

CILIATES FROM MARINE WOOD-BORING MOLLUSCS

by

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Reprinted from

T R E U B I A

Museum Zoologicum Bogoriense, Bogor, Indonesia
Volume 28, Part 2, p.p. 41 - 58 (1973)

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ABSTRACT.

Seven species of ciliates, collected from the mantle cavity of the shipworms, *Nausitora hedleyi*, *Teredo furcifera* and the pholad *Martesia striata*, of the South West coast of India are described. One new genus *Thigmozoon* has been created to accommodate the new species *Thigmozoon fencheli* under the order *Thigmotrichida*. Two more new species, *Trichodina balakrishnia* and *Nyctotherus marina*, are described under *Peritrichida* and *Heterotrichida* respectively. Of the remaining four species only *Boveria teredinidi* has been recorded from shipworm. Others are new records from the wood-boring molluscs.

Observations on the behaviour of the ciliates outside and inside the body of the hosts are described.

INTRODUCTION.

The first record in the literature pertaining to ciliates living in the mantle cavity of bivalves is by EHRENBERG (1938), who found a *Conchophrythirus* in a species of *Unio* and described it under the name of *Leucophrys unodontae*. Later, QUENNERSTEDT (1867) noticed and described an *Ancistrum mytili* from mussels taken on the west coast of Sweden, but he classified it as an *Opalina*. MAUPAS (1883), too, found this species from mussels in the Mediterranean, and erected the genus *Ancistrum* to include the species described by Quennerstedt. FABRÉ-DOMERGUE (1888) detected *Hemispeira asteriasi* on asterids from the coast of France. ISSEL's memoir (1903), represents the first comprehensive systematical work on ciliates from bivalves and gastropods. CHATTON and LWOFF (1921) initiated publishing a series of papers on the ciliate fauna of molluscs. This series was concluded in a memoir on the morphology and systematics of the thigmotrich ciliates (CHATTON and LWOFF 1949, 1950). They created the order *Thigmotrichida*. CHEISSIN (1931) described an endemic fauna of hemispeirids from the molluscs in Baikal sea. RAABE (1933 a, 1933 b,

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1963 b), JAROCKI (1934, 1935), KIDDER (1933 a, 1933 b) and KOZLOFF (1945, 1961) have substantially contributed to the knowledge of thigmotrich ciliates. In recent years it has been shown that the peritrich urceolariids play an important role as commensals in molluscs (HAMPL 1955; UZMAN and STICKNEY 1954; RAABE and RAABE 1959, 1961; FENCHEL 1965).

It was NELSON (1923) who first noticed and published a new species, *Boveria teredinidi*, from the gills of the wood-boring molluscs, *Bankia gouldi* and *Teredo navalis*.

The ciliate fauna from wood-boring lamellibranchs, especially from *Teredo furcifera* VON MARTENS, *Nausitora hedleyi* SCHEPMAN and *Martesia striata* LINNAEUS, consist representatives of the Thigmotrichida, Peritrichida and Heterotrichida.

It is noteworthy that, except *Boveria teredinidi* (GANAPATI and NAGABHUSHANAM 1955) and *Nucleocorbula adherens* (SANTHANUMARI and NAIR 1970), all are new records and three of them are new species.

MATERIAL AND METHODS

The host animals studied were collected from underwater timber structures along the coast, such as coconut piles, fenders, discarded country canoes, dealwood boxes and test panels. The host animals were carefully removed from their burrows and washed in filtered seawater. Then the body cavity was opened and examined for specimens. With a view to preparing permanent mounts, the most effective method however was the application of the smear method. After smearing the Mayer's albumen, a few drops of suspension of ciliates were spread. After the excess fluids were drained off the slides were rapidly dipped into the fixing fluid (vide infra). A comparative study of the different available methods revealed that the smear method produces little distortion of the animals and it was a much easier way to obtain well differentiated preparation of the organisms concerned.

The fixing agents used were: Schaudin's, Bouin's, Zenker's, formalin and Da Fano's cobalt nitrate-formalin.

Among the various stains employed, the following were specially noteworthy: Delafield's haematoxylin and Mallory's triple connective tissue stain.

Of the silver impregnation techniques, that of CHATTON and LWOFF's as described by CORLISS (1953) and KLEIN (1958) used with success for the study of the scopula of *Trichodina*.

ORDER THIGMOTRICHIDA CHATTON AND LWOFF 1922

SUBORDER ARHYNCHODINA CORLISS 1957

THIGMOPHRYIDAE CHATTON AND LWOFF 1923

Thigmozeon new genus

Oval Thigmophryids living in mantle cavity of shipworms. Macronucleus branched and a small spherical micronucleus present between branches of macronucleus. Mouth situated on posterior quarter of body.

