively straight, almost parallel to border membrane. Anterior blade surface smooth and straight, extending to Y+1 axis. Apophysis of blade present and obvious as result of lower part of blade with indentation. The posterior surface smooth and nearly parallel to anterior blade surface. Blade connection relatively thin. Central part not well developed with rounded point fitting tightly into preceding denticle, extending past half way to Y-1 axis. Width of central part 2.0—3.0μm (2.1 ± 0.5). Ray relatively thin, and length of ray 6.0—8.0μm (7.9 ± 0.7). Ray apophysis present and prominent especially in the division cells (Plate I-4). Ratio between denticle above and below X axis about one. Adoral ciliary spiral turns about 400° around peristomial disc. Macronucleus C-shaped, and micronuclear oval situated in +Y position.

Division: During ontogeny, new blades are in process of development whilst old ring is being resorbed according to observation from the silver impregnated specimens of *T. ngoma* (Plate I-4). Apophysis of blade and ray can be clearly observed from the old denticles during divison. In terms of shapes of denticle, old denticles shapes in the division cell show some variations with adult individuals (sickle-shaped *vs.*quadrangular), however, the shape of new forming blades showes some resemblance to some extent.

Discussion: *T. ngoma* was firstly discovered from freshwater fish, *Hemigrammocharax multispine* of South Africa by Van As & Basson in 1992. It is an organism with widely parasitic positions, such as it can be found from the skin, fin and gills of *Hemigrammocharax multispine*. However, our population was only been found from the gill of the host, *Carassius auratus*. Except that data of border membrane and length of denticle are larger than the original population, the other morphometric data of our population including body size, diameter of adhesive disc fall into the range of *T. ngoma* from South Africa as described by Van AS & Basson (1992) [20].

Since *T. ngoma* was first found from *Hemigrammocharax multifasciatus* of Lake Lisikili, belonging to Zambesi River System which locates in Eastern Caprivi, South Africa by Van As & Basson in 1992, it has not been reported again up to now. Our study has supplemented the data of division, and *Carassius auratus* is also established as a new host for *T. ngoma*. Above all, this is not only the first record in China, but also a new distribution in Asia for this taxon.

***Trichodina reticulata* Hirschman & Partsch, 1955 (Plate III, IV)**

Host and site: Gills of *Carassius auratus*.

Locality: Chongqing, China.

Prevalence: 1/21 (4.76%).

Population 1 from Baishiyi County of Chongqing, China.

Date of sampling: April, 2003.

Description (Plate III-9,10; Plate IV-15): Large-sized freshwater *Trichodina*; body hat-compressed in lateral view; and cell diameter 52.0—65.0μm (56.2 ± 3.3). Diameter of adhesive disc 42.0—56.0μm (49.3 ± 4.3). Central zone of adhesive disc relatively clear and with about 12 uniform and spherical granules. Border membrane 5.0—6.0μm (5.2 ± 0.4) in width. Diameter of denticulated ring 27.0—36.0μm (32.3 ± 3.4). Number of denticles about 24—31 (25). Number of radial pins per denticle 8—10 (25). Span of denticle 12.0—16.0μm (14.6 ± 1.3). Length of denticle 6.0—8.0μm (6.8 ± 0.5). Blade shape broad and club-shaped, 6.0—8.0μm (6.9 ± 0.7) in length. Distal blade surface round and smooth, higher than or at the same line as tangent point. Anterior blade surface straight and smooth, the lower part of anterior blade surface forming concave in some specimens (Plate III-9,10). Apex of blade always extending past Y+1 axis. Apophysis of blade present. Central part well developed with rounded point fitting tightly into preceding denticle and width of central part 2.0—3.0μm (2.1 ± 0.4). Ray connection stumpy, inconspicuous and barely distinguishable from ray; ray apophysis present in some specimens and length of ray 5.0—7.0μm (5.8 ± 0.7). Macronucleus U-shaped, and micronucleus oval, situated in $-Y^1$ position. Adoral ciliary spiral turns about 370°—390° around peristomial disc.

Population 2 from Huilongba County of Chongqing, ChinaDate of sampling: April, 2004.

Description (Plate III-11, 12; Plate IV-16): Medium-sized freshwater *Trichodina*; cell diameter 45.0—50.0μm (47.3 ± 2.3). Diameter of adhesive disc 36.0—42.0μm (38.4 ± 2.9). Central zone of adhesive disc with smaller oval granules. Border membrane 4.0—5.0μm (4.6 ± 0.3) in width. Diameter of denticulated ring 25.0—29.0μm (26.4 ± 2.5). Number of denticles about 24—31 (16). Number of radial pins per denticle 7—10 (16). Span of denticle 11.0—14.0μm (12.4 ± 1.2). Length of denticle 5.0—7.0μm (5.9 ± 0.6). Upper and lower part of blade broad and middle part relatively narrow, and blade 5.0—7.0μm (5.8 ± 0.6) in length. Distal blade surface straight, nearly at the same leval as tangent point. Anterior blade surface with a little concave in most specimens. Apex of blade always extending past Y+1 axis. Apophysis of blade present. Central part not well developed and width of central part 1.0—2.0μm (1.3 ± 0.4). Ray relatively thin and not smooth, and with needle-shaped top in some individuals (Plate III-12). Length of ray 4.0—6.0μm (4.9 ± 0.5). Ray apophysis not obvious and rarely seen. Macronucleus U-shaped, and micronucleus oval, situated in +Y position. Adoral ciliary spiral turns about 370°—380° around peristomial disc.

Population 3 from Huilongba County of Chongqing, China

Date of sampling: April, 2004.

Description (Plate III-13,14; Plate IV-17): Medium to large-sized freshwater *Trichodina*; cell diameter 50.0—55.0μm (52.6 ± 1.8). Diameter of adhesive disc 42.0—47.0μm (43.6 ± 2.1). Central zone of adhesive disc relatively clear and with uniform and oval granules. Border membrane 4.0—5.0μm (4.4 ± 0.5) in width. Diameter of denticulated ring 25.0—30.0μm (27.6 ± 2.3). Number of denticles about 30 (12). Number of radial pins per denticle 8—10 (12). Span of denticle 14.0—17.0μm (15.4 ± 1.3). Length of denticle 5.0—7.0μm (6.0 ± 0.6). Blade broad and rectangular, 6.0—7.0μm (6.4 ± 0.5) in length. Distal blade surface straight and smooth, nearly at the same leval as tangent point. Anterior and posterior blade surface straight and parallel to each other. Apex of blade always extending past Y+1 axis. Apophysis of blade unseen. Central part relatively developed and width of central part 1.0—2.0μm (1.7 ± 0.2). Ray relatively thin and not smooth and nearly parallel to Y axis. Length of ray 5.0—8.0μm (6.8 ± 1.3); ray apophysis rarely seen. Macronucleus horseshoe-shaped, and micronucleus oval, situated in +Y position. Adoral ciliary spiral turns about 370°—390° around peristomial disc.

Discussion: Up to the present time, *T. reticulata* has been described from different hosts in the world, mostly cyprinids and in the majority of cases from the genus *Carassius*. It was originally described from the USA by Hirschmann & Partsch (1955) [21], then, *T. reticulata* has been reported from USSR, Eastern Europe, Iran, North Korea, Japan, China, Indonesia, Israel and South Africa[22]. Nevertheless it is recognized as an Asian species that has distributed widely via the introduction of *Carassius* species [23]. However, the different populations of *T. reticulata* reported before show some variation in body dimensions. Not only the same case can be found in our populations, but also morphology variation of adhesive disc can be observed in our populations.

Among our three populations, population 1 is the largest one in body size. No matter what body size, granules of central zone, and denticle morphology, population 1 corresponds well with the descriptions by Lom (1960), Kazubski (1982) and Basson *et al.* (1983, 1993) [22, 24-26], and this is a most common and widely distributed population.

Population 2 is the smallest one in body size relatively, morphometric data of the population fall well within the range of population provided by Basson & Van As (1993) [22]. However, based on the granules of central zone and denticle morphology, this population shows minor difference from those ones as mentioned above. With exception with some spherical granules, there are minor and oval ones in central zone in this population. In terms of shapes of blade, the character with upper and lower part of blade broad and middle part relatively narrow presents in this population. Nevertheless, population 2 corresponds well to the other populations in overall morphology of adhesive disc.

