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Studies on Ciliates from Mollusks of Iowa

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I. Introduction

The ciliates which live a commensal life on the gills and in the mantle cavities of mollusks include several species of peritrichs, but most of them belong to a suborder of holotrichs called Thigmotricha (Chatton and Lwoff, 1922). Very little work has been done on the geographical distribution and host-specificity of the ciliates of mollusks. Emphasis has been placed rather upon the taxonomic and morphological features.

Most of the early work was published in Germany (Ehrenberg, 1838; Stein, 1861; Englemann, 1863; Quennerstedt, 1867; and Schuberg, 1889). Chatton and Lwoff (1922, 1923, 1926, 1949, 1950) have made significant contributions to the taxonomy and morphology of the ciliates from mollusks of France. Issel (1903) has made observations on ciliates from gastropods and amphineurans in Italy, and Jarocki (1934, 1935) and Raabe (1933, 1934) have studied ciliates from the terrestrial snails of Poland. Uyemura (1935) has investigated ciliates from mussels of Japan, and Rossolima and Jakimowitsch (1929) have studied the life cycle of *Myxophyllum steenstrupi* from terrestrial snails of Russia. Kidder (1933, 1934), Kozloff (1945, 1946), Reynolds (1936), MacLennan and Connel (1931) and others have made observations on the mollusks of the east and west coast of the United States. DeMorgan (1925) and Mackinnon and Ray (1930) have studied the mollusks of Great Britain, and Ghosh (1918) has made observations on ciliates from the mollusks of India. These and other workers have contributed much to our knowledge of the ciliate fauna of mollusks. However, there are many species of mollusks in all parts of the world whose ciliate fauna has not been examined.

This report presents data on the ciliate fauna of nine species of mollusks collected in Iowa. Emphasis is placed upon morphology, reproductive activities, and host-parasite specificity. There has been previous work on the species of ciliates reported, but as far as is known these ciliates have not been previously reported from the mollusks listed herein.

II. Materials and Method

The mollusks used in this study were collected in Iowa. Table I gives a summary of the species of mollusks collected, the number

examined, the locality, and the parasites found. One snail, *Triodopsis multilineata* (Say) was collected in City Park, Iowa City, Iowa. Another snail, *Helisoma trivolvis* (Say), was collected by Mr. Henry Turner, Iowa State College, Ames, Iowa, in Ledges State Park, Boone County, Iowa. The mussels, *Lampsilis siliquoides* (Barnes) and *Anodonta grandis* (Say), and the snails, *Oxyloma decampi gouldii* Pilsbry, *Lymnaea palustris* (Müller), *Physa gyrina* (Say), and *Zonitoides arboreus* (Say) were collected from several locations near

Table 1

Host	No. Examined	Iowa Locality	Organism found
Mussels			
Anodonta grandis (Say)	150	Trumble Lake Clay County	*Conchopthirius curtus* Englemann
Lampsilis siliquoidea (Barnes)	45	Miller's Bay Dickinson Co.	*Conchopthirius curtus* Englemann
Terrestial Snails			
Anguispira alternata (Say)	74	Milford Woods Dickinson Co.	*Myxophyllum steenstrupi* (Stein)
Triodopsis multilineata (Say)	100	City Park Iowa City Johnson County	*Myxophyllum steenstrupi* (Stein)
Oxyloma decampi gouldii Pilsbry	70	Silver Lake Fen Dickinson County	*Myxophyllum steenstrupi* (Stein)
Zonitoides aboreus (Say)	55	Lakeside Lab. Dickinson Co.	No organisms
Aquatic Snails			
Lymnaea palustris (Müller)	13	Crossroad Pond Dickinson Co.	No organisms
Physa gyrina (Say)		Crossroad Pond Dickinson Co.	*Trichodina* sp.
Helisoma trivolvis (Say)	39	Ledges State Park, Boone Co.	*Trichodina* sp.

the Iowa Lakeside Laboratory in northwestern Iowa (see Table 1).

The mussels were brought to the laboratory and placed in tanks supplied with lake water which was changed daily. The ciliates were obtained by opening the shell, and pouring the contents of the mantle cavity into a Petri dish. A fine pipette and a dissecting microscope were used to select individuals for study and staining.

The organisms were very active; they usually moved over the bottom surface of the container, and remained alive from twenty to twenty-four hours in the fluid from the mantle cavity of the mollusks.

Myxophyllum steenstrupi (Stein) was found in the body slime of the following snails: *Oxyloma decampi gouldii* (Say), *Anguispira alternata* (Say), *Triodopsis multilineata* (Say).

The shells of these terrestial snails were carefully removed, and 0.6 percent saline solution was added to the slime. The ciliates

were removed from the material with a fine pipette to a coverglass for examination. Fixation of the organisms was accomplished by placing some of the fluid containing them on a coverglass, and pipetting off the excess liquid, then adding the fixative. For the study of the nucleus and general body form, organisms were stained with Mayer's haemalum following fixation in Schaudinn's sublimate alcohol and glacial acetic acid.