Thigmozeon fencheli new species

Body shape constant, oval when viewed from dorsum or ventre, thin in lateral view with convex dorsal and concave ventral surface; body covered with thick, clear, semi-rigid pellicle and fine cilia originating in basal bodies arranged in close set rows; ciliary rows parallel to each other; lateral ciliary rows meet in a line extending down on dorsum (Fig. 2). Body contraction not observed, although ciliate flexes somewhat during body movements.

Ectoplasm entirely covered by cuticle, containing granular structures; endoplasm finely granular, clear; numerous food particles surrounded by food vacuoles, appearing in posterior and middle regions; streaming of endoplasm discernible in living animal during feeding periods; cytoplasm containing glistening glycogenous globules (Fig. 3), yellowish brown when stained with iodine.

Mouth ventral, placed in pocket situated at posterior quarter of body (Fig. 2); adoral ciliature difficult to make out in living condition.

Macronucleus highly branched, situated in middle region, occupying more or less major portion of body (Figs. 4, 5 and 6); central stem of macronucleus single, curved at ends; branches of macronucleus extending from central stem, enclosed in single continuous membrane; nature of macronucleus branching varying individually (Fig. 6). Among order Thigmatrichida, similar branching nature occurring in *Conchophthirius caryoclada* (KIDDER 1933). Micronucleus single, as dense sphere, in region between branches of macronucleus.

Body size: length 80-100 μ ; width 60-80 μ .

Discussion. This species obviously belongs to the family Thigmophryidae of the suborder Arhynchodina. However, some characters, such as the general shape of the body and the branched macronucleus, are similar to those of *Conchophthirius caryoclada* KIDDER (1933) (*Conchoph-*

thiriidae). The latter is larger in size (140 - 250 μ in length and 90 - 160 μ in width) and possesses fewer ciliary rows.

The systematics of Thigmophryidae has been treated by CHATTON and LWOFF (1949), RAABE (1953) and FENCHEL (1964, 1965).

Genus *Boveria* STEVENS 1901.

Mouth situated at posterior pole. Adoral rows performing 360° arc around posterior pole. One of somatic kineties following undulating membrane for some distance.

Boveria teredinidi NELSON 1923.

Body shape elongated and subcylindrical, varying in size and form (Fig. 9); pellicle as thin layered protective covering; cilia of two distinct types: body or somatic cilia, arranged in parallel, slightly oblique, of equal length, spaced equidistantly in longitudinal rows, extending from posterior adoral ciliary spiral to posterior end where they converge (Fig. 7); two types of cilia distinguishable on truncate peristomal field; innermost circle of oral cilia closely set in single line, longer adoral cilia arising from basal granules forming double, parallel dextrotropic spiral peripherally, ending distally in liotropic spiral terminus; oral cilia slightly shorter and more active in feeding than those of adoral whorls.

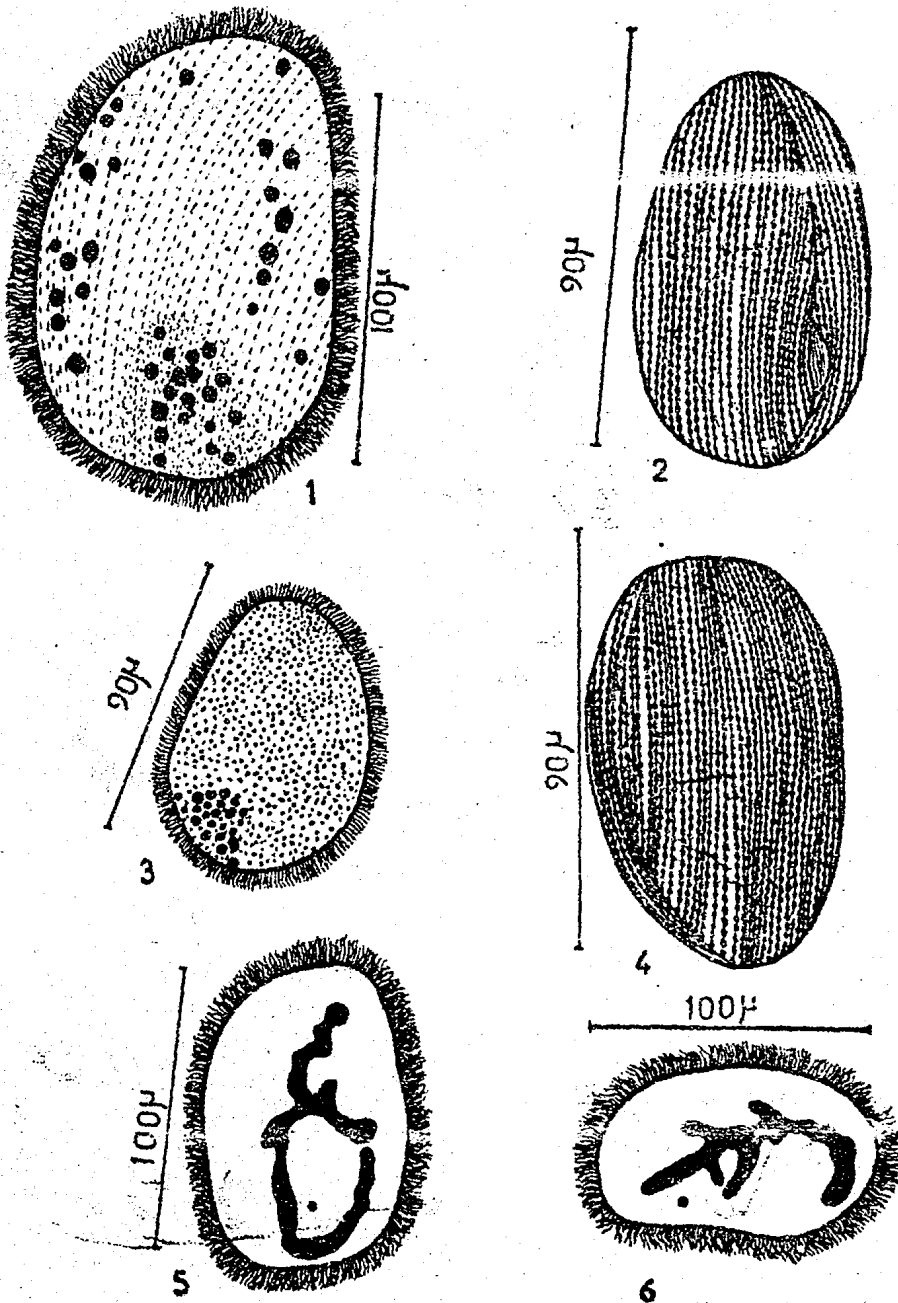
No distinct line of demarkation between ectoplasm and endoplasm; cytosome variable in size; endoplasm alveolar, somewhat coarser near macronucleus than at body ends; single contractile vacuole discernible dorsally near outer end of dorsal ciliary zone (Fig. 8); food vacuoles in oral end of body in crowded situation, near macronucleus in elongated specimen.