Population 3 in our study corresponds well to the population described by Chen (1963) in body size and denticle morphology, such as in these two populations, and the smooth denticle, approximately rectangular blade and slim ray can be clear observed[27], so this is relatively uncommon population.

Despite of those intro- or inter-populations variation present in three populations, *T. reticulata* can be also easily recognized by the diagnostic feature that there are 8 to 16 spherical or oval granules in central zone of adhesive disc and this important point can be clearly

proved in our study.

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重庆地区鲫外寄生车轮虫形态分类学研究及一新种的描述

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摘要: 在对重庆地区进行鱼类寄生虫学的调查过程中, 从鲫鳃表获得 3 种隶属于车轮虫属 *Trichodina* Ehrenberg, 1830 的外寄生车轮虫, 其中含一新种, 短棘车轮虫 *Trichodina brevicirra* sp. nov, 其典型的鉴别性特征为其短细的齿棘。另外两种车轮虫分别为沃玛车轮虫 *Trichodina ngoma* Van As & Basson, 1992 与网状车轮虫 *Trichodina reticulata* Hirschman & Partsch, 1955; 沃玛车轮虫为亚洲新记录种。文章对网状车轮虫的 3 种群进行了详尽的比较讨论研究, 尽管该三种群间存在着细微的种群内差异, 但网状车轮虫的典型特征则显而易见地存在于三种群间, 即为附着盘中央具有 8—16 个球状或卵圆形的中央颗粒。