To study the fibrillar system, the Corliss (1953) modification of the Chatton-Lwoff technic was used with good results. The organisms were first fixed in Champy's fluid for three minutes, and then in Da Fano's fluid from three to twenty-four hours. After imbedding in saline gelatine, the organisms were placed in a solution of 3 percent silver nitrate for thirty minutes in a cold, moist chamber. Reduction of the silver was accomplished by means of sunlight or an ultraviolet ray lamp. The time of exposure to light was from ten to fifteen minutes.

Examination of aquatic snails was made in a manner similar to that used for the terrestrial forms. The only ciliate found was *Trichodina* sp. in limited numbers. No study has been made of these ciliates.

III. Descriptions

Thigmotricha (Chatton and Lwoff 1922)
Family Conchopthiriidae (Reichenow 1929)

Conchopthirius curtus (Englemann 1862)

Conchopthirius curtus was found in large numbers in the mantle cavities of *L. siliquoidea* and *A. grandis*. All of the mussels examined were heavily infected.

The body outline of the ciliate as seen from the dorsal aspect (Fig. 1) is ovoid, somewhat rounded at the anterior and posterior ends. Eighty living individuals ranged from 46 μ to 92 μ in width, and from 91 μ to 153 μ in length, averaging 71 μ by 117 μ. The ciliate is dorso-ventrally flattened, with a concave ventral surface.

The peristomal area is located near the right margin, in the anterior third of the body (Figs. 1, 3). Two rows of long cilia extend from the margin of the cytostome to a tubular pharynx (Fig. 1). The gullet extends from the pharynx, curving toward the left side and extending almost to the posterior end of the body, ending in the cytoplasm (Figs. 1, 22). The peristomal basket is a long, narrow area at the posterior margin of the pharynx, which is lined by a network of fibers (Figs. 3, 19).

The cilia are disposed in 60 to 63 rows on the dorsal surface, and

in 57 to 64 rows on the ventral surface (Figs. 18, 21). Each row contains approximately 89 cilia. The total number of cilia on the dorsal surface is estimated at 5400, and on the ventral surface at 5100. These figures were computed by using the average number of cilia per row in five individuals, and multiplying by the number of rows.

The cytoplasm is colorless, and the region anterior to the gullet contains a zone of endoplasmic granules (Figs. 1, 22), which extends posteriorly along the anterior margin of the gullet. Very few granules are observed posterior to the gullet. The granules are highly refractile, and stain intensely with Mayer's haemalum. Food vacuoles are numerous in most specimens (Figs. 21, 22), and are found only in the area posterior to the gullet. Attempts to study their formation by the addition of carmine to the medium were unsuccessful.

The macronucleus is located in the middle of the ciliate; its shape is somewhat variable, ranging from ovoid to spheroid (Figs. 1, 22). The micronucleus (Fig. 22) is difficult to distinguish in stained preparations. Kidder (1934a) describes it as imbedded in a macronucleus, but positive identification of its position and size could be determined only after a detailed study of specimens in division.

The contractile vacuole lies in the middle region of the body, posterior to the micronucleus toward the right side (Fig. 1). A large, slit-like pore is found laterally and to the right of the vacuole (Figs. 1, 18, 19). The connecting canal, described by Schuberg (1889), was not observed. The maximum size of the vacuole at systole ranges from 10 μ to 14 μ. Timing of the pulsation of the contractile vacuole of 13 individuals gave an average interval between discharge of 13.7 seconds.

Fibrillar System

The fibrillar system includes closely set ciliary rows, which originate in an anterior suture (Fig. 18), and terminate in the posterior suture (Figs. 2, 18). The anterior suture extends from the dorsal portion of the peristome around the margin of the anterior end. This suture is marked by two fibrils which are united at their peristomal ends and connected by cross fibrils along their length. The anterior suture becomes the pre-oral connecting fiber in the region of the peristome, and gives rise to the fibers of the peristomal net (Figs. 3, 19). These fibers give off secondary fibers in the peristomal field, which form the numerous fine fibers of the peristomal basket. The posterior suture extends from the peristome along the left margin almost to the posterior end.

The ciliary rows are more closely spaced around the peristome, but have their origin in the anterior suture and their termination in the posterior suture (Fig. 19). Two rows end at the pore of the contractile vacuole (Figs. 4, 19). The attached end of each cilium is imbedded in a basal granule, which is connected by fibrils to the basal granules of other cilia in the same row, and to the internal fibrillar system. Measurements show the granules to be 1.0 μ to 1.5 μ apart in the rows, and the rows to be approximately 1.5 μ to 2.0 μ apart.

Division

Division stages were found frequently in preparations of material fixed and stained with Mayer's haemalum or impregnated with silver. Initially, there is a gradual rounding up, elongation and constriction of the macronucleus, which may be easily identified, both in living and stained ciliates undergoing fission. Early division stages show an elongate nucleus, with a compact, densely staining core of chromatin. Subsequent elongation and constriction result in the formation of a central ball (Fig. 6), which Kidder (1933a) has termed the residual mass. This mass disintegrates rapidly in the cytoplasm of one of the daughter cells; it was not present in newly divided daughter cells.