Mouth and pharynx seen only during feeding activities.

Macronucleus large, oval, somewhat centrally placed in interphase, 9 - 20 μ at long axis. Micronucleus between macronucleus and posterior end of animal, spherical, 1 - 2 μ in diameter.

Body size: length 26 - 226 μ ; width at oral en 11 - 33 μ .

Conjugation. A detailed account of the conjugation is given by IKEDA and OZAKI (1918) for *B. labialis*. In the case of *B. teredinidi* also conjugation has been noticed repeatedly during the present study (Figs. 14 and 15). Two conjugants may come into mutual attachment with their aboral ends. The changes undergone by the nuclei during conjugation has not been followed in detail.



Figs 1—6. 1—6, *Thymozoon fencheli* new species: (1) typical shape of living specimen; (2) showing mouth, adoral ciliature, etc. (silver impregnated specimen); (3) showing glycogenous bodies (silver impregnated); (4) arrangement of ciliary rows, macro- and micronucleus; (5 and 6) variation in shape of macronucleus.

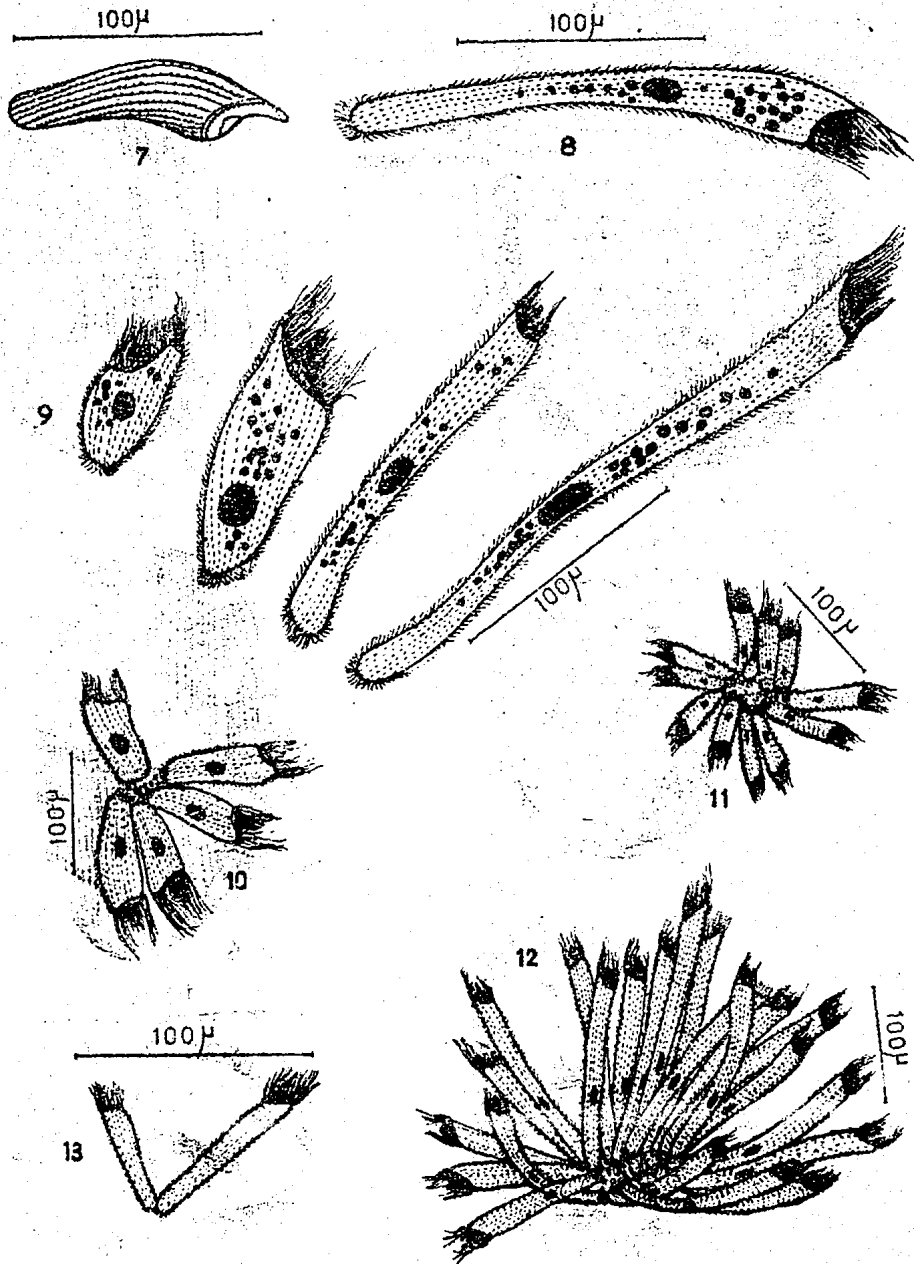


Fig. 7—13. 7—13. *Boveria teredinidi*: (7) showing ciliary pattern (silver impregnated specimen); (8) showing macro-, micronucleus, contractile- and food vacuoles (living specimen); (9) variation in body size and form; (10—12) different types of colony; (13) colony with two individuals.

Encystment. Cysts have been noticed in the tissues of the mantle of shipworms and it is probable that these may be the encysted condition of the ciliates probably that of *B. teredinidi*. The figures of encystment of *B. labialis* is very similar to the cysts that have been observed in the mantle of these shipworms (Figs. 16 and 17).

Colony formation. The most common but hitherto unnoticed feature in *Boveria* is an interesting type of its colony formation. The individuals in the colony may vary in number. They attach together by their aboral end and the members assume a rosette-like appearance (Figs. 10, 11 and 12). Observations indicate that this so-called colony is not a permanent one. The individuals could automatically be detached and the colony dismantled by keeping them in a container for 12-18 hours. Within about 24 hours colony possessing 18 individuals got separated and the individuals moved off independently. The reason for the separation of individuals from the association is however, not clear.

Observations on live *B. teredinidi* outside the host animal. The host animals were carefully removed from their burrows in the wood. When the gill chamber is opened for close observation, *Boveria*, if present, may be seen with its aboral end in contact with the gills or mantle and the body extending radially out into the mantle cavity. This position is maintained by the animal with effective strokes of the body cilia. When disturbed, *Boveria* may swim rapidly away in any direction. The body possesses great flexibility even though a characteristic form is maintained at all times. Swimming is characteristically with the aboral end foremost with a slight flexion of the oral part of the body in a course which is spiral when viewed from the oral end of the animal. This movement is clockwise. *Boveria* may appear as single individuals or in colonies. Single individuals execute circular movements in an irregular fashion in clockwise direction and may simultaneously rotate their body in a clockwise direction. After performing these circular movements for a few seconds, they may suddenly turn their circular rotations in an anti-clockwise direction, simultaneously rotating their bodies too anti-clockwisely. Very often it resorts to effortless somersault movements in an acrobatic fashion. After performing these, it may swim very rapidly, rotating its body in a clockwise direction, in a zig-zag fashion. During this type of rapid swimming, it may execute a wavy course instead of the usual circular movement. The oral end of the body may pendulate in wider diameter than during normal movements. Adhering to the substratum by its aboral end and body extending radially, it moves in circles.

