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Journal

Invertebrate Biology, 110(3)

ISSN

1077-8306

Authors

Snyder, Richard A
Ohman, Mark D

Publication Date

1991-07-01

DOI

10.2307/3226658

Peer reviewed



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Author(s): Richard A. Snyder and Mark D. Ohman

Source: *Transactions of the American Microscopical Society*, Vol. 110, No. 3 (Jul., 1991), pp. 237-243

Published by: Blackwell Publishing on behalf of American Microscopical Society

Stable URL: <http://www.jstor.org/stable/3226658>

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Description of a New Species of Strombidinopsidae (Ciliophora: Choreotrichida) from Coastal Waters of Southern California, U.S.A.¹

RICHARD A. SNYDER² AND MARK D. OHMAN

Marine Life Research Group 0227, Scripps Institution of Oceanography,
La Jolla, California 92093, U.S.A.

Abstract. The planktonic ciliate protist *Strombidinopsis cheshiri* n. sp. is described from Protargol-stained preparations of cultured cells. The specimen reacted unsatisfactorily to commonly used fixatives; 87% disintegrated in Bouin's fluid, leaving only oral polykinetids. Cell shape changed from spherical in recently divided cells to long and tapered in pre-division cells. The range of cell size was 34–110 μm (l) \times 32–60 μm (w). Fourteen to sixteen external oral polykinetids had oral membranelle cilia 14–16 μm long. Four inner oral polykinetids were found within the infundibulum. Twelve to fifteen somatic kineties extended for the entire length of the cell, with 18–32 dikinetids per kinety. Both kinetosomes of each kinetid bore cilia 4 μm long. Incomplete kineties were common. Two roughly ovoid macronuclei and one micronucleus were found per cell. *S. cheshiri* was algivorous in laboratory culture.

Interest in marine planktonic microbial processes has focused attention on the confused state of existing systematics for the “naked” planktonic choreotrichs and the inaccurate taxonomic identification of these organisms accompanying ecological research. Recent works by Lynn & Montagnes (1988), Lynn et al. (1988), Lynn et al. (1991), and Montagnes et al. (1990) took up the dual challenge of establishing criteria for taxonomic assessment based on Protargol silver-stained specimens and of reconciling these findings with historical descriptions.

Morphological descriptions of species of the genus *Strombidinopsis* were compiled by Maeda (1986). Lynn et al. (1991) proposed a revision of criteria for taxonomic assignment within the genus *Strombidinopsis* using both historical records and morphological data on six species (four new), based on Protargol-stained specimens. They revised the diagnosis of the family Strombidinopsidae Small & Lynn, 1985 and the genus *Strombidinopsis* Kent, 1881 as follows: “Free swimming, aloricate ciliates. Many (usually >10) ciliated somatic kineties typically extending the entire length of the cell and composed of dikinetids. Typically two similarly shaped spherical to ovoid macronuclei.”

The present paper provides a description of a new species isolated from the near-shore environment of Southern California, U.S.A.

¹ We express thanks to J. R. Wilkinson for maintaining laboratory cultures, and to Dr. D. H. Lynn for comments on an earlier version of the manuscript. Supported by NSF grant OCE-8812123 and NOAA grant NA89AA-D-SG138 to M. D. Ohman.

² Present address: Center for Environmental Diagnostics and Bioremediation, University of West Florida, USEPAERL Sabine Island, Gulf Breeze, Florida, U.S.A.

MATERIALS AND METHODS

All culturing was conducted at 15°C with glass-fiber filtered (Whatman GF/F) natural seawater. The ciliates were isolated from surface water collected approximately 3 km offshore above the La Jolla Canyon. They were established as a clonal culture and maintained on suspensions of *Heterocapsa triquetra* and *Isochrysis galbana*, both grown in a modified IMR/2 medium (Eppley et al., 1967), and diluted with glass-fiber filtered seawater for use. The modifications to the IMR/2 medium involved omission of silicate, halving the trace metal concentrations, and doubling the chelating agent (EDTA) concentration.

Ciliates were fixed using a modified Bouin's fluid picric acid fixative (Lee et al., 1985) (20 ml culture suspension was added to 1 ml of concentrated fixative) and stained using the Protargol (albumous silver) method (Lee et al., 1985). Additional fixatives tested were 2% acid Lugol's iodine, 2% and 36% formaldehyde, and 2% (final concentration) glutaraldehyde in seawater. Fixation was observed under a dissecting stereomicroscope by micropipetting individual cells into fixatives. Measurements (rounded to the nearest 1.0 μm) were made with an eyepiece micrometer reticule. Sketches were made of stained specimens using a drawing tube; a composite drawing was compiled from collected sketches. Photomicrographs were made on a Zeiss Axiophot® light microscope with a 63 \times planapochromat oil immersion lens and panchromatic technical film (Kodak Tech Pan®).

TAXONOMIC ACCOUNT

Family Strombidinopsidae Small & Lynn, 1985

Genus *Strombidinopsis* Kent, 1881

Strombidinopsis cheshiri n. sp.

(Figs. 1, 2)

Description. Cell long, tapered to a point posteriorly, 34–110 μm long, 32–60 μm wide (Bouin's fluid-fixed cells). Recently divided cells may be more spherical, bluntly tapered posteriorly. External oral polykinetids forming a radially continuous circling, 14–16 in number, oral membranelle cilia 14–16 μm long. Oral depression containing four inner oral polykinetids, orientation to long axis of the body variable. Somatic kineties 12–15 in number, extending to cell posterior, 18–32 dikinetids per kinety. Incomplete kineties common, lacking either posterior or anterior ends. Dark-staining line adjacent to somatic kineties, may be continuous or broken. Some partial kineties may be present. Both kinetosomes ciliated, cilia 4 μm long. Two roughly ovoid macronuclei and one micronucleus per cell. Algivorous.

Type locality. La Jolla Canyon surface waters, California, U.S.A. 32°52'N, 117°17'W.

Type specimen. A Protargol-stained holotype on slide USNM 42454 was deposited in the ciliate type specimen slide collection, United States Museum of Natural History, Smithsonian Institution, Washington, D.C., U.S.A.

Etymology. The specific epithet *cheshiri* refers to the ability of the Cheshire cat in Lewis Carroll's *Alice in Wonderland* to disappear leaving only its smile,