The micronucleus is extremely small, and the details of chromosomes during division are very difficult to observe. During early prophase the micronucleus moves out of its pocket in the macronucleus (Fig. 6), divides, and each daughter micronucleus takes up a position in the region of the cell between the corresponding daughter macronucleus and the division plane (Fig. 7). Eventually, each daughter micronucleus enters the corresponding daughter macronucleus.

The peristome occupies a position in the non-dividing individual in the anterior third of the body near the right margin (Fig. 1). During division, the peristomal area is reorganized: there is lengthening, constriction and fusion of the peristomal groove in the midregion of the old mouth. Two new mouths are formed at the point of constriction. The two new mouths move apart at the time the daughter nuclei are separating (Fig. 6). The old contractile vacuole is retained in the posterior daughter, and a new one forms in the anterior daughter soon after fission has started.

No evidence of conjugation was observed, but some organisms show fragmentation of the macronucleus (Figs. 9, 10). This suggests that either conjugation, autogamy, or some type of nuclear reorganization has taken place.

Myxophyllum steenstrupi (Stein)

This species was placed in the genus *Conchopthirius* by Stein when he described the species in 1861, however, the species is sometimes ascribed to Raabe who erected the genus *Myxophyllum* for this single species in 1934. *M. steenstrupi* was found in the body slime of the following terrestrial snails: *A. alternata, T. multilineata,* and *O. d. gouldii.* The body of this ciliate is elliptical, flattened dorsoventrally, and the ventral surface is concave (Fig. 11). Forty living specimens ranged from 76 μ to 107 μ in width, and from 107 μ to 153 μ in length, averaging about 88 μ by 128 μ. The body appears to be easily deformed but readily returns to normal shape.

The peristome is located on the ventral surface of the animal, near the right posterior part of the body (Figs. 11, 23). A well defined gullet extends into the cytoplasm of the body up to the region of the contractile vacuole, which is centrally located (Fig. 23). The ciliary system consists of 72 to 79 rows on the dorsal surface, and 54 to 60 rows on the ventral surface. The total number of cilia on the dorsal surface is approximately 4000, and on the ventral surface 5500. These figures were computed by counting the number of cilia in a square and multiplying by the number of squares in the entire surface of the animal.

The cytoplasm is colorless, and contains a few scattered granules (Fig. 11). Food vacuoles are dispersed in all parts of the organism (Fig. 11). There are generally seven macronuclei (Figs. 11, 23); Stein has reported nine to twenty. A count of the macronuclei in 100 individuals gave the following numbers: 1 with 5; 13 with 6; 76 with 7; 9 with 8; 1 with 9.

When viewed from the dorsal side, a single micronucleus is found between the posterior two macronuclei and the right margin (Fig. 23). The contractile vacuole lies posterior to the macronuclei, near the middle area of the body (Figs. 11, 23). A tubular canal extends from the vacuole to the gullet; this is barely visible in Fig. 23. Pulsation times of the contractile vacuole ranged from two to four minutes.

Fibrillar System

The fibrillar system includes closely set ciliary rows, which originate in the anterior suture (Fig. 25). This suture begins near the left margin in the anterior third of the body, and extends around the anterior end to the right margin. The posterior suture extends from the lower third of the right margin, across the posterior end, into the peristome (Figs. 12, 25). The ciliary rows originate at the anterior suture and terminate in the peristome and in the posterior suture. Both sutures contain numerous cross fibrils.

The cilia arise from basal granules, which are connected by fibrils to other basal granules in each row, and to the internal fibrillar system, as in *C. curtus*.

Reproduction

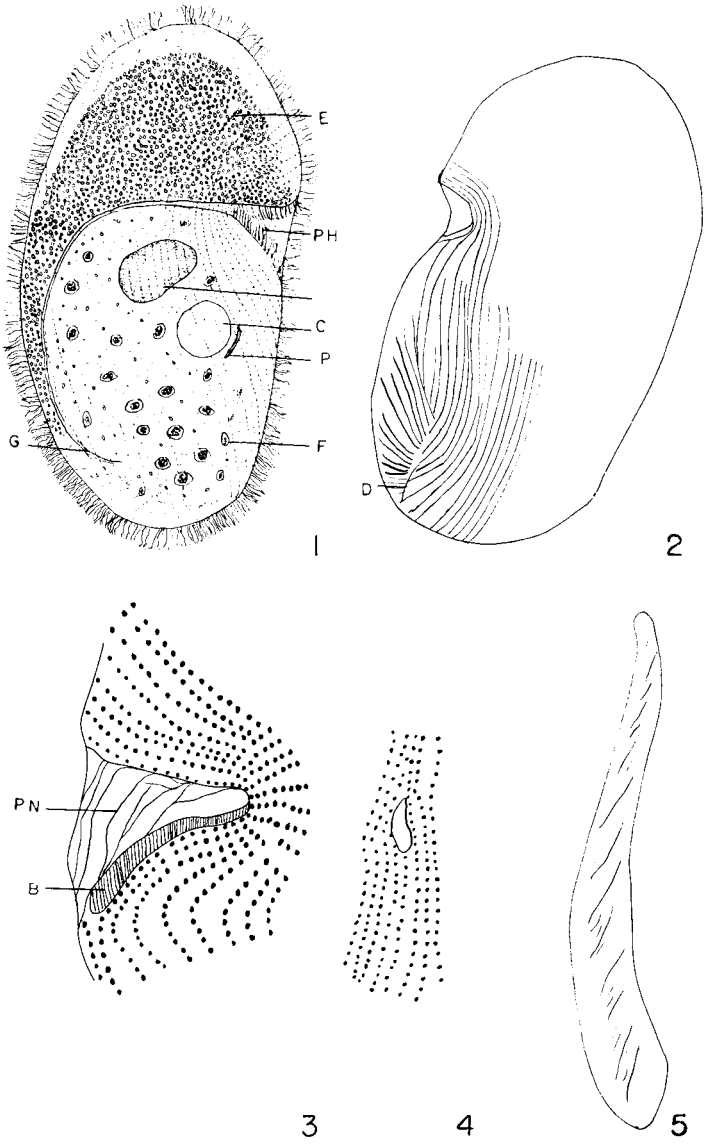
Organisms in the early stages of division in this species are difficult to identify under the dissecting microscope, so that these stages are present in preparations purely as a matter of chance.

