

COCHLIOPHILUS DEPRESSUS GEN. NOV., SP. NOV. AND COCHLIOPHILUS MINOR SP. NOV., HOLOTRICHOUS CILIATES FROM THE MANTLE CAVITY OF PHYTIA SETIFER (COOPER)

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INTRODUCTION

Examination of specimens of the pulmonate snail *Phytia setifer* (Cooper)<sup>1</sup> from salt marshes bordering San Francisco Bay disclosed the presence of two closely related species of flattened holotrichous ciliates within the mantle cavity. A new genus, *Cochliophilus*, is proposed to include these ciliates, which will be described herein as *Cochliophilus depressus* gen. nov., sp. nov. and *Cochliophilus minor* sp. nov.

I wish to express my appreciation to Professor S. F. Light and Professor Harold Kirby for their interest and helpful advice during the progress of this investigation.

TECHNIQUE

*Phytia setifer* occurs under matted vegetation and debris in salt marshes and in the vicinity of brackish water ponds on the Pacific Coast of central and northern California. Material for this study was collected at several localities along the east shore of San Francisco Bay at Oakland and Berkeley.

For observation of the living ciliates the shell of the snail was carefully removed and the anterior part of the animal crushed in a drop of sea water on a slide. Fixation of the organisms for permanent preparations was accomplished by liberating them in this manner on a coverglass and then dropping the coverglass smear-side down onto the surface of the fixative in a Petri dish.

Staining with iron hematoxylin gave good results following the fixatives of Schaudinn, Champy, Bouin, and Heidenhain ("susa"). For a study of the ciliary system the method devised by Bodian (1936, 1937) for impregnation with activated silver albumose (protargol) was used after fixation in Hollande's cupric-picroformol mixture. The Feulgen nuclear reaction was tried with success on material fixed in a saturated aqueous solution of mercuric chloride with 5 per cent of glacial acetic acid.

DESCRIPTION OF SPECIES

There is no agreement among protozoologists in regard to the orientation for descriptive purposes of compressed ciliates in which the cytostome is situated along the margin of the flattened body or displaced to the surface opposite that in

<sup>1</sup> Dall (1921) has implied that the species described by Cooper (1872) is distinct from *Phytia myosotis* (Drap.) of Europe and the Atlantic Coast of North America. No conclusive evidence has been presented to support or to refute this contention.

contact with the substrate. Hentschel (1924), writing of *Entodiscus* (*Cryptochilum*) *borealis*, stated that "since convention dictates that the side on which the mouth is situated shall be called ventral, we must say that the animal is flattened from side to side." Reichenow (1927-29) applied this scheme to *Conchophthirus*, as did also Kahl (1931, 1934) and Raabe (1932, 1934b).

De Morgan (1925), in his description of *Kidderia* (*Conchophthirus*) *mytili*, considered the concave under-surface to be ventral and the position of the cytostome to be lateral. Kidder (1933b) recognized the oral surface of *Kidderia mytili* as the "physiological ventral surface," but for purposes of clearness accepted De Morgan's plan of orientation. In the present paper I will follow De Morgan and Kidder in referring to that surface of the body most often found in contact with the substrate as ventral. The lateral margin on which the cytostome is situated will be referred to as the oral margin, and the opposite side as the aboral margin.

*Cochliophilus depressus* gen. nov., sp. nov. (Figs. 1 and 2)

The body outline as seen from the dorsal or ventral aspect is ovoid, often somewhat truncate at the posterior end. A view from the oral or aboral margin shows this ciliate to be much flattened, the ventral surface being slightly concave and the dorsal surface convex. In some individuals the curvature of the dorsal surface appears to be less regular than in others.

Twenty living individuals taken at random ranged from 70  $\mu$  to 107  $\mu$  in length and from 47  $\mu$  to 77  $\mu$  in width, averaging about 93  $\mu$  by 63  $\mu$ . The thickness varied from 11  $\mu$  to 16  $\mu$ . The relation of the length to the width is not the same in all specimens. Fixation of the organisms on coverglasses produced some shrinkage and frequently also distortion of shape due to compression.