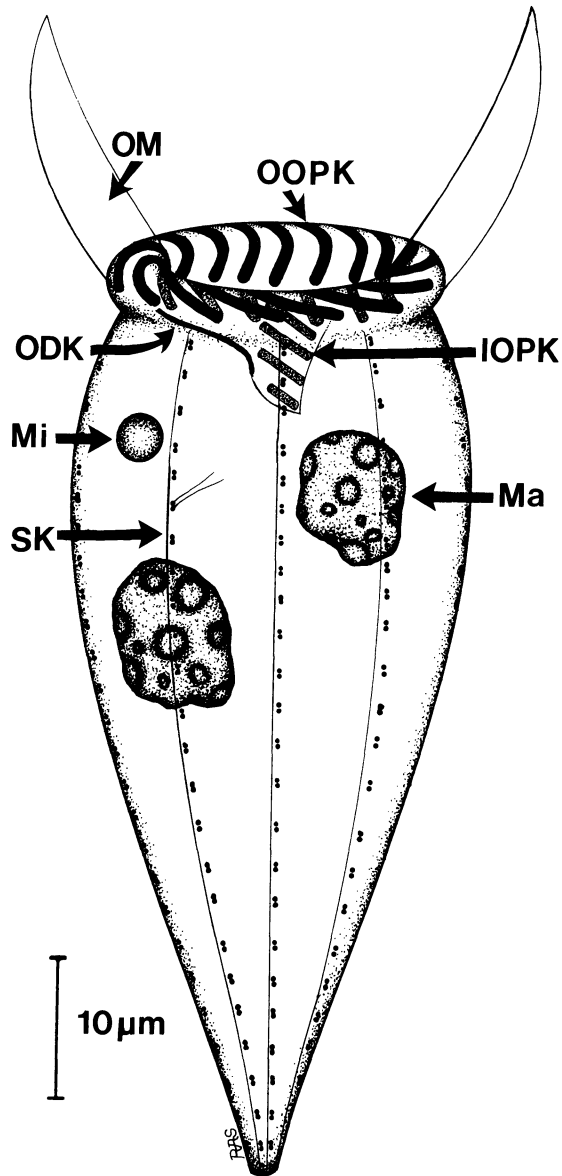


FIG. 1. Composite drawing of *Strombidinopsis cheshiri* n. sp., from Protargo-stained specimens. IOPK, inner oral polykinetid; Ma, macronucleus; Mi, micronucleus; ODK, oral dikinetid; OM, oral membranelles; OOPK, outer oral polykinetid; SK, somatic kinety. Scale bar represents 10 μm .

as this ciliate was frequently observed to do. In reaction to fixation procedures, the ciliate would disappear completely leaving only its oral membranelles.

DISCUSSION

Specimens of *Strombidinopsis cheshiri* did not preserve well with commonly used fixatives. Most cells lysed, but intact oral membranelles (Fig. 2a, b) from