As previously described, the non-dividing animal usually has seven or more macronuclei and one micronucleus. At the beginning of division, each of the macronuclei begins to elongate and forms a spindle-like body (Fig. 14). These spindles increase in length, and a small mass of chromatin is detached centrally from each macronucleus (Figs. 14, 16). Constriction of the cytosome begins as soon as the spindles have elongated, and the attachment between the daughter macronulcei is broken (Fig. 15). The small mass of chromatin between the halves of each spindle-shaped macronucleus is absorbed into the cytoplasm of the daughter cells.

It is extremely difficult to observe the details of the chromatin structure of the micronucleus. It also elongates and divides, along with the macronucleus, moving apart into the presumptive daughter regions of the cells.

A new mouth is formed at the point of division of the daughter cells, and the old mouth remains in its original position (Fig. 26). Subsequent elongation of the cell and reorientation of the ciliary rows in the area of the mouths occur simultaneously with nuclear migration. A new contractile vacuole is formed in the anterior daughter in the area behind the macronuclei (Fig. 27).

Many apparent instances of nuclear reorganization were observed in this ciliate (Fig. 17); this indicates that other patterns of reproduction are to be found.



EXPLANATION OF PLATES

All drawings of fixed material were made with the aid of a camera lucida. Photographs were made at a uniform magnification of x 534. Anterior end toward the top of the page except where indicated.

PLATE I

Figs. 1-5. *Conchopthirius curtus* Englemann

Fig. 1. Dorsal view. x 790.

Fig. 2. Pattern of cilia in the posterior suture area. Silver preparation. x 790.

Fig. 3. Ventral view of the peristomal area. Silver preparation. x 1560.

Fig. 4. Pore area. Anterior end toward the bottom of page. Silver preparation. x 1560.

Fig. 5. Diagrammatic representation of side view.

(B-peristomal basket; C-contractile vacuole; D-posterior suture; E-endoplasmic granule zone; F-food vacuole; G-gullet; P-pore of contractile vacuole; Ph-pharynx; PN-peristomal net)