关键词: 车轮虫; 鲫; 短棘车轮虫; 新种; 重庆

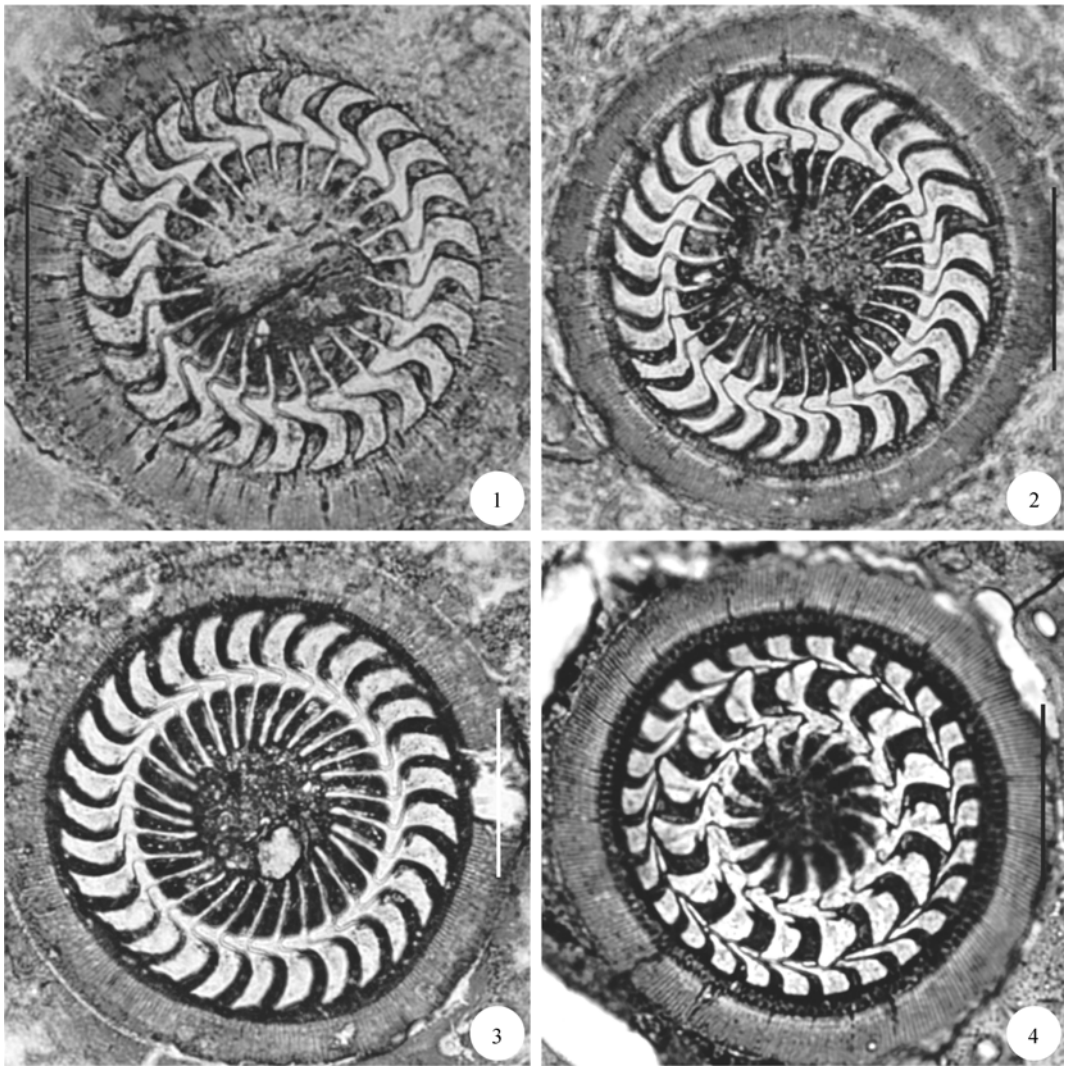

Plate I Photomicrographs of silver impregnated adhesive disc of trichodinids

1, 2. *Trichodina brevicirra* sp. nov.; 3, 4. *Trichodina ngoma* Van As & Basson, 1992: 3. Adult cell with developed denticles; 4. Daughter cell: blades in process of development whilst old ring is being resorbed. (Scale bar = 20 μm)

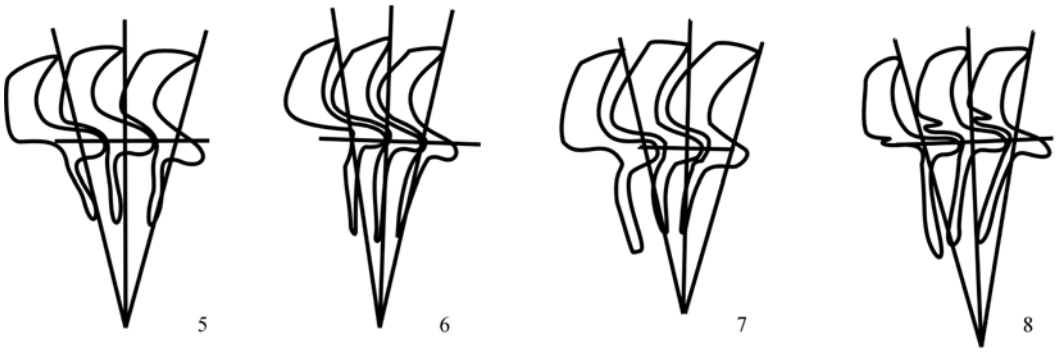

Plate II Diagramatic drawing of the denticles of trichodinids

5. *Trichodina brevicirra* sp. nov.; 6. *T. paranigra* Tang, Zhao & Chen, 2005; 7. *T. cobitis* Lom, 1961; 8. *T. ngoma* Van As & Basson, 1992