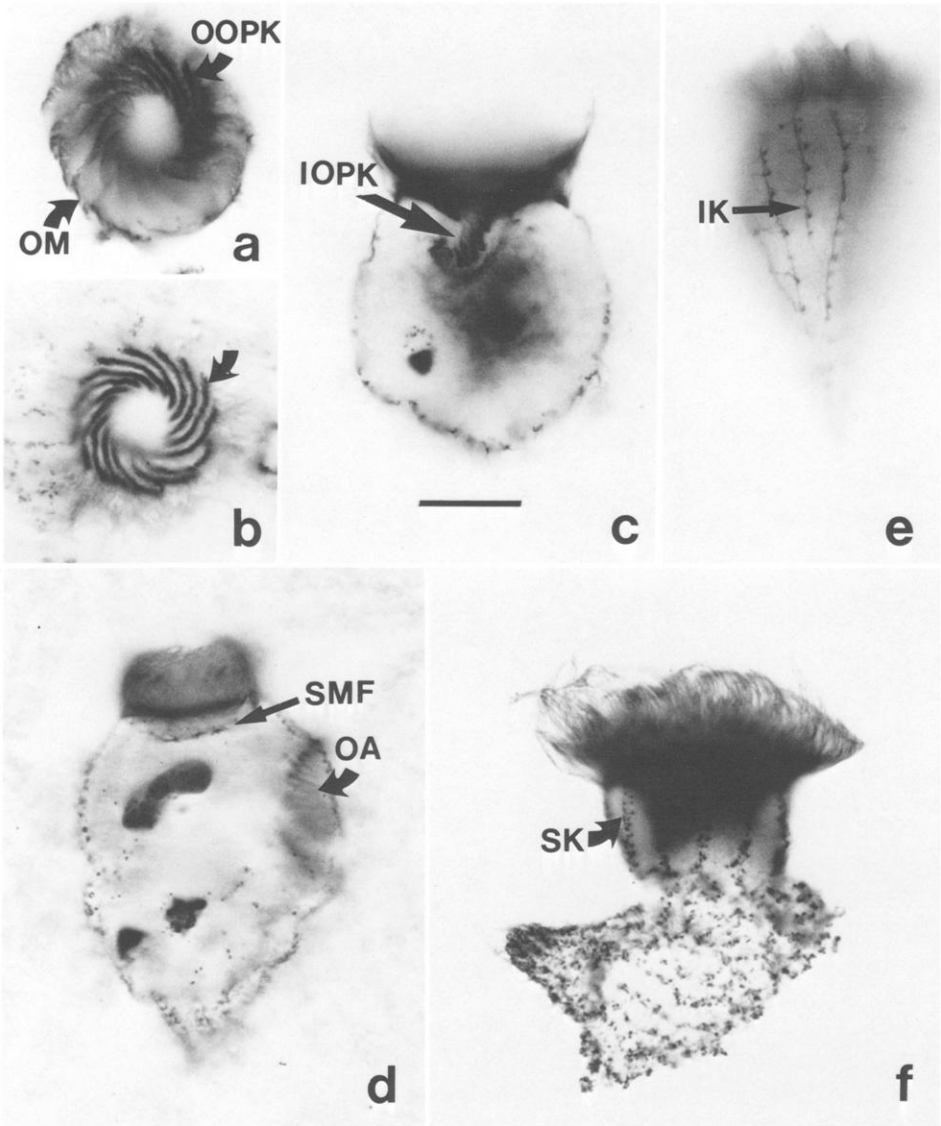


FIG. 2. Protargol-stained specimens of *Strombidinopsis cheshiri* n. sp. a. Outer apical oral membranelles (OM) and outer oral polykinetids (OOMK). b. Outer oral polykinetids. c. Inner oral polykinetids (IOPK) found within the oral depression. d. Internal stomatogenesis (OA) and submembranellar fibrils (SMF). e. Somatic kineties of a well-fixed specimen showing an incomplete kinety (IK). f. Somatic kineties (SK) showing fragmentation. Scale bar represents 10 μ m.

lysed cells were found in stained preparations. By counting intact membranelles and entire cells in Protargol preparations, a conservative estimate of the loss of cells with Bouin's fixation is approximately 87%. The proportion of cells that disintegrated entirely without leaving identifiable membranelles is not known. A few partial membranelle remains were seen.

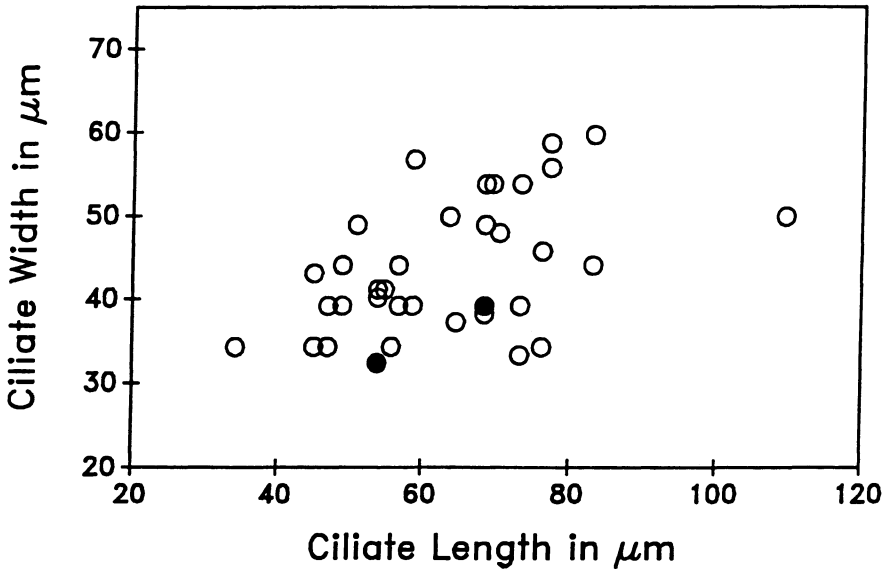


FIG. 3. Variation in cell length and width of Bouin's fluid-fixed and Protargol-stained specimens ($n = 37$). Solid circles represent overlapping observations.

Fixations monitored under a dissecting stereomicroscope revealed that disintegration occurred 0–2 sec after the cells became immobilized either in 2% formaldehyde or 2% acid Lugol's. In 2% glutaraldehyde, fixed cells remained intact somewhat longer, but disintegrated in less than 30 sec. Cells pipetted into 36% formaldehyde appeared to fix the best. This suggests that the 13% intact cells remaining after fixation in Bouin's fluid may have resulted from the initial contact of specimen sample (20 mls) with the concentrated fixative (1 ml). As more sample was added, the fixative would have become more dilute, resulting in poorer fixation. Poor fixation of members of the Strombidinopsidae, Strobilidiidae, and Oligotrichida reported in this and other studies (Choi & Stoecker, 1989; Beers, pers. comm.) indicates that abundances of these organisms probably are underestimated in environmental counts.