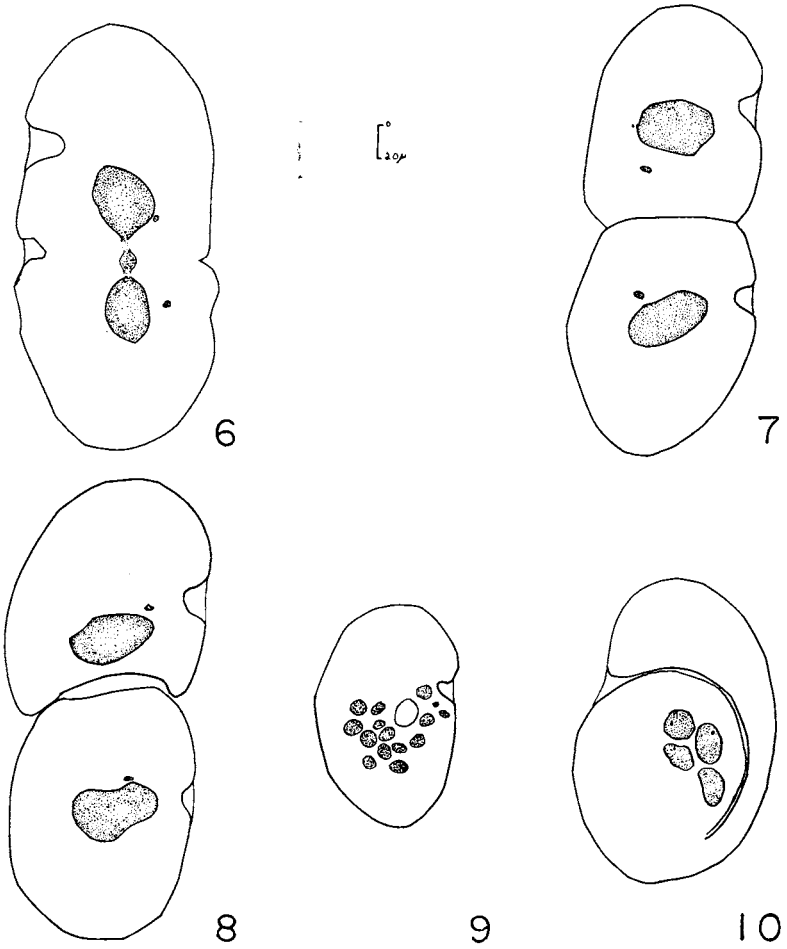


PLATE II

Figs. 6-10. *Conchopthirius curtus* Englemann. Schaudinn's: Mayer's haemalum x 477.
Fig. 6. Early division.
Figs. 7-8. Late stages of division.
Figs. 9-10. Nuclear fragmentation.

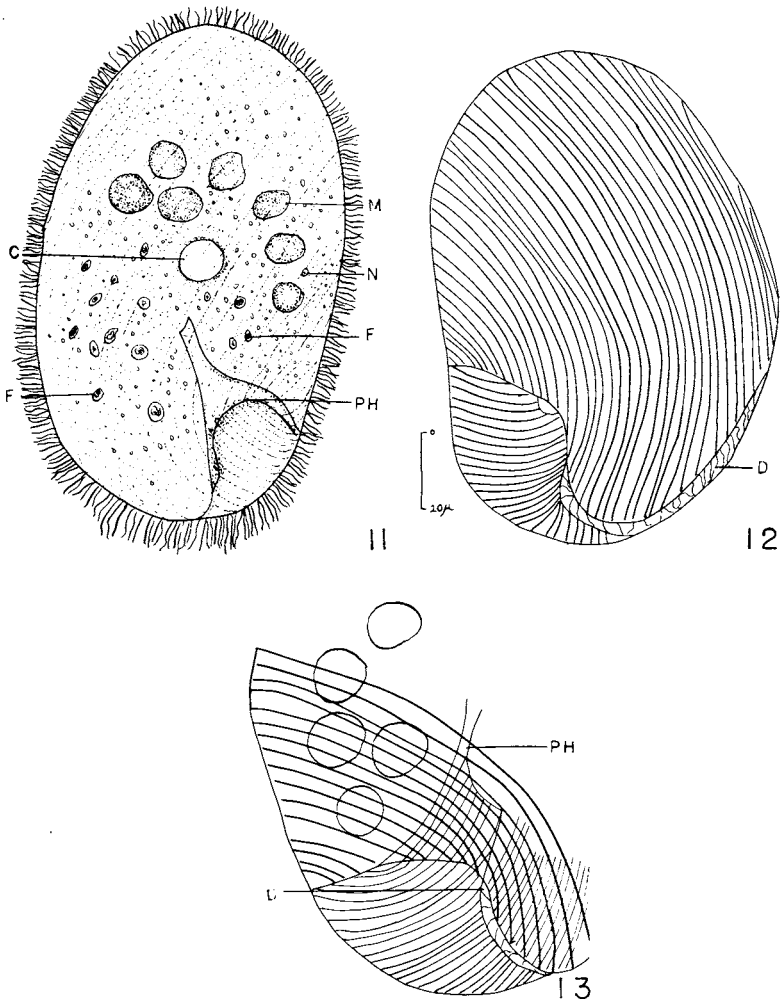


PLATE III.

Fig. 11-13. *Myxophyllum steenstrupi* (Raabe)

Fig. 11. Dorsal view.

Fig. 12. Ventral view to show ciliary pattern. Not all rows indicated. Silver preparation.

Fig. 13. Ventral view of peristomal area. Not all rows indicated. Silver preparation.

(C-contractile vacuole; D-posterior suture; F-food vacuole; M-macronucleus; N-micronucleus; PH-pharnyx)

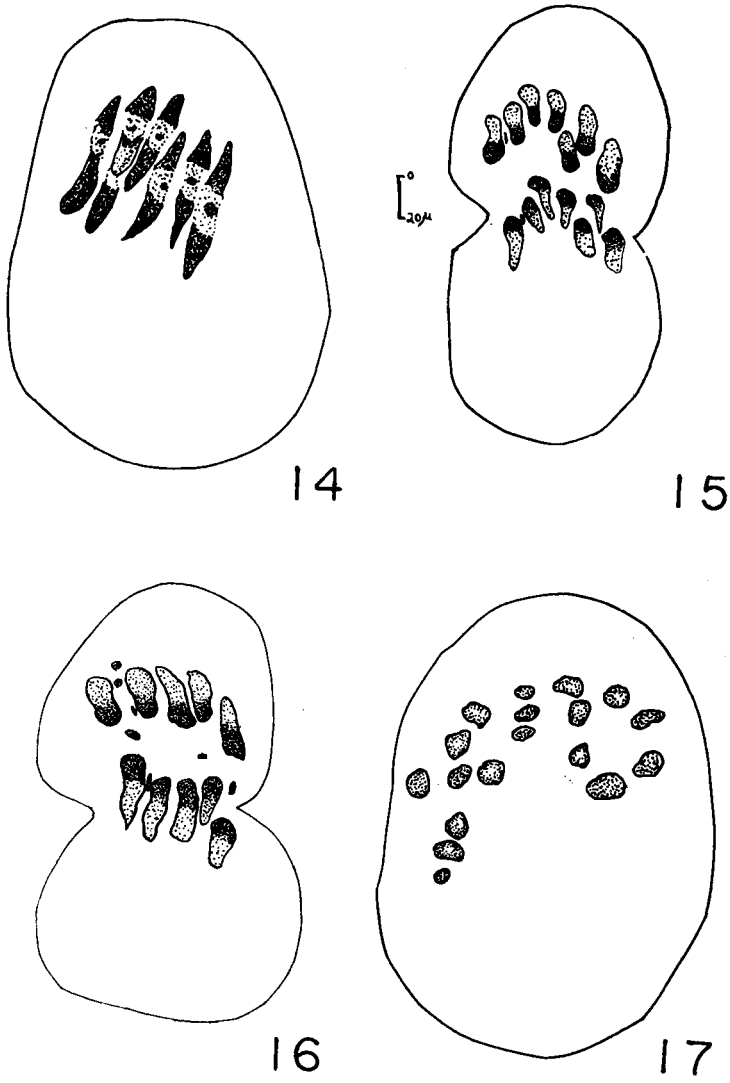


PLATE IV

Figs. 14-17. *Myxophyllum steenstrupi* (Raabe) Schaudinn's: Mayer's haemalum.
Fig. 14. Early division stage, with spindle-like macronuclei.
Figs. 15-16. Late division stage.
Fig. 17. Nuclear reorganization.

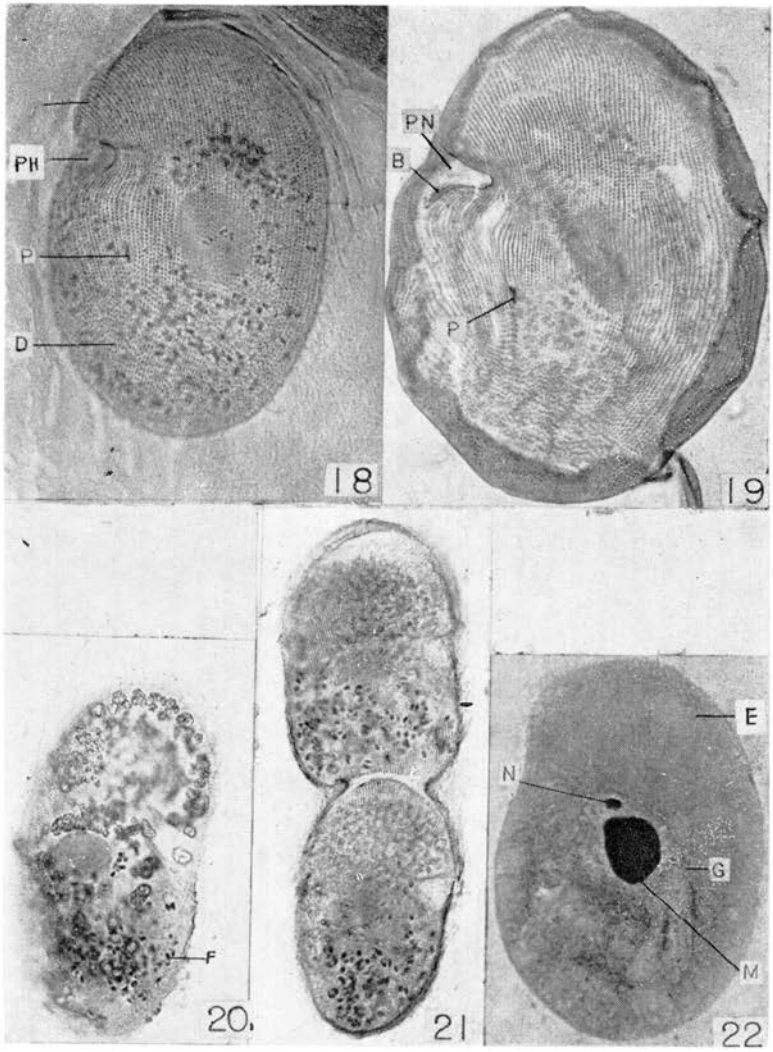


PLATE V

Figs. 18-22. *Conchopthirius curtus* Engl. x 534.
Fig. 18. Ventral view. Silver preparation.
Fig. 19. Ventral view to show peristomal area. Silver preparation.
Fig. 20. Dorsal view. Silver preparation.
Fig. 21. Dorsal view of a division stage. Silver preparation.
Fig. 22. Ventral view. Schaudinn's: Mayer's haemalum.
(B-peristomal basket; D-posterior suture; G-gullet; M-macronucleus; N-micronucleus; P-pore of contractile vacuole; PH-pharynx; PN-peristomal net)