Very often individuals come together to form temporary association giving the appearance of a sort of colony formation. The individuals forming a colony may vary in numbers from 2 to 126. This colony forming tendency has been noticed in all size groups, i.e. from very small individuals to large members. In many cases, it was interesting to note that the size of the individuals forming a colony is, more or less the same. But in the case of association between two individuals the partners are very often remarkably dissimilar in size. If one individual approaches another closely facing their aboral ends, they may soon join. In a colony of three or more individuals the movement is different. In this instance, oral end of one individual may move backwards with the simultaneous movement of the adjacent individual in identical manner. This may lead to a clockwise rotation and they move slowly. In the case of an association between two individuals the partners make a V-shaped configuration (Fig. 13) and move with the oral end forwards. At times one individual may drag the other for a pretty long distance. The colonies are apparently very reluctant to leave the substratum unlike single individuals which are of a wandering type.

Behaviour of *B. teredinidi* inside the host animal. Within the body of the host animal *Boveria* seems to be apparently much less active than when they are removed to a shallow dish of sea water from the host. Very often they remain attached to the gills, or to the mantle or to other organs in the mantle cavity of the host with their aboral end. During such times cilia on the oral end can be seen moving slowly. The colony is certainly not permanent by being composed of individuals capable of independent life. This is evident from the fact that the composing individuals of a colony may detach from the association when subjected to starvation or some other environmental change.

Discussion. The specimens discussed resemble *B. teredinidi* of NELSON (1923) and that discussed by PICKARD (1927) in all such important respects as the number of ciliary rows, size and shape of the body, etc. NELSON (1923) and PICKARD (1927) have stated that *Boveria* confined entirely to the gills of the host. But in the present study it has been noticed not only in the gill and mantle cavity but also in the caecum and the gonad of shipworms, *Teredo furcifera* and *Nausitora hedleyi*. However, in the case of the pholad, *Martesia striata*, this form has been noticed only in the gill and mantle cavity.

ORDER PERITRICHIDA STEIN 1859

SUBORDER MOBILINA KAHL 1933

URCEOLARIIDAE DUJARDIN 1841

TRICHODININAE RAABE 1963

Genus *Trichodina* EHRENBERG 1836

Cilia short, forming wide arc of 330° to 450° . Denticles heavily built with well developed spikes and hooks. Macronucleus sausage-shaped

Trichodina macomarum RAABE and RAABE 1959

Body shape disc-like (Fig. 18); denticles 22 - 30 in number; each with 6 - 9 radial pins; denticle ring 25 - 37 μ (average 31 μ); hooks well developed, of characteristic hatchet-like shape (Fig. 19); spikes often uneven in length.

Macronucleus sausage-shaped. Micronucleus near end of macronucleus (Fig. 20).

Body size: diameter 40 - 60 μ (average 50 μ); height 15 - 20 μ (average 18 μ); diameter of adoral spiral averaging 42 μ .

RAABE and RAABE (1959) found this species in *Macoma baltica* from various spots of the Gulf of Gdansk. FENCHEL (1965) found this species from the same host from Askø and Presund.

Trichodina balakrishnia new species

Body shape variable from very small to a high cylinder (Fig. 21); height of cylinder considerably larger than diameter.

Scopula (adhesive disc), complicated organelle of attachment, situated at aboral pole, concave (Fig. 21), with thickened edge; skeletal complex consists of striated membrane, underlying denticulate ring, and border membrane; diameter varying from 33 to 55 μ (average 44 μ); denticulate ring 20 - 35 μ (average 28 μ) in diameter, with 22 - 30 denticles, (24, 26, 27 and 28 as dominating numbers in various populations), surrounded by triple crown of cilia; hook (measured from centre of denticle) 4 - 7 μ in length; spike 4 - 7 μ long; radial pins 8 - 10, between two neighbouring denticles; in fine structure denticles and radial rods built of scleroproteins (FAURÉ-FREMIT *et al.* 1956).

Macronucleus peculiar in shape, with 4 elongated rod-like structures extending nearly from oral to aboral end (Figs. 22 and 23); height of

macronucleus up to 85 μ . Micronucleus located at aboral end near end of macronucleus.

Adoral zone lying in plane parallel to adhesive disc performing a 400°, along body surface, anterior to mouth vestibulum; plunging deeper, separated from outside by low lip; contractile vacuole situated some distance from central body axis.

Cytoplasm containing large amount of fine grains in middle part, transparent and clear in peripheral region.

Body size: height up to 120 μ ; diameter 25 - 65 μ . (average 45 μ). Adoral spiral 30 - 75 μ in diameter (average 53 μ).

Discussion. The particular structure of the macronucleus, which has not been reported before, and the shape of the body are the distinctive features by which *T. balakrishnia* can be recognized from the other *Trichodina* species. The horse shoe-shaped macronucleus has so far been recorded in the genus *Trichodina* with a slight variation of the same in *Trichodina baltica* RAABE (1959). These entitle to diagnose *Trichodina balakrishnia* as a separate species.

