Morphology and Life History of *Trichodina reticulata* from Goldfish and Other Carps

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(Received March 17, 1977)

日本のキンギョとコイの体表、鰭および鰓の粘液から得られた *Trichodina reticulata* の詳細な形態と生活史を検討した。

体は一般にドーム形で直径 50~90 μ 、体高は 15~35 μ である。吸着盤は直径 44~80 μ 、歯状突起環の直径 42~72 μ 、馬蹄形の太核は 22.5~49 μ 、その厚さは 6~7 μ である。歯状突起の数は 24 と 27 が最も多かった (範囲 20~31)。各歯状突起当りの線条数は 10 が最も多かった (範囲 7~10)。歯状突起の計測値は以下の通りである：外側突起の長さ 4.5~7.5 μ 、内側突起の長さ 5.5~7.5 μ 、中央円錐部の長さ 2.5~4 μ 、歯状突起の長さ 5~11 μ 。境界膜の幅は 3.5~6 μ である。小核 (7~15 $\times$ 2~5 μ) は大きく、紡錘形で、太核の右腕の外側の浅いくぼみの中にある。吸着盤の中心部はいくつかの細胞様構造に分かれている。収縮胞および食胞は明瞭である。縁膜は繊細で透明である。運動は縁膜と口外繊毛と小膜によって行なわれる。2本の繊毛帯は通常と同じである。口外繊毛帯は約 370° のらせんを形成している。

二分裂はまず虫体が拡大することから始まる。次に、太核は短かく厚くなり、クロマチンが任意に分布するようになり、2つの垂鈴形の核に等分する。小核は大きくなり、有糸分裂する。分裂の開始に先だって、新しい歯状突起の原基が旧環と吸着盤の辺縁の間にあらわれる。亀裂が縁膜中にあらわれ、縁膜の下の方の線条帯中に裂け目ができる。収縮胞は分裂して、各娘細胞に対し、1個の収縮胞を形成する。分裂後、半数の歯状突起を持つ旧環はしだいに消失し、その約2倍の歯状突起が新たにその外側に発達してくる。線条と繊毛も同様にものと数を有するようになる。

同形および異形の接合がしばしば認められた。経接合個体は大きく、1個の機能を有する核、即ち融合核を持ち、それは3回分裂し、8個の小核を形成するが、そのうちの7個は原基であり、1個が小核としての機能を有する。細胞分裂は各娘細胞が1個の大核原基と1個の小核を有するまで続く。この場合、娘細胞の新しい環に形成される歯状突起の数は旧環の時と同じである。

Trichodina reticulata HIRSCHMANN and PARTSCH (1955) was isolated from the mucous of the body surface, fins and gills of goldfish (*Carassius auratus*), crucian carp (*Carassius auratus*) and coloured carps (*Cyprinus carpio*) of Japan by the author (AHMED, 1976). DOGEL (1940), ŠTEJN (1954) and LOM (1960) also reported this trichodinid from crucian carp. These investigators were dealing mainly with the taxonomical features of the ciliate and transmission experiments. Detailed morphology and reproduction of only a few trichodinids are known, and those of *T. reticulata* is yet to be given. In his previous paper the author (AHMED, 1976) reported on the general morphology, taxonomy and the disease (Trichodiniasis) caused by *T. reticulata*

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to the goldfish and other carps of Japan and also some of the control measures.

The present paper is concerned with the detailed morphology and life history of *T. reticulata*. The study was undertaken also to elucidate the economic importance and the biological aspect of the parasite.

Different aspects of the morphological features, separating the species from the closely related forms e.g. *T. domerguei*, *T. pediculus* and *T. tenuidens*, were noticed. *T. reticulata* reproduces principally by binary fission and sometimes by conjugation.

Materials and Methods

Trichodina reticulata, isolated from the mucous of body surface, fins and gills of goldfish and other carps were collected regularly from goldfish farms of Edogawa, Tokyo and

occasionally from other farms of Japan, for about two years.

The fish were caught by dip-nets and were kept in the laboratory aquarium of Tokyo University for a short period until observations were made. The parasites were preserved (with infected gills, fins and body slime) in 4% neutral formalin.

Live specimens were also studied. Smear preparations made from the scrapings of the parasitized fish were fixed in Schaudinn's solution, and were stained with Heidenhain's iron hematoxylin and counter stained with eosin. Sections of the ciliates were made by introducing them in summer orange cells. Paraffin sections varying from 2–5 μ in thickness were made in the usual way and stained with the former stains. For precise identification of the ciliate, Chatton-Lwoff's silver nitrate impregnation technique was employed.

Results

Morphology

Shape of the body: The general shape of this trichodinid ciliate is dome-shaped or hemispherical when free, but disc-like when attached and sucking the host (Figs. 1, 2 and 5). The convex, adoral (anterior) side is elevated or dome-shaped while the concave, aboral (posterior) side forms a complicated attachment organ, the sucking or adhesive disc. The diameter of the body ranges from 50–90 μ with an average of 76 μ . The height of the body is 15–35 μ .

Structure of sucking disc: The disc (Figs. 1, 3 and 4) is the most prominent part of the organism and is formed of two sets of structures; (i) The very complicated sucking (cuticular) skeletal structure, arranged in the

form of three concentric rings and (ii) the locomotor structures including membranelles, cilia (cirri) and the velum. The diameter of the disc is 65 μ (range 44–80 μ) in average.

The denticulate ring or corona: It is the most conspicuous part located on the inner surface of the disc and is made up of 24, 27 (range 20–31) horny elements or denticles, arranged like beads in a necklace (Figs. 3 and 4). Each denticle is shaped like a hollow cone with the smaller end of the cone inserted into the cavity of the adjacent denticle, an arrangement admirably adapted to give both flexibility and strength to the ring as a whole. The diameter of the ring is 45 μ (42–72 μ). The length of the denticle is 5–11 μ and the width of the central conical part is 2.5–4.5 μ .

Blade (hook): On the outer side toward the circumference of the disc each denticle bears a flattened, blade-like structure, the blade or hook (Fig. 3). It is concave on one edge and convex on the other. The concave side is thicker and stains more intensely, while the convex side is thin. The length of the blade is 4.5–7.5 μ (dispersion 3.5–8.5 μ).

Ray: On the inner side of the denticle and approximately opposite the blade is a tooth-like process, the ray (thorn) which projects toward the center of the disc (Figs. 3 and 4). The length of the thorn is 5.5–7.5 (dispersion 3–12.5 μ). The center of the disc is filled with protoplasm of cell-like appearance (Fig. 3) which may also be seen in the living material. This separation of the central light zone into several fields of cell-like structure (distinct when impregnated with AgNO_3) is the most characteristic feature of the species.

Radial pins on the striated membrane:

Text figures: The process of conjugation in *T. reticulata*. 1, Start of conjugation. Macronucleus fragmenting. 2, Micronucleus dividing and macronucleus fragmented into spherical and oval-shaped bodies of various sizes. 3, Gametic nuclei in an early stage of fusion to give rise to the synkaryon and cytoplasmic continuity between the conjugants is established. 4, Cytoplasmic contents of the microconjugant pass into the macroconjugant. The new denticulate ring and vacuole develop while the old ring is reabsorbed. 5, Exconjugant (Macroconjugant) with large zygotic nucleus. 6, Development of seven macronuclear anlagen indicates that three divisions of the zygotic nucleus take place, the eighth, remaining as functional micronucleus, is dividing. 7a and 7b, Daughter cells produced by first cell division resulting into three and four macronuclear anlagen distribution. 8a and 8b, Daughters resulting from second division. 9a and 9b, Daughters resulting from third division, with macro and micronuclei. One showing the development of other structure.

The circular ribbon-like structure overlapping the blades on the adoral side extending toward the outer margin of the disc, known as the striated band appears as a series of radiating line extending from the denticulate ring to near the edge of the disc. These lines are formed by long, slender pins (rods) lying just above the blades and extending for about half their length beyond the ends of these structures (Fig. 4). The outer edge of each pin is attached to the membrane which covers the surface of the adhesive disc. The number of radial pins per denticle is about 10 (7-10). The distinct line at the outer edge of the striated band, separating it from the third and outer ring, forms the border of the adhesive disc or main cuticular ring (Fig. 3).

The border membrane or outer cuticular ring: It is a thin flexible membrane joining the striated band by an articulation upon which it moves freely (Figs. 3, 4 and 5). The membrane is $3.5-5\ \mu$ wide, densely and finely striated than the striated membrane, but striations are not continuous with those of striated band and the number is usually somewhat greater. It serves for a faster adherence of the ciliate to the substrate, contracting towards middle.

Velum: A thin marginal fold of granular cytoplasm forming the outer edge of the body proper, the velum (Figs. 3 and 5), is a delicate transparent membrane lying just anterior to the base of marginal cilia.

Cilia: The cilia occur as (i) the adoral ciliary girdle or oral ciliary ring (double rows) at the oral end of the body, arise from the floor of a narrow, deep groove which surrounds the base of the dome (Figs. 1 and 5). The adoral cilia continue into the cytopharynx, and are arranged in the form of membranelles. The cilia, except the inner tuft, beat continuously and function only to draw food particle into the body. (ii) Marginal cilia (Figs. 4 and 5). Under the lower velum margin there is a shallow groove to which is attached a band of short and delicate cilia forming the so called marginal ciliary band. (iii) Aboral ciliary girdle (Fig. 5), occurring beneath the marginal cilia, consists of two rings: one inner ring of shorter and

softer cilia placed closely behind the border membrane, and the other ring of strong and long cilia, successively arranged membranelles. These cilia are the main locomotor organelles.

Locomotion: The organelles responsible for locomotion are the membranelles, the vibratile aboral cilia (cirri) and the velum. *T. reticulata* which sucks the body surface and tips of the gills, attaching itself by the powerful sucking disc, often leaves the host and swims freely in water. It moves rapidly with the aboral surface forward. This broad side method of swimming enables the ciliate to affix itself to any new host without delay. The membranelles provide for gliding movement over the surface, the cilia provide for an entirely different three-dimensional motion through the water, and the velum contributes to the slow revolving motion of the ciliate.

The vestibule or mouth (Figs. 1 and 5) is conical, the opening being elliptical, proceeds inward from the edge of the peristomal disc and curves to the left. The vestibule opens into a small bulb-like cytopharynx (gullet) which extends some distance into the body (Fig. 5).

Vacuoles: The contractile vacuole (Fig. 6) is large, conspicuous and clear vacuole, situated slightly excentrically and usually near the end of cytopharynx. The wall of the vacuole is composed of modified endoplasm and is connected with the vestibule by a narrow excretory duct. The food vacuoles (Fig. 5) are usually numerous throughout the body, generally located more toward the periphery than near the center of the cell, and contain granules of various sizes consisting of cellular particles and sometimes erythrocytes from the host.

Course of adoral zone: The cytopharynx bears to parallel rows of long hair-like cilia, following spiral course and are continued through the vestibule into a groove which also follows a spiral course clockwise around the adoral surface, known as the adoral spiral (Fig. 7). This plays a certain role in generic determination in *Trichodina* and in *T. reticulata* it forms a spiral of about 370° .

Nuclear apparatus: The large regularly horse-shoe shaped macronucleus (Figs. 7 and

11) is $22.5\text{--}49\ \mu$ ($45\ \mu$) in diameter and $6\text{--}7\ \mu$ thick. It is the most conspicuous object in the endoplasm and is filled with fine granular chromatin material. The open ends enclose the vestibule and it is so located that the cytopharynx and the duct leading from the contractile vacuole lie between the open ends of the horse-shoe.

The micronucleus (Figs. 6, 7, 9 and 11) is unusually large ($7\text{--}15\ \mu \times 2\text{--}5\ \mu$), compact, spindle shaped and the value of y distance being $+5\text{--}25\ \mu$. It is located adjacent to the right arm of the macronucleus in a shallow depression on the outside and contains a deeply staining central body (endosome) and separated from the delicate membrane by a clear area (Fig. 6).

Reproduction

Binary fission: The first indication of division is the enlargement of the ciliate accompanied by the increase in the homogeneity of the cytoplasm. Gradually re-differentiation of organelles takes place, new structures appear while the old are still functioning, e.g. the denticulate ring. The process can conveniently be followed by tracing the fate of each organelle individually:

Macronucleus. After the enlargement of the cell, the macronucleus loses its shape, becomes shorter and thicker and begins to condense, while the chromatin granules become smaller in size and randomly distributed in the matrix. The form becomes more and more irregular, finally becoming spherical. The macronucleus then divides, becoming dumbbell-shaped, and then pulls apart into two equal halves (Fig. 8).

Micronucleus. As the macronucleus begins to change in shape, the micronucleus swells, eventually becoming spherical (Fig. 8). It then divides mitotically. The division continues as the macronucleus separates, and the two daughter micronuclei are formed prior to the complete division of the macronucleus. The whole process of fission is probably initiated by the micronucleus.

Denticulate ring. Prior to the onset of cell division, the anlage of the new denticulate ring (corona) of the prospective daughter cells appear as a delicate ring close to the

posterior border of the adhesive disc between the old corona and circumference of the disc. When the cell constricts each ring forms a figure 8 (Fig. 9) and after division the old inner ring, with half the normal number of denticles, gradually disappears.

Velum. It gradually becomes thickened and the margin somewhat irregular. This is accompanied by the appearance of slight indentation in the velum and the aboral side, which marks the beginning of the constriction. This constriction eventually divides the animal into two equal parts (Fig. 10).

Striated band. While the above changes are taking place, breaks appear in the striated band immediately beneath the dentations in the velum which eventually extends to the denticulate ring. The plane of division coincides with that of nuclei and divides the ciliate into two equal halves (Figs. 9 and 10).

Vacuole. During division the old large contractile vacuole disappears, while just before separation a new one appears and apparently divides to produce one for each daughter cell.

The two daughter individuals resulting from the fission are identical with the parent excepting that they are only one half the size and contain only half the number of parental skeletal parts. The adoral and aboral ciliary bands are retained but the vestibule and cytopharynx disappear during the fission. The macro and micronuclei regain their original shape and position (Fig. 11). The old corona is divided into two parts in such a way that each piece contains approximately the same number of denticles. In the daughters the broken ends round up so as to form almost complete circles again (Figs. 10 and 11). About twice as many denticles are developed in the new outer corona as are present in the old inner parent ring and thus the original number is restored. At first the cone is formed, then the outer blade and lastly the rays (Figs. 11 and 12). Simultaneously the old ring is pushed into the central plug of the cytoplasm and are gradually absorbed, eventually disappearing entirely. The possession of two coronas is a criterion for identifying recently formed daughters. Again, as the

striated band is also divided into one-half in number during the fission, obviously the number of pins in the band is reduced accordingly. The original number, however, is restored by the formation of new pins between those of the original bands. A faint line appears between each striation (Fig. 12) which gradually increase in density until it is indistinguishable from the older striations on either side.

Conjugation: Reproduction by conjugation was observed in *T. reticulata*, usually in autumn and spring. In general, the process resembles very much to those described by PANDOS and NIGRELLI (1942) in *T. spheroidesi* and DAVIS (1947) in different fresh water species of *Trichodina*. The sequence of events in the process of conjugation is shown diagrammatically in the text figure.

The aboral surface of the microconjugant (when anisogamous) is fitted over the adoral surface of the macroconjugant (Fig. 13). After the conjugants have assumed their respective position, the micronucleus then begins to swell, becomes vesicular and passes from the original posterior to the more central location in the cell, and eventually divides mitotically (Text figs. 1 and 2). At the same time the macronucleus shows signs of vacuolization and breaks down into large coarse fragments. This continues to break up into smaller and smaller parts until minute spherical bodies with deeply staining granules are formed (Text figs. 2 and 3). During the final fragmentation of the macronucleus, two micronuclear divisions take place in each conjugant. With the micronuclear divisions the protoplasm of the two conjugants, becomes continuous and the contents of the microconjugant passes into the macroconjugant (Text figs. 3 and 4). It is assumed that the gametic nuclei then combine to form the synkaryon, and the remaining nuclei are reabsorbed. The remains of the microconjugant collapse and the ensuing processes of conjugation is confined to the single large exconjugant (Fig. 14 and Text fig. 5).

The exconjugant now contains only one functional nucleus, the synkaryon and re-

mains of the broken down macronucleus, and degenerating micronuclei. The zygotic nuclei is assumed to divide three times, resulting in the formation of eight micronuclei. The old corona persists at least until the end of the second division and is then reabsorbed. Seven of the micronuclei become the macronuclear anlagen and one functional micronucleus (Text fig. 6). The latter divides in the usual way and in the division of the cell the macronuclear anlagen are distributed between the daughter cells. The most frequent distribution is three (Text fig. 7a) and four (Fig. 15 and Text fig. 7b), but sometimes may be two and five or even one and six. In any case cell division continues until each of the daughter individuals contain one macronuclear anlagen and a micronucleus. The macronucleus then increases in size and assumes the characteristic horse-shoe shape (Fig. 16 and Text figs. 9a and 9b).

It may be mentioned here that reorganization of the denticulate ring occurs in the macroconjugant shortly after fusion of the protoplasmic contents of the two daughter conjugants. A new ring is formed outside the old in the same manner as in fission, but in this case the number of denticles in the new ring of the daughters is the same as in the old (Fig. 17).

Discussion

More than sixty seven species of the genus *Trichodina*, excluding eight somewhat different forms, have been described. But detailed morphology and life history of only a few viz. *T. pediculus* (FULTON, 1923), *T. domerguei* (MACLENAN, 1939), *T. urechi* (NOBLE, 1940), *T. spheroidesi* (PANDOS and NIGRELLI, 1942), *T. discoidea*, *T. symmertica* and *T. californica* (DAVIS, 1947) and *T. branchicola* (TRIPATHI, 1948) have been studied. *T. reticulata* was often confused with *T. domerguei*, *T. pediculus* and *T. tenuidens* and sometimes used as a synonym of *T. domerguei* f. *megamicronucleata*. But the development of Klein's and Chatton-Lwoff's AgNO₃ impregnation technique has solved this problem. Besides, their differences in biometrical measurements, the AgNO₃ impregnated sucking disc

of *T. domerguei* has a central clear area with undulated border, dotted with more or less numerous dark granules. While the central area of *T. reticulata* is partitioned into several fields of cell-like structures (Fig. 3). *T. pediculus* has a small, clear central area and comparatively longer rays of the denticles. *T. tenuidens* has some similarities in the arrangement of the central area, cell-like structure being present, but the higher number of denticle (28-36), the shape of the thorn which is quite thin at the center and broad at its end, the slender central conical part and the elongated blades are quite different from those of *T. reticulata*. Again, considering the host specificity observed by LOM (1960) the author considers *T. reticulata* a completely different species.

The sucking disc has been termed by many authors (MACLENNAN, 1939; DAVIS, 1947 etc.) as basal or adhesive disc. But as the author (AHMED, 1976) has already established the sucking habit of the parasite, both the terms of sucking as well as adhesive disc could be used in case of *T. reticulata*. MACLENNAN (1939) used the term cuticular rings for rings in the adhesive disc.

The large spindle-shaped micronucleus, situated in a shallow depression at the external surface near the end of the right arm of the macronucleus (Fig. 7) is also a characteristic feature of *T. reticulata*. It resembles *T. pediculus* and sometimes *T. domerguei* in respect of its situation, but in the latter cases the micronuclei are shorter and oval in shape and the macronuclei are thicker than *T. reticulata*.

Binary fission is the principal method of multiplication in *T. reticulata* and was observed throughout the year. The rate of fission increases during the summer, when individuals undergoing fission are very common. This may be due to the fact that increase in temperature to certain level increases the rate of fission. In the laboratory condition (in aquarium) it was observed by the author that in 18-23°C temperature range *T. reticulata* undergoes rapid fission.

Binary fission in some other trichodinids was described by several authors (PESHKOW-

SKY, 1923; DILLER, 1928; NOBLE, 1940; PANDOS and NIGRELLI, 1942 and DAVIS, 1947). The author's observations are essentially the same as the above authors excepting the following points:

PESHKOWSKY (1923) reported that during the fission of *T. steinii* and *T. mitra* the adoral cilia, gullet and contractile vacuole were absorbed. Careful study of the silver nitrate impregnated material shows the retention of adoral and aboral cilia in *T. reticulata*. DILLER (1928) and TRIPATHI (1948) reported the persistence of the old vestibule in one of the daughter cells of *Trichodina* sp. from tadpole and *T. branchiocola* respectively. In the present study the fate of vestibule and cytopharynx however could not be traced. The contractile vacuole reappears and divides with the macronucleus at the same time. According to FULTON (1923), DILLER (1928) and PANDOS and NIGRELLI (1942) the new denticulate ring is laid down before the onset of division. While NOBLE (1940) observed no indication of denticulate ring before or during actual cleavage. The author had findings like the former investigators (Fig. 10).

Conjugation. PANDOS and NIGRELLI (1942) emphasized the fact that the conjugation in *T. spheroidesi* is anisogamous. While DILLER (1928) found isogamous type of conjugation in *Trichodina* sp. from tadpole. The author observed both the conditions. It seems probable that where the conjugants differ in size, this difference is due to their relative age. Microconjugant is younger than the macroconjugant since the striations in the striated band stains deeply in the macroconjugant. On the other hand the condition of striated band in isogamous conjugants indicates that they are approximately of the same age.

DILLER (1928) described the nuclear changes in *Trichodina* sp. which he believed to be endomixis. The author is in complete agreement with the conclusions of PANDOS and NIGRELLI (1942), that is the endomictic stages described by DILLER conform closely to the exconjugative stages in *T. reticulata* and that exconjugation was misinterpreted as endomixis.

Summary

Detailed morphology and life history of *Trichodina reticulata*, isolated from the mucous of body surface, fins and gills of goldfish and other carps of Japan, were studied.

Generally dome-shaped body measures from 50–90 μ in diameter and height of the body is 15–35 μ . The diameter of sucking disc is 44–80 μ and that of the denticulate ring 42–72 μ and the horse-shoe shaped macronucleus 22.5–49 μ , the thickness being 6–7 μ . Number of denticle is most often 24 and 27(20–31) and radial pins per denticle is 10(7–10). Dimensions of denticle: length of blade 4.5–7.5 μ ; length of ray 5.5–7.5 μ ; width of central conical part 2.5–4 μ and length of denticle 5–11 μ . Width of border membrane 3.5–6 μ . Large spindle-shaped micronucleus (7–15 $\times$ 2–5 μ) lies in a shallow depression on the outside of right arm of the macronucleus. The central part of the sucking disc is separated into several fields of cell-like structures. Both contractile and food vacuoles are distinct. Velum is delicate and transparent. The ciliate moves by means of velum, aboral cilia and the membranelles. Both ciliary girdles lie in usual manner. Adoral zone forms a spiral of about 370°.

Binary fission starts with the enlargement of the ciliate, changes in shape of the macronucleus, becoming short and thick with randomly distributed chromatin; finally dividing into two dumbbell-shaped equal halves. The micronucleus swells and divides mitotically. Prior to the onset of fission the anlage of new denticulate ring appears between the old ring and circumference of the disc. Indentations appear in the velum and breaks appear in the striated band below the velum. Contractile vacuole divides to produce one for each daughter cell. After division the old ring with half the number of denticles gradually disappears and about twice the number of denticles are developed in the new outer ring. Similarly radial pins and cilia also restore original number.

A few cases of conjugation were found where both iso and anisogamous types were observed. The large exconjugant contains one func-

tional nucleus, the synkaryon, which divides three times and thus forming eight micronuclei, seven of them micronuclear anlagen and one functional micronucleus. The cell division continues until each of the daughter individuals contain one macronuclear anlage and a micronucleus. In this case the number of denticles in the new ring of the daughters is the same as in the old ring.

Acknowledgements The author acknowledges his sincere gratitude to Professor S. EGUSA of the Department of Fisheries, Faculty of Agriculture, University of Tokyo, for his guidance and advice throughout the work. The author is also much indebted to Dr. H. WAKABAYASHI of the above department and Dr. K. MUROGA of the Department of Fisheries, University of Hiroshima, for their technical assistance.

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Explanation of Figures

Abbreviations: ab.c., aboral cilia; ad.c., adoral cilia; ad.s., adoral spiral; b, blade; b.m., border membrane; c.c.p., central conical part; cp., cytopharynx; c.v., contractile vacuole; dt., denticle; dt.r., denticulate ring; f.v., food vacuole; ma., macronucleus; Ma.c., macroconjugant; m.c., marginal cilia; m.c.r., main cuticular ring; mi., micronucleus; Mi.c., microconjugant; mng., macronuclear anlagen; n.r., new ring; o.r., old ring; r., ray; r.p., radail pin; ve., velum; vt., vestibule.

- Fig. 1.** Microphotograph of the side view of a fresh *T. reticulata* showing shape of the body and other structures. $\times 400$.
- Fig. 2.** *T. reticulata* (live) attached to the body surface. $\times 310$.
- Fig. 3.** AgNO_3 impregnated adhesive disc with distinct denticles and central cell-like structures. $\times 460$.
- Fig. 4.** A fresh specimen with distinct denticulate ring (corona), radial pins, marginal cilia and border membrane. $\times 650$.
- Fig. 5.** Vertical section through cytopharynx showing body shape, food vacuole, ciliary girdles and other morphological structures. Stained. $\times 860$.
- Fig. 6.** Section through micronucleus and contractile vacuole. Stained. $\times 420$.
- Fig. 7.** A stained preparation showing macro and micronuclei and adoral spiral. $\times 660$.
- Figs. 8-12.** Successive stages in binary fission.
- Fig. 8.** Condensed macronucleus dividing, constriction almost complete. Micronucleus spherical. Stained. $\times 500$.
- Fig. 9.** 8-shaped body showing the division of denticulate ring. Micronucleus and macronucleus already divided. Fresh. $\times 550$.
- Fig. 10.** Daughter cells almost separated and denticulate rings closing. Fresh. $\times 380$.
- Fig. 11.** A recently formed daughter cell with distinct macro and micronuclei. New denticulate ring reorganizing with almost double the number of blades, overlapping the old ring. Fresh. $\times 500$.
- Fig. 12.** AgNO_3 impregnated adhesive disc of an older daughter with well developed blades and radial pins. Rays yet rudimentary. The old ring is being reabsorbed. $\times 740$.
- Fig. 13.** Conjugating pair (anisogamous) viewed from low lateral side. The macroconjugant is an adult while the microconjugant is a juvenile. Fresh. $\times 420$.
- Fig. 14.** Exconjugant with initial stages in the development of macronuclear anlagen. Stained. $\times 350$.
- Fig. 15.** A daughter cell with four macronuclear anlagen, a functional micronucleus and rounded fragments of original macronucleus. Stained. $\times 550$.
- Fig. 16.** A daughter cell with horse-shoe shaped macronucleus. Stained. $\times 570$.
- Fig. 17.** A later stage than Figure 16 in which a new denticulate ring is developed with the same number of denticles as the old. Fresh. $\times 550$.