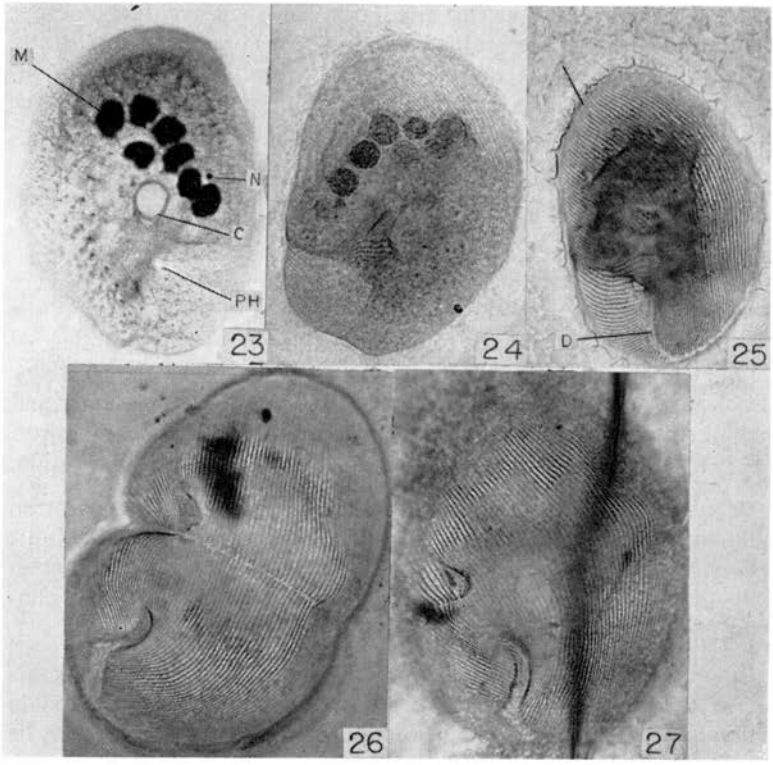


PLATE VI

Figs. 23-27. *Myxophyllum steenstrupi* (Raabe) x 534.
Fig. 23. Dorsal view. Schaudinn's: Mayer's haemalum.
Figs. 24-25. Ventral view to show peristomal area. Silver preparation.
Fig. 26. Ventral view of an organism in division showing the formation of the mouth. Silver preparation.
Fig. 27. Ventral view showing mouth and contractile vacuole. Silver preparation.
(C-contractile vacuole; D-posterior suture; M-macronucleus; N-micronucleus; PH-pharynx; V-anterior suture)

IV. Discussion

Conchopthirius curtus from the mussels examined for this paper is identical with *C. curtus* as described by Kahl (1930) and as reported by various authors from other mussels: by Kidder (1933a, 1934b), from *Anodonta implicata, A. cataracta, Lampsilis radiata, L. cariosa,* and *Elliptio complanatus;* by Uyemura (1935) from *Anodonta lauta.* In northwestern Iowa, as elsewhere, it appears to be limited to mussels. Evidence points to a world-wide distribution for this parasite in various species of mussels. It has been reported from fresh-water pelecypods in India, in Europe, in Japan, and in the eastern United States.

Structurally, the organism is well adapted for existence in mus-

sels. The characteristic elongated cilia and concave body permit easy attachment to the host. Kidder (1934a) found *C. curtus* and *C. magna* on all exposed surfaces within the shell of the host. Members of the family Conchopthiriidae exhibit certain trends in the evolution of thigmotacticism. Fauré-Fremiet (1950) has pointed out the following significant features of the Thigmotricha: (a) the anterior location of thigmotactic cilia, (b) the posterior movement and independence of the buccal cilia in certain forms (Hemispeira), and (c) adaptation to parasitism, which leads to the regression of cilia, and the appearance of entirely new organs such as suckers in the thigmotactic area of certain other forms (Rhynchodea). While the members of the family Conchopthiriidae possess a well-defined thigmotactic area, which is identical to that of other thigmotrichs, they are primitive in respect to the position of the peristome and lack of regression of body ciliature. Accordingly, Chatton and Lwoff (1939) have placed the Conchopthiriidae first in their classification.

Myxophyllum steenstrupi appears to be restricted to terrestrial pulmonate snails, and occurs in snails from a variety of habitats. The structure of *M. steenstrupi* studied in this investigation agrees with that of the same form as described by Stein (1861), by Schuberg (1889), and by Raabe (1933, 1934).

It is generally concluded that these ciliates are commensals rather than true parasites. Numerous food vacuoles were found in individuals freshly removed from the host, and there appeared to be no significant increase in the number of vacuoles in organisms which had remained outside the host for prolonged periods. Kidder (1933a) observed that food vacuoles of many Conchopthiriidae contained algae, bacteria, and even sperm cells of the host.