One obvious reason for poor fixation of these organisms is the paucity of cytoskeletal elements underlying the membrane of the cell body, which in other ciliates provide some support for the cell. Some indirect evidence suggests that the biochemical composition of the cell membrane also may play a role. For a strain of *Strombidium* sp. (also under cultivation), exponentially growing cells exhibited more fixation damage than did starved, stationary phase cells using the same fixatives noted above.

Average cell length and width of Bouin's fluid fixed specimens ($n = 37$) was 63 μm and 43 μm , respectively, with ranges of 34–110 μm and 32–60 μm , respectively (Fig. 3). These ranges were recorded from cells throughout the growth cycle; i.e., from small recently divided cells, to large, pre-division cells.

A wide range of variation in the position of the polykinetids in fixed specimens suggested considerable flexibility of the oral region, indicating an ability to

rotate the polykinetids around the peristomial lip. The location of the polykinetids varied from an internal, contracted position to an external position with maximum membranellar exposure. The four short polykinetids within the oral cavity occupied positions ranging from perpendicular to the long axis of the cell to a parallel alignment with that axis. An oral dikinetid occurred within the oral cavity (Fig. 1).

Stomatogenesis was internal (Fig. 2d). Internal fibers associated with the oral polykinetids as mentioned in Lynn & Montagnes (1988) for strobilidiids were observed in a few specimens (Fig. 2d), but no distinct patterns of taxonomic interest were apparent.

Somatic kineties (12–15/cell, $n = 30$) extend the entire length of the cell from just beneath the oral apical membranelles to the posterior tip (Figs. 1, 2e, f), although partial kineties (Fig. 2e) were common, with either the anterior or posterior portions missing. Both kinetosomes of each dikinetid bear equal cilia $4 \mu\text{m}$ long. Each kinety was associated with a dark-staining line adjacent to the kinetosomes (Fig. 2e). In most fixed specimens, this dark-staining material was broken up into segments of varying length (Fig. 2f). Kineties in some specimens were not arranged into discrete linear kineties. This condition also was reported in the description of *Strobilidium marinum* Fauré-Fremiet, 1924, which Lynn et al. (1991) suggested may be a species of *Strombidinopsis*. For *Strombidinopsis cheshiri*, however, the dispersion of the dark-staining material and the kineties appears to be a result of fixation trauma. Those fixed specimens whose shape most closely matched living cell structure had somatic kinetosomes aligned into linear kineties with the dark-staining material present as a continuous line from anterior to posterior.

Strombidinopsis cheshiri appears to be most closely related to *Strobilidium marinum*, and to *Strombidinopsis acuminatum* Fauré-Fremiet, 1924 (as re-described by Lynn et al., 1991). The shape of the cells and arrangement of the membranelles are similar. The somatic kineties of all three species extend the entire length of the cell, sometimes dispersed as noted above. The length range of *S. cheshiri* (34–110 μm) overlaps with the reported lengths for both *S. acuminatum* (110–115 μm , Fauré-Fremiet, 1924; 70–124 μm , Lynn et al., 1991) and *Strobilidium marinum* (100 μm , Fauré-Fremiet, 1924). However, the number of apical oral polykinetids differ for *S. cheshiri* (15–16, $n = 50$) and *S. marinum* (30–32). The number of apical oral polykinetids for *S. cheshiri* and *S. acuminatum* are within the same range (14–16 and 15–16, respectively), although there is not a one-to-one relationship of somatic kineties to oral membranelles in *S. cheshiri* as reported for *S. acuminatum* (see Fauré-Fremiet, 1924). In the strain of *S. acuminatum* studied by Lynn et al. (1991), 15–16 somatic kineties were found, and this agrees with the description of Fauré-Fremiet (1924). The range of 12–15 found in *S. cheshiri*, although more variable and overlapping the range found in *S. acuminatum*, does not correspond to the number of oral polykinetids. In addition, Lynn et al. (1991) reported three inner oral polykinetids for *S. acuminatum*, whereas four were recorded for *S. cheshiri*. Considering these differences, we believe that the new species herein described is justified.

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