ORDER HETEROTRICHIDA STEIN 1859

SUBORDER HETEROTRICHINA STEIN 1859

STENTORIDAE CARUS 1863

Genus *Stentor* OKEN

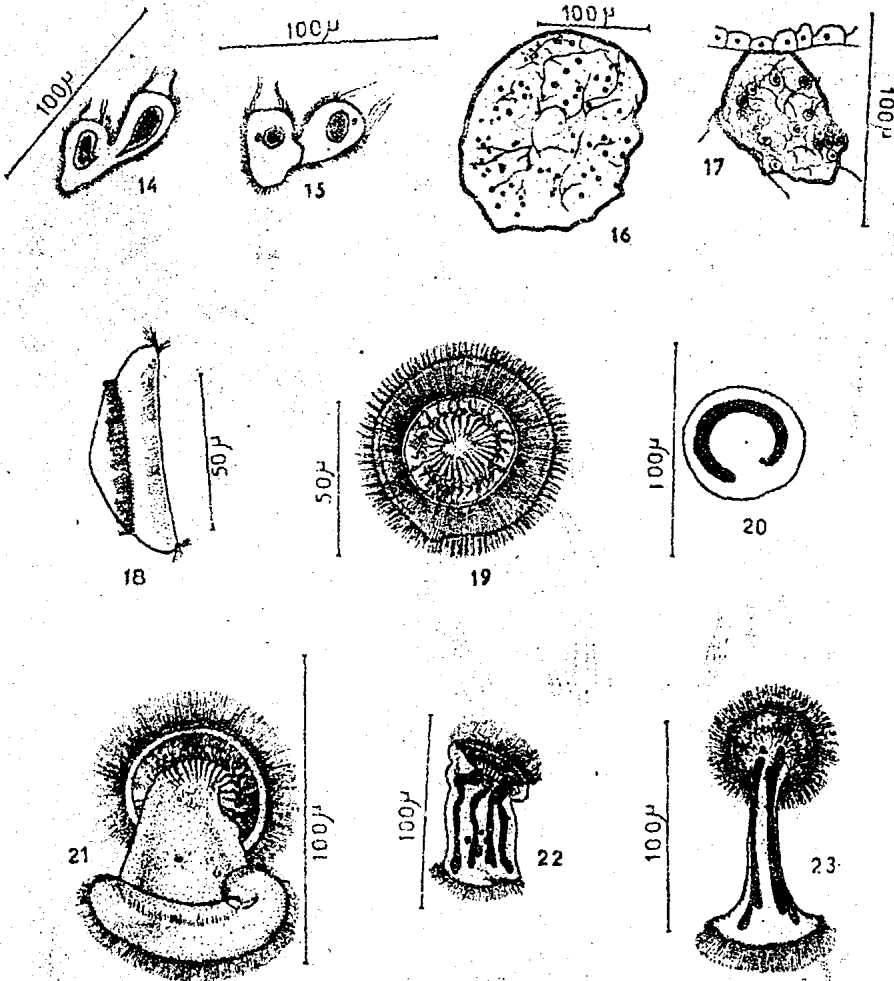
Body trumpet-shaped or cylindrical when extended; highly contractile. Some forms with mucilaginous lorica. Adoral zone encircles peristome in a spiral form, leaving narrow gap on ventral part.