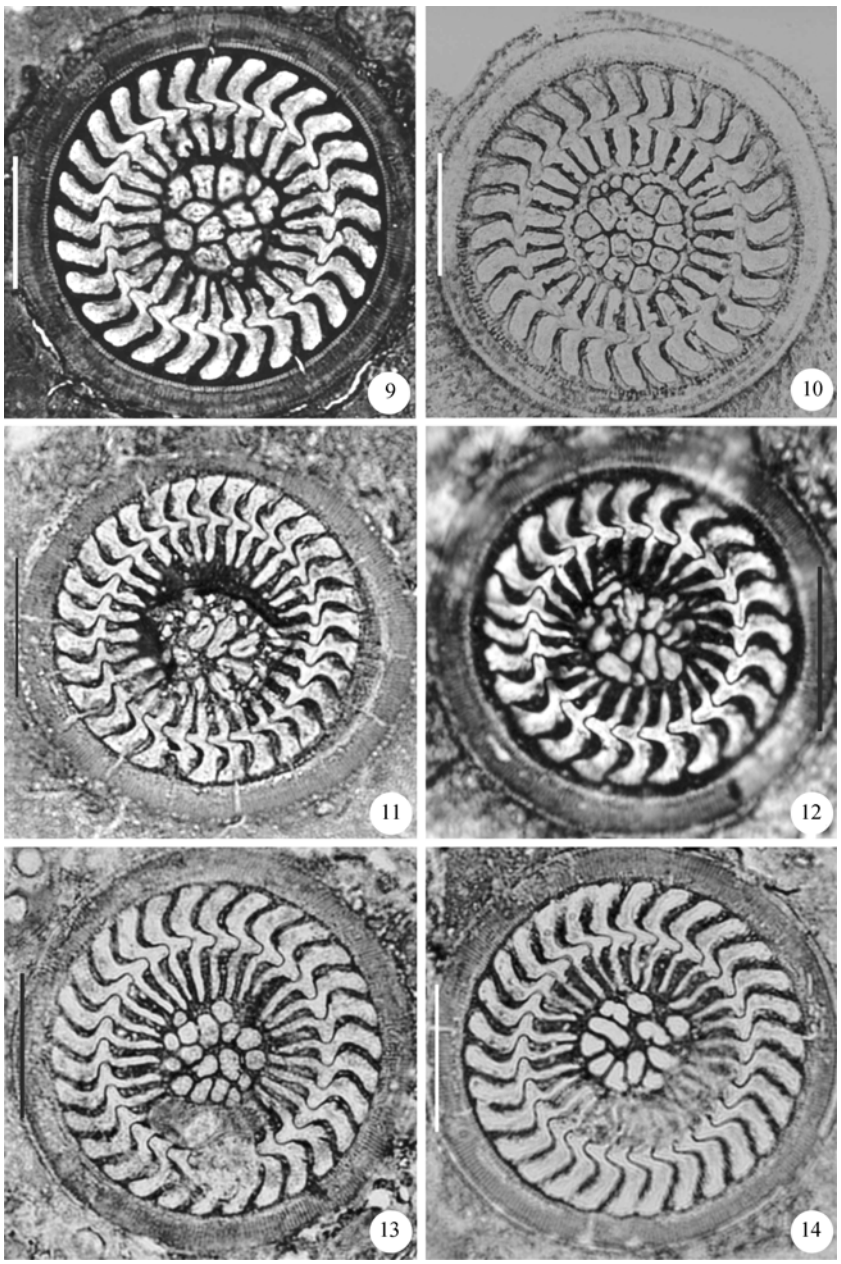

Plate III　Photomicrographs of silver impregnated adhesive disc of *Trichodina reticulata* Hirschman & Partsch, 1955
9, 10. Population 1; 11, 12. Population 2; 13, 14. Population 3 (Scale bar = 20 μm)

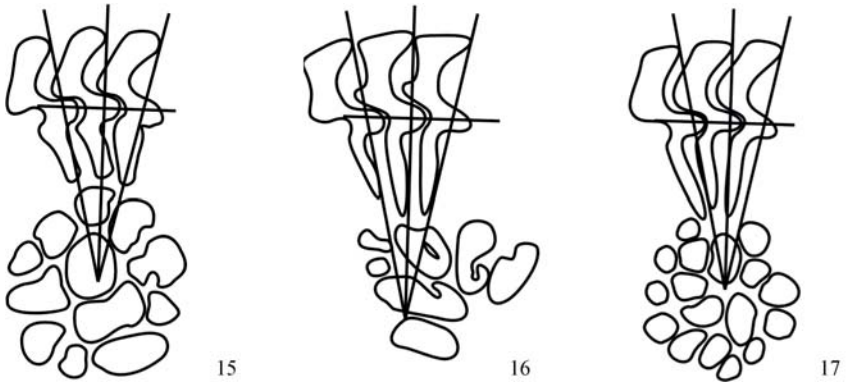

Plate IV　Diagramatic drawing of the denticles of *Trichodina reticulate*
15. Population 1; 16. Population 2; 17. Population 3