The elongated peristomal area is situated in the posterior fourth of the body. Specialized ciliary elements which will be described presently extend from the anterior end of the peristomal indentation to the cytostome. That part of the peristomal area lying posterior to the cytostome is naked.

A well-defined pharynx is not present. I prefer to regard the irregular tubular structure which passes from the cytostome into the cytoplasm as the gullet. This gullet is difficult to see in living individuals, but in fixed material is demonstrable following staining in iron hematoxylin. As it approaches the macronucleus the gullet widens out and its boundaries become inexact.

A thin pellicle covers the body. Flexure of the pellicle in this ciliate is rarely noted, and then only when the animal comes in contact with solid obstructions in its path of movement. Trichocysts are absent.

The cilia of the body are disposed in 52 to 56 longitudinal rows and beat metachronously. The cilia on the dorsal and ventral surfaces are somewhat longer than those along the margin. The ventral cilia are thigmotactic, but not strongly so. On the ventral surface at the anterior end is a transverse suture (anterior field) from which the ventral rows of cilia extend backward, and from which the dorsal rows curve upward and continue posteriorly. Most of the dorsal rows converge in a characteristic pattern towards the posterior end. A definite unciliated area is evident between the longer dorsal rows and the ventral rows which curve upward a short distance over the posterior end.

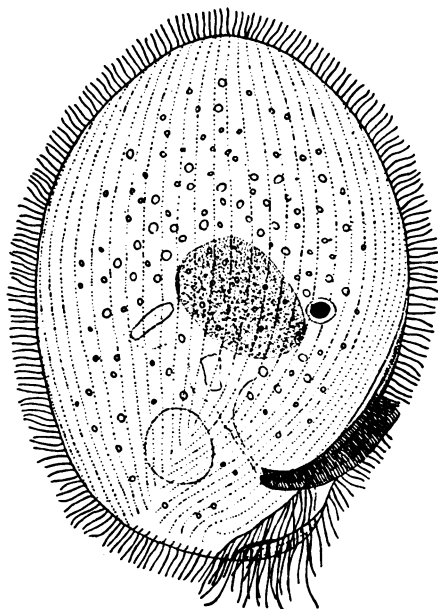


FIGURE 1. *Cochliophilus depressus* gen. nov., sp. nov. Dorsal aspect. Heidenhain's fixative ("susa")-iron hematoxylin. Drawn with aid of camera lucida.  $\times 900$ .

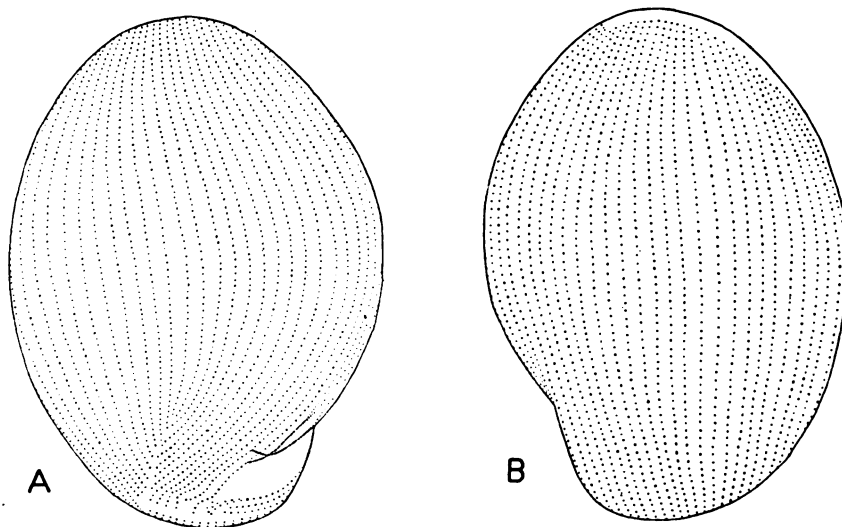


FIGURE 2. *Cochliophilus depressus* gen. nov., sp. nov. Distribution of ciliary rows. Hollande's fixative-protargol. Drawn with aid of camera lucida. A. Dorsal aspect. Though distorted somewhat due to compression, this individual shows well the arrangement of ciliary rows entering the peristomal indentation.  $\times 670$ . B. Ventral aspect.  $\times 950$ .

Three ventral rows of cilia close to the oral margin turn dorsally near the end of their course to delimit the naked part of the peristomal area posteriorly. The first of these rows is ordinarily seen to ramify into an incomplete double or triple series of cilia. The post-peristomal extensions of the ventral rows and the terminal part of the dorsal row which borders the peristome above bear cilia which are two to three times as long as the peripheral cilia elsewhere on the body.

The specialized peristomal cilia arise from two series of closely-set basal granules, each of which is seen to be a continuation of two rows of peripheral cilia essentially lateral in position, lying between the three ventral rows of cilia and one dorsal row marking off the peristomal area. The cilia of the upper peristomal row are appreciably longer than those of the lower row and appear in living individuals to form a membrane-like structure which beats up and down as a unit. The cilia of the lower row are much thicker and do not beat synchronously. The activity of the peristomal cilia ceases soon after the organism is dissociated from the host.

The cytoplasm is colorless. Greenish granules appearing as highly refractile bodies are distributed through the cytoplasm. These are most numerous around the macronucleus and following fixation stain intensely with iron hematoxylin.

The macronucleus is centrally located. In outline it varies from oblong to round, and in life is conspicuous as a clear granular body surrounded by food inclusions and cytoplasmic granules. The micronucleus is greenish in color and difficult to detect in living individuals. It is easily demonstrated by iron hematoxylin or the Feulgen nuclear reaction. The micronucleus is commonly situated close to the macronucleus, between the latter and the oral margin. Upon fixation it shrinks considerably and draws away from the membrane by which it is invested.

The contractile vacuole lies in the posterior fourth of the body behind the gullet, and apparently opens to the exterior at a point between the convergence of the shorter dorsal ciliary rows. I have been unable to distinguish a permanent opening in the pellicle.

When free in water, *Cochliophilus depressus* swims actively, generally in circles and with its concave ventral surface in contact with the substrate. Occasionally, however, it follows an erratic course, rotating on its longitudinal axis. The transverse anterior field is always at right angles to the direction of movement. In the presence of pieces of tissue from the host *Cochliophilus depressus* will sometimes seek refuge among them or cling to epithelial surfaces by means of its ventral thigmotactic cilia.

I have found *Cochliophilus depressus* to be present in the mantle cavity of nearly all specimens of *Phytia setifer* which I have examined. It occurs in small numbers and is usually less common than the following species.

*Cochliophilus minor* sp. nov. (Figs. 3 and 4)

The shape of this species resembles in general that of *Cochliophilus depressus*, except that the posterior end is rather pointed, never truncate, and the dorso-ventral dimension in relation to the length and breadth is comparatively greater. In addition, the curvatures of the ventral and dorsal surfaces are more pronounced in *Cochliophilus minor*.

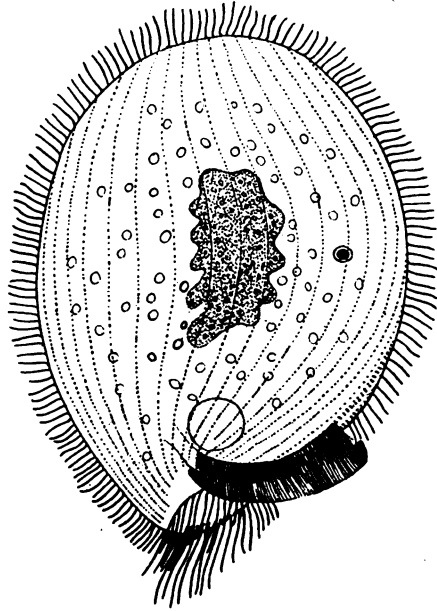


FIGURE 3. *Cochliophilus minor* sp. nov. Dorsal aspect. Heidenhain's fixative ("susa")-iron hematoxylin. Drawn with aid of camera lucida.  $\times 1250$ .

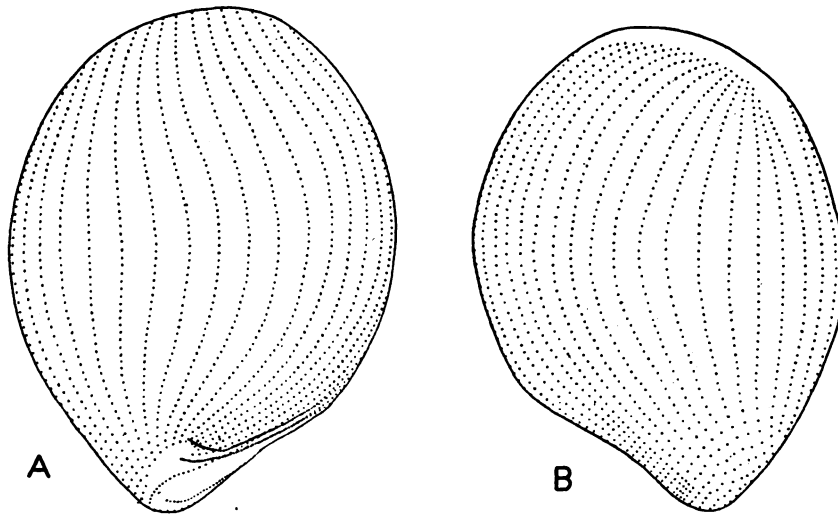


FIGURE 4. *Cochliophilus minor* sp. nov. Distribution of ciliary rows. Hollande's fixative-protargol. Drawn with aid of camera lucida. A. Dorsal aspect.  $\times 1250$ . B. Ventral aspect.  $\times 1250$ .

Twenty living individuals taken at random ranged from  $51\ \mu$  to  $80\ \mu$  in length and from  $33\ \mu$  to  $56\ \mu$  in width, averaging about  $63\ \mu$  by  $45\ \mu$ . The thickness varied from  $11\ \mu$  to  $18\ \mu$ .

The peristomal area is situated in the posterior fourth of the body. Two rows of specialized cilia extend from the anterior end of the peristomal indentation to the cytostome. That part of the peristomal area posterior to the cytostome is naked.

An irregular gullet may sometimes be traced a short distance from the cytostome, but it is not as easily discerned as the comparable structure in *Cochliophilus depressus*.

The cilia are disposed in 36 to 38 longitudinal rows and beat metachronously. The cilia on the ventral and dorsal curvatures are slightly longer than those along the margin. The ventral cilia are weakly thigmotactic. The ventral rows extend from an anterior transverse suture to the posterior tip of the body. The basal granules of most of the ventral rows come to lie farther apart towards the posterior end, while those of three or four rows near the oral margin lie closer together. The dorsal rows of cilia pass from the transverse suture over the anterior end of the body and continue backward to terminate in a conformation homologous with that found in *Cochliophilus depressus*. The posterior dorsal unciliated area of *C. depressus* has no exact homologue in this species. There exists, nevertheless, an unciliated area between the converging dorsal rows and the dorsal row bordering the peristomal area above.

One or two rows of cilia following the oral margin curve dorsally near the end of their course to delimit the naked part of the peristomal area posteriorly. These extensions and the terminal part of the most nearly lateral dorsal row on the oral side bear exceptionally long cilia.

The peristomal ciliary apparatus consists of a membrane-like structure of long, fine cilia which curves downward over a row of closely-set, rather thick cilia extending from the anterior end of the peristomal indentation to the cytostome. The membrane-like structure appears to be non-motile and to function as a funnel directing food particles into the cytostome.

The cytoplasm is colorless. Refrangent cytoplasmic granules are present, but to a lesser extent than in *Cochliophilus depressus*.

The size and shape of the macronucleus are highly variable. In living as well as fixed individuals it is nearly always seen to be ramified, although ovoid or round macronuclei are occasionally noted in this species. Reorganization stages in which two or more smaller and round macronuclei are present are not infrequently met with. The micronucleus ordinarily occupies a position between the macronucleus and the oral margin. In fixed and stained preparations it is considerably shrunken.