Conchopthirius curtus and *M. steenstrupi* have certain similarities such as origin of the ciliature, the presence of thigmotactic cilia, and feeding habits, but differ markedly in other structural features. Since the position of the mouth is so different in the two species the morphogenetic movements which occur during fission may give some clue to the origin of structural differentiation found among ciliates in general. The mouth of more generalized ciliates is anterior, therefore, the location and origin of the mouth in *C. curtus* represent a more primitive condition, and in *M. steenstrupi* an advanced condition. The centrally located mouth of *C. curtus* gives rise to the old and new mouth, as in *Paramecium* (Downing, 1951), but in *M. steenstrupi* the new mouth also originates in a central position, and has no connection with the old mouth which is located posteriorly. It appears as if some agent is at work in the cortex which controls the origin of the oral system. Lwoff (1950) believes that cyclical dedifferentiation occurs, and the old structures disappear and the properties of the predividing organisms are such that phylogeneti-

cally primitive conditions and the corresponding structures are formed.

The fibrillar systems of *C. curtus* and *M. steenstrupi* are basically alike. Each cilium originates from a basal granule; these are connected by longitudinal and cross fibrils, so that the fibrillar structure has a characteristic cross-hatched appearance. The structure of the peristome is quite different in the two forms. *C. curtus* possesses a peristomal basket (or undulating membrane according to Raabe, 1934), whereas, such a structure is lacking in *M. steenstrupi.* Cilia originate in anterior suture in both ciliates, but with the migration of the oral system posteriorly, as in *M. steenstrupi,* most of the ciliary rows have their termination in the peristome.

Generally, contractile vacuoles are of two types: (a) vesicle-fed vacuoles, and (b) canal-fed vacuoles, however, some may be fed by diffusion. The contractile vacuole of *M. steenstrupi* is permanently located in the cell, and appears to have neither canals nor vesicles, but this point needs further investigation. The vacuole has no permanent pore on the external surface, but discharges its contents into the canal leading to the gullet. The contractile vacuole of *C. curtus* is vesicle-fed (Kidder, 1934a), and has a permanent slit-like pore. The faster pulsation of the vacuole in this species is probably due to a difference in the medium in which the ciliates live, since body fluids of terrestrial animals usually have a higher osmotic pressure than do fresh-water forms. It has been found that the blood and pericardial fluid of *Anodonta cygnea* are equivalent osmotically to 0.1 percent NaCl and the urine to 0.06 percent NaCl (Picken, 1937). The land snail, *Helix pomata,* has a blood equivalent to 0.069 percent NaCl when active (Kamada, 1935). If these conditions hold for other mussels and terrestrial snails, ciliates found in the mantle cavity of a mussel would be exposed to lower osmotic pressure than those in the body fluids of a terrestrial snail.

The elimination of macronuclear material by the two ciliates during fission appears to be an example of a common occurrence among members of this particular family; this phenomenon has also been observed in a number of other ciliates; *Uroleptus halseyi* (Calkins, 1930); *Euplotes patella* (Turner, 1930); *Conchopthirius anodonta, C. magna* (Kidder, 1934a); *Colcodium colpoda, C. campylum* (Kidder and Diller, 1934); *Blepharisma undulans* (Young, 1939); and *Tillina magna* (Beers, 1946). Kidder is of the opinion that the process is probably the elimination of the waste substances of prolonged cell division. Dass (1950), on the other hand, believes the mass to be surplus desoxyribonucleic acid about to be converted by the cytoplasm to ribonucleic acid necessary for active growth. Available evidence does not permit definite conclusions in regard to this phenomenon, but its widespread occurrence among ciliates probably

points to a genetic and physiological reorganization within the macronucleus.

Multiple macronuclei usually fuse before fission, but the seven macronuclei of *M. steenstrupi* do not fuse into one mass and each gives the appearance of dividing mitotically. The seven macronuclei might have originated in the following way: after conjugation or autogamy the fusion micronucleus divides three times to form eight micronuclei, one of which remains a micronucleus and the other seven become macronuclei, which, however, retain some of the properties of the micronucleus—i.e. they divide at every fission in a manner reminiscent of mitosis.

V. Summary

1. Seven species of mollusks examined were found to harbor endozoic ciliates.
2. Two species of ciliates, which belong to the family Conchopthiriidae, are described: (1) *Conchopthirius curtus* Englemann, from the gills and mantle cavity of *Lampsilis siliquoidae* Barnes and *Anodonta grandis* (Say); (2) *Myxophyllum steenstrupi* (Stein), from the slime of the following terrestrial snails; *Anguispira alternata* (Say), *Triodopsis multilineata* (Say), and *Oxyloma decampi gouldii* Pilsbry.
3. The chief structural differences between *C. curtus* and *M. steenstrupi* is the position and structure of the mouth, and the number of macronuclei.
4. The fibrillar systems, consisting of the peristome, cilia, internal and external fibrillar systems are described.
5. The contractile vacuoles of the ciliates are described.
6. The reproductive activities of *C. curtus* and *M. steenstrupi* are described.
7. The formation of the mouth occurs in the region of the old mouth in *C. curtus,* and in an entirely new region in *M. steenstrupi.*
8. No observations were made on *Trichodina sp.* found ectozoic on two species of aquatic snails: *Physa gyrina* Say and *Helisoma trivolvis* (Say).

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