Stentor coeruleus EHRENBERG 1838

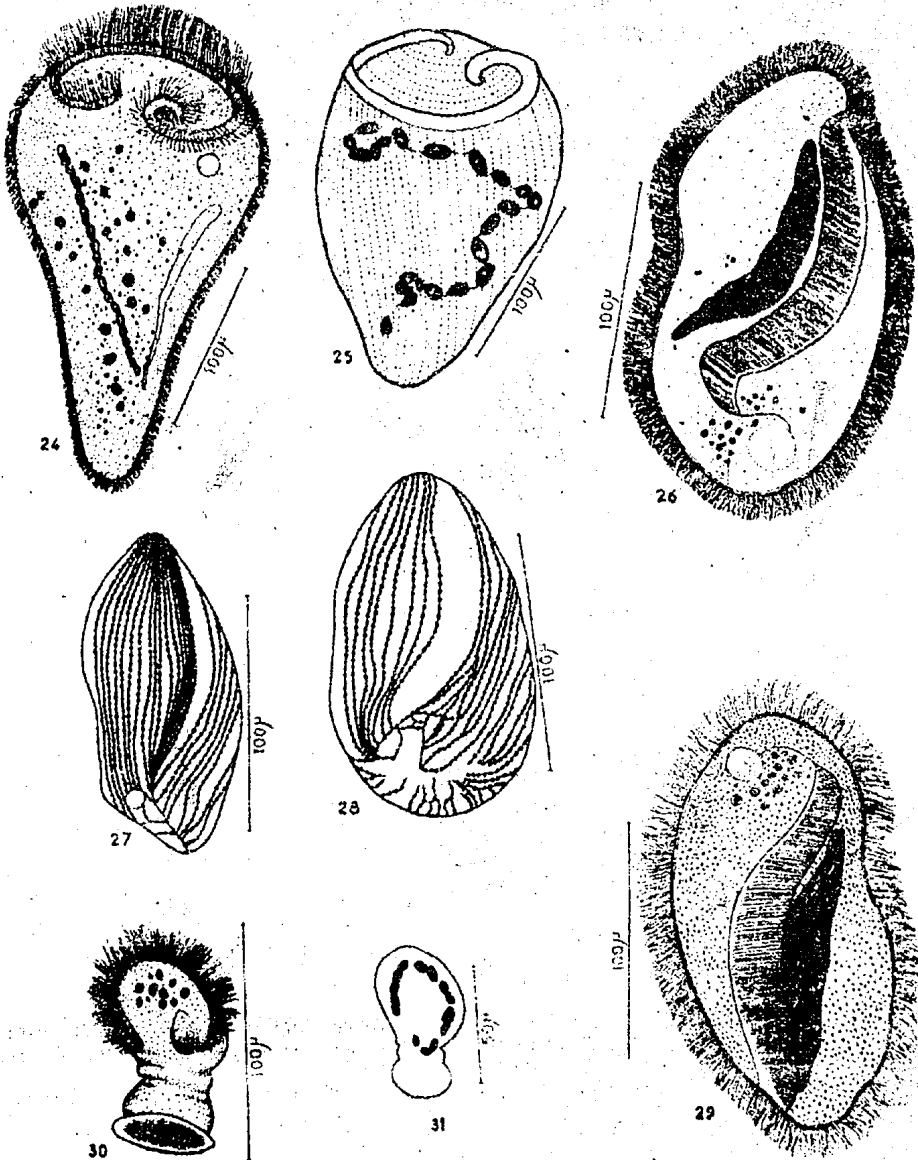
(Figs. 24 and 25)

Body trumpet-shaped when extended, reaching 1 - 2 mm. long in full extension; anterior end of body fully expanded; highly contractile and may assume even spherical shape when disturbed; usually oval to pyriform while swimming. Blue colour of body caused by pigment stentorin occurring as granules in ectoplasm.

Peristomal field conspicuous; adoral zone encircling peristome in spiral form, leaving narrow gap on ventral part; adoral zone and peristomal field sink toward cytostome; adoral zone continuing into cytopharynx.



Figs. 14—23. 14—17, *Boveria teredinidi*: (14 and 15) conjugation; (16 and 17) cysts in mantle of shipworm. 18—20, *Trichodina macommarum*: (18) side aspect; (19) details of denticle radial pins, hooks, etc.; (20) macro- and micronucleus. 21—23, *Trichodina bulakrishnia* new species: (21) typical shape of living specimen; (22 and 23) structure of macronucleus.



Figs. 24—31. 24—25, *Stentor coeruleus*: (24) typical shape of living specimen; (25) details of ciliary pattern and macronucleus (silver impregnated specimen). 26—29, *Nyctotherus marina* new species: (26) structural details; (27 and 28) ciliary pattern (silver impregnated); (29) living specimen treated with iodine. 30—31, *Licnophora* species: (30) typical shape of living specimen; (31) details of macronucleus.

Macronucleus moniliform; nodes of macronucleus containing different concentration of thymonucleic acid. Contractile vacuole at anterior end.

Discussion. *Stentor coeruleus* is previously recorded only as a free living form. But here it was noticed in the mantle cavity of the shipworm *Nausitora hedleyi* during the very low saline period. It suggests that the association of *Stentor* and the shipworm is very casual and it is not an obligate commensal.

PLAGIOTOMIDAE BÜTSCHLI 1887

Genus *Nyctotherus* LEIDY 1849

Body oval or reniform, compressed. Peristome beginning at anterior end, turning slightly to right, bending in cytostome midway between two ends; cytopharynx as long tube with undulating membrane running dorsally and posteriorly. Ciliary rows longitudinal, close set.

Endoplasm with discoid glycogen bodies, especially in anterior region; contractile vacuole and cytophyge terminal.

Macronucleus massive in anterior half. Micronucleus single. In some specimens nuclei suspended by karyophore.

Nyctotherus marina new species

Body oval in outline, with both ends rounded, posterior end wider than anterior end; dorsal surface slightly convex, ventral surface slightly concave. Anterior end of body differentiated by possession of knob-like projection. Pellicle thick, covering entire body; body cilia small, covering whole body, ranging from 8 to 15 μ in length, helping in locomotion of organism; ciliary rows somewhat parallel to long axis of body, can be seen clearly, even in living animal; arrangement of ciliary pattern clearly seen in impregnated specimen (Figs. 27 and 28).

Peristome beginning at anterior end of body on ventral part; cytopharynx as continuation of peristome, extending from cytostome in transverse disposition proceeding posteriorly to form about right angle (Fig. 26); undulating membrane present, extending from apical end of peristome to end of pharynx.

Numerous discoid glycogen bodies in endoplasm (Fig. 29), more concentrated in posterior region of body.

Contractile vacuole opening to body surface (Fig. 26), three in number, one at posterior, two at anterior region; contractile vacuoles begin to

enlarge (about double) within half hour when animal reaching moribund outside host.

Body size: length 135 - 220 μ ; width of posterior end 45 - 80 μ .

Binary fission. The individual multiplies by binary fission. Transverse binary fission has been noticed in the case of this form. At first the animal gets lengthened and then a constriction appears in the middle of the body. Simultaneously, the macronucleus, cytopharynx, undulating membrane and other structures get elongated and finally the constriction at the middle of the body deepens and the animal gets divided into two.

Behaviour of *Nyctotherus marina* outside the body of host animal. When *Nyctotherus* is removed from the caecum or mantle cavity of the host and kept in sea water in a slide, the organism performs rotating movements. After sometime, it swims without showing the rotation and after that it attaches ventral side to the slide. Always it has a tendency to hide under dirty or any other material seen in the mantle cavity or the caecum. When it is disturbed from there, it speedily swims away with the oral end directed forward. More often it shows gliding movement. Very often it shows clockwise rotation. Rarely, it moves in anti-clockwise direction also. The change of direction of clockwise to anti-clockwise is very sudden.

Remarks. This species has been collected from the mantle cavity and caecum of the shipworms *Nausitora hedleyi* and *Teredo furcifera*. It has been noticed very rarely from the caecum of the animal.

So far, there is no record of the presence of a *Nyctotherus* species in the marine habitat. Usually it is parasitic chiefly in oligochaetes and in frogs. It has been recorded from other invertebrates, vertebrates and also from man.

SUBORDER LICNOPHORINA CORLISS 1957

LICNOPHORIDAE BÜTSCHLI 1887

Genus *Licnophora* CLAPAREDE 1867

Ciliates without somatic ciliature but with strongly developed adoral zone of membranelle. Basal disc somewhat cup-shaped, surrounded by several concentric undulating membranes, serving for attachment to host.

Licnophora was first described by CLAUS (1862), but not named. CLAPAREDE (1867) created the genus *Licnophora* for two species, *auerbachii* COHN and *cohnii* CLAPAREDE. Literature on this group is not extensive. Reference to the older literature can be found from the papers of STEVENS

(1901), BALAMUTH (1941 and 1942) and VILLENEUVE - BRACHON (1940). FENCHEL (1965) was the first who noticed *Licnophora* species from lamellibranch.

Licnophora species

This is a very rare ciliate in the mantle cavity of the wood-boring bivalves, *Martesia striata*, *Teredo furcifera* and *Nausitora hedleyi*, and also found moving on the shells of *Martesia striata*.

Body elongated, divided into three distinct regions: attachment disc (basal disc), neck and oral disc (Fig. 30); attachment disc (efficient means of attachment to substratum) somewhat circular in outline, consisting of circular cup surrounded by ciliary membrane; velum present outside ciliary membrane; neck flattened dorsoventrally, flexible, showing conspicuous wrinkles and burrows on both dorsal and ventral parts; oral disc oval in outline; adoral zone of membranelle strongly developed, on concave ventral surface, beginning near region between neck and oral disc, extending around anterior end of disc; food vacuoles numerous.

Macronuclear chain consisting of 12 - 13 segments broken into sections (Fig. 31). Micronucleus not noticed.

Body size: length 80 - 105 μ ; width 56 - 60 μ ; diameter of basal disc 40 - 55 μ , of velum 45 - 50 μ .

Behaviour of *Licnophora* species outside the host animal. When it was taken out from the mantle cavity of the host and placed in water in a cavity slide, it remains attached for a considerably long time by means of its basal disc. In the attached state itself it slowly rotates in clockwise direction on the longitudinal axis. During the movement, the ciliary membrane beats against the slide. The oral cilia move with the effective stroke toward the mouth. The organism bends by its neck even when the oral cilia is at rest.

After this sort of clockwise rotation it swims forward for a short distance. Then again it attaches to the substratum. The attachment is so strong that it is very difficult to remove an attached *Licnophora* with the pipette. If we detach the specimen with the help of a needle from the substratum it will simply lie in the water for two to three seconds and then show somersault movements repeatedly and again it attaches to another site.

The behaviour of this species inside the host animal has not been observed.

ACKNOWLEDGEMENTS

The authors are deeply indebted to Prof. Tom Fenchel, Zoological Institute, University of Aarhus, Denmark, for valuable discussions about the identification of the species. Funds were provided by the Council of Scientific and Industrial Research for the execution of the scheme "Studies on the biological aspects of marine borer problem in India".

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(Received 11 April, 1972)