The contractile vacuole is situated anterior to the cytostome. It opens to the exterior between the convergence of the shorter dorsal ciliary rows. I have not detected a permanent opening in the pellicle.

When separated from its host *Cochliophilus minor* swims in circles or proceeds forward rotating on its longitudinal axis. Its movements are in general slower than those of *Cochliophilus depressus*.

*Cochliophilus minor* is found in association with *Cochliophilus depressus* in the mantle cavity of *Phytia setifer*. It is usually more numerous than *C. depressus*.

## SYSTEMATIC POSITION

On the basis of certain features of the morphology of the two species of *Cochliophilus* which I have described it may be justifiable to allocate this genus to the sub-order *Thigmotricha* Chatton and Lwoff, although in view of the deficiencies of the systems of classification of holotrichous ciliates currently recognized I must defer a conclusive statement with regard to its position. The organization of the peristome of *Cochliophilus* hints its affinity with *Kidderia* Raabe, represented by *K. mytili* (De Morgan) from *Mytilus edulis*. Raabe (1936) retained *Kidderia* in the family Conchophthiridae Reichenow,<sup>2</sup> but removed to the family Thigmophryidae Chatton and Lwoff *Myxophyllum* and *Conchophyllum*, genera created by him to accommodate, respectively, Stein's species *Conchophthirus steenstrupi*, commensal on various terrestrial pulmonate molluscs, and *Conchophthirus caryoclada* Kidder, from the bivalve *Siliqua patula*. It is interesting to note, in passing, that a specific character of *Conchophyllum caryoclada* is its branched macronucleus, of which the macronucleus of *Cochliophilus minor* is reminiscent.

The presence of a membrane-like structure in the peristome of *Cochliophilus* could be the basis for objections to the inclusion of this genus in the *Thigmotricha*. Very similar ciliary elements have been observed, however, in certain species of the family Ancistrumidae Issel. Raabe (1932, 1934b) has stressed the presence of an undulating membrane in *Conchophthirus*, although Kidder (1934), after studying species of *Conchophthirus* from fresh water mussels in this country, was unable to corroborate Raabe's findings, and suggested that Raabe may have mistaken the fibers of the peristomal basket for an undulating membrane.

*Genus Cochliophilus gen. nov.*

Diagnosis: Flattened holotrichous ciliates, ovoid in outline as seen in dorsal or ventral view. The peristomal area is elongated and is situated on the right lateral margin in the posterior fourth of the body. A membrane-like structure of fine cilia overlies a series of thick cilia extending from the anterior end of the peristomal indentation to the cytostome; that part of the peristomal area posterior to the cytostome is naked. The peripheral cilia are disposed in longitudinal rows extending from a ventral transverse suture at the anterior end of the body. The dorsal rows converge in a characteristic pattern posteriorly. Thichocysts are absent. The macronucleus is centrally located; the micronucleus is usually situated near the macronucleus, between the latter and the oral margin. The contractile vacuole opens to the exterior between the convergence of the shorter dorsal ciliary rows; no permanent opening in the pellicle is discernible. Genotype: *Cochliophilus depressus* gen. nov., sp. nov. Two species, commensal in the mantle cavity of *Phytia setifer* (Cooper).

*Cochliophilus depressus gen. nov., sp. nov.*

Diagnosis: Average size about 93  $\mu$  by 63  $\mu$ , the thickness being about one-sixth the length. The ciliary rows are 52 to 56 in number. The peristomal membrane-like structure is motile. The macronucleus is round or oblong. Syntypes are in the collection of the author.

<sup>2</sup> Reichenow (1927-29) was apparently the first to use the name Conchophthiridae, although Raabe credits Kahl (1931) with establishing this family.

*Cochliophilus minor* sp. nov.

Diagnosis: Average size about  $63\ \mu$  by  $45\ \mu$ , the thickness being about one-fourth the length. The ciliary rows are 36 to 38 in number. The peristomal membrane-like structure is apparently immobile, serving as a funnel directing food particles into the cytostome. The macronucleus is characteristically ramified. Syntypes are in the collection of the author.

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