Mytilophilus pacificae n. g., n. sp.: A New Mytilid Endocommensal Ciliate (Scuticociliatida)[1]

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Abstract. *Mytilophilus pacificae* n. g., n. sp. is described as an endocommensal scuticociliate found on the foot and mantle of the Pacific coastal mussel *Mytilus californianus*. *M. pacificae* is distinctive in its buccal apparatus which, while pleuronematine in organization, is dominated by the third membranelle. The third membranelle is a characteristic of the genus and distinguishes *M. pacificae* from other pleuronematine scuticociliates. The general size, body form, dense ciliation, and nuclear configuration are reminiscent of *Peniculistoma mytili*, which probably occupies a similar ecological niche in the bay mussel *Mytilus edulis*. Based on the strong correlation of characters of general morphology, buccal structure and habitat with *P. mytili*, *M. pacificae* is placed within the Family Peniculistomatidae. The close correspondence between these two organisms and their respective host species suggests that investigation of possible *Mytilophilus–Peniculistoma*–host interrelationships may provide instructive data for our understanding of speciation events and ciliate evolution.

The general morphology, buccal structure, and occurrence of a new pleuronematine scuticociliate, *Mytilophilus pacificae* n. g., n. sp., are described. *M. pacificae* is a large ciliate found on the foot and mantle of *Mytilus californianus* Conrad, a common littoral mussel of the Northeast Pacific Basin. The new species shares a suite of morphological characters, including size, shape, ciliation pattern, and nuclear configuration with *Peniculistoma mytili* (De Morgan, 1925) Jankowski, a ciliate found in the circumtemperate littoral mussel *Mytilus edulis* L. (see Beers, 1959; Fenchel, 1965; Kidder, 1933; Raabe, 1949). The new ciliate's marked similarity in morphology and habitat to the relatively well-known *P. mytili* may explain its past anonymity. However, silver-stained specimens of *M. pacificae* demonstrate its unique buccal structure, which distinguishes it from *P. mytili* and all other known ciliates. Thus, we have erected a new genus *Mytilophilus* n. g., with a single included new species, *M. pacificae* n. sp., for this unique scuticociliate.[3]

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[3] The generic name *Mytilophilus* first appeared in Corliss' text on the ciliated protozoa as a *nomen nudum* in anticipation of the present publication. See his discussion of such treatments (pp. 207, 209 in Corliss, 1979).

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Materials and Methods

Host Collection and Examination

Individuals of *Mytilus californianus* were collected during low tides from large, well-established mussel beds at several locations between Pigeon Point, California and Astoria, Oregon. Mussels were pried open to allow for the insertion of a Pasteur pipette filled with filtered seawater. The seawater was squirted vigorously into the mantle cavity with attention to washing off the fleshy parts of the mantle and foot. The rinse fluid was collected in a Syracuse dish, and, after 2–3 repetitions, ciliates were removed individually by micropipette. We have not made a systematic investigation of the autecology of this organism, but *Mytilophilus pacificae* was recovered from greater than 80% of the specimens at most localities examined; the numbers of individuals found within each mussel appeared to vary significantly.

Cytological Techniques and Mensurations

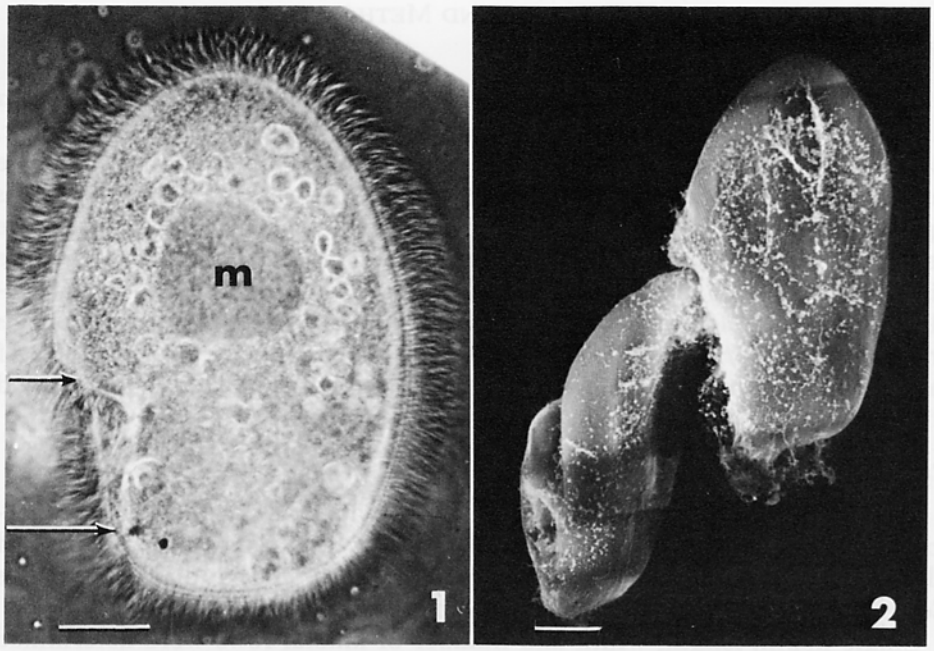
Live specimens were examined and photographed with the aid of phase-contrast and differential interference-contrast optics (Zeiss). Two methods of silver impregnation were used: the $AgNO_3$ method of Chatton & Lwoff (see Corliss, 1953) and the Protargol method of Bodian (see Kirby, 1950).

Ciliate depth, length, width and number of kinetal rows were determined for 25 similarly oriented, Protargol-stained specimens from each of two locations. Length and depth were measured with a calibrated ocular micrometer at 400× for both living and Protargol-stained organisms. Width was determined by focusing through the ciliate and counting the 2-μm units of the fine focusing knob of the microscope. The manufacturer's calibration was verified to be accurate by tests on a stack of coverslips which had been measured with a die-maker's micrometer gauge and engraved on each side to allow for visualization. Due to the extremely dense ciliation of this organism, the number of kinetal rows could only be counted on selected specimens with great difficulty. Therefore, in order to achieve an accurate and unbiased estimate, the following sampling protocol was devised. Since the organism is approximately elliptical in median transverse section, the perimeter of each ellipse was calculated using the width and depth measurements achieved (see above). Two quadrants were selected, each 6 μm wide, one just above the nucleus on the right side and the other just below the nucleus on the left side. The mean number of kinetal rows in the two quadrants was extrapolated to give the total number of kinetal rows around the ciliate. Standard counts made on each of two extraordinarily well-stained and oriented specimens fell within the range of kinetal rows calculated for the 25 specimens from that locality and within 5% of the computed values for these two specimens.

Taxonomic Account

Order Scuticociliatida Small, 1967

Family Peniculistomatidae Fenchel, 1965



FIGS. 1, 2. *Mytilophilus pacificae* n. g., n. sp. Fig. 1. Left aspect of living organism as seen by phase-contrast microscopy. Note extended, relaxed ciliature in this stationary, feeding organism. Arrows delimit the buccal cavity, although membranelle 1 and the paroral membrane begin just below the anterior suture (see also Fig. 4). m; macronucleus. Scale bar represents 20 μm. Fig. 2. Low-power scanning electron micrograph of conjugating pair. Anterior suture of organism to left is in contact and fuses with the anterior end of the oral area of the organism to the right. Scale bar represents 40 μm.

Mytilophilus pacificae n. g., n. sp.
(Figs. 1–8; Table I)

Description—Living Specimens

Organism laterally flattened and densely ciliated.[4] Organism kidney-shaped, as seen from right or left side, with somewhat pointed anterior end and broadly rounded posterior end (Fig. 1). Length 99–143 ($\bar{x}$ = 116 ± 9.8) μm; depth 44–77 ($\bar{x}$ = 65.6 ± 11.2) μm; length-to-depth ratio (overall estimate of shape and variations) 1.26–2.63 (measurements based on 25 living specimens from Pigeon

[4] We accept the convention used by Raabe (1967) and others which considers the surface on which the mouth lies to be ventral.

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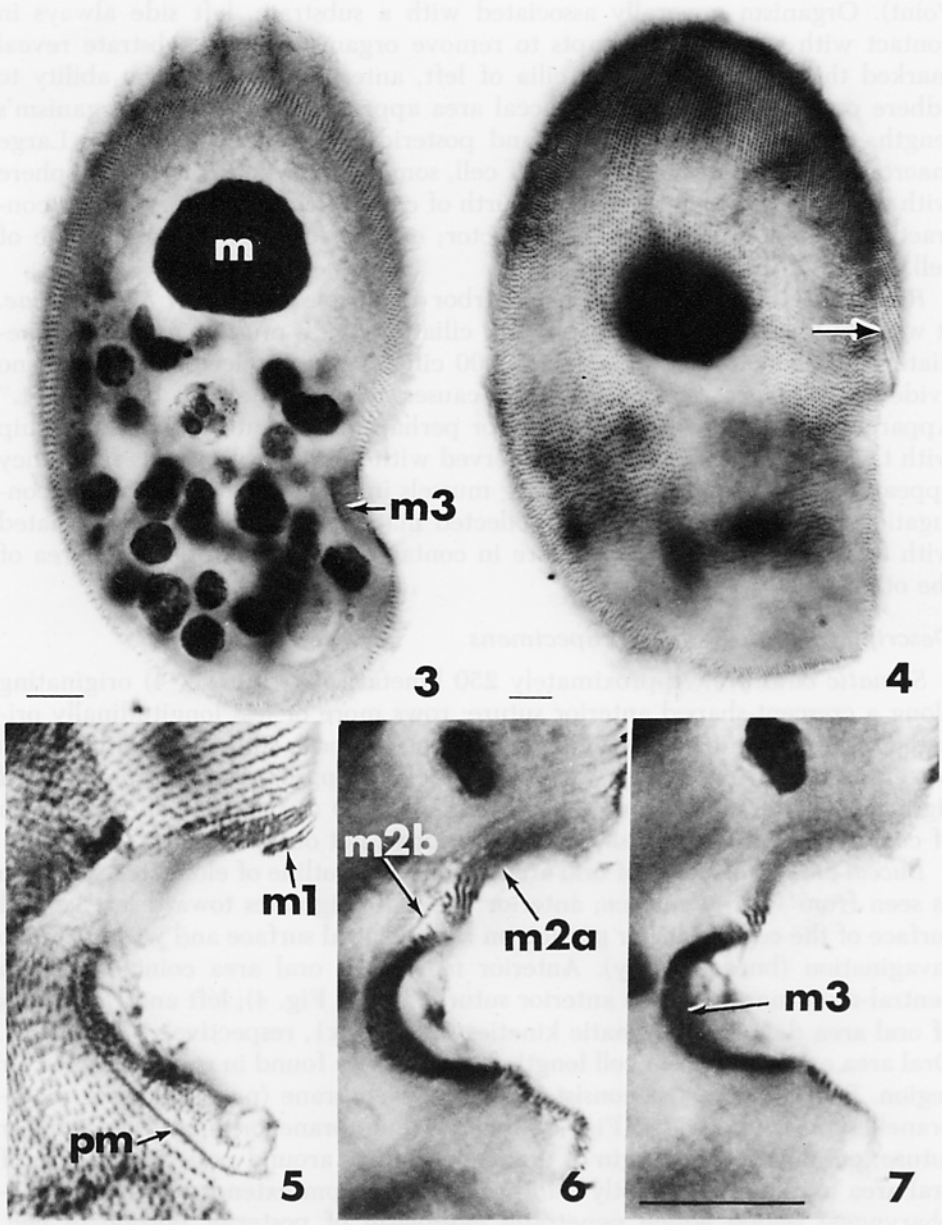
FIGS. 3–7. Protargol-stained specimens of *Mytilophilus pacificae* n. g., n. sp. Fig. 3. Ciliature and overall aspect as seen from the right side of the organism. Depression at membranelle 3; m3 is buccal overture. Scale bar represents 10 μm. Fig. 4. Ciliature and overview of left side of the



organism as seen from the *right* side. Arrow indicates ventral origin of anterior suture which courses over the anterior end of the organism and the origin of the paroral membrane and membranelle 1, both of which course posteriorly. Scale bar represents 10 μm. Figs. 5–7. Through-focal series of the oral area of an early divider. At this stage, only somatic structures have been seen to replicate; the slight change in the shape of the cell provides an orientation favorable for photomicrography of these normally flattened organisms. Focus is from right to left in the series. The organism is seen from the right side. pm, paroral membrane; membranelles m1, m2a, m2b and m3 are indicated. Scale bar on Fig. 7 represents 5 μm and applies to Figs. 5–7.

Point). Organism generally associated with a substrate, left side always in contact with substrate; attempts to remove organisms from substrate reveal marked thigmotactic abilities; cilia of left, anterior ciliature have ability to adhere organism to substrate. Buccal area approximately ⅓–½ of organism's length; occurs along the middle and posterior parts of cell (Fig. 1). Large macronucleus in anterior one-half of cell, somewhat posterior flattened sphere with diameter approximately one-fourth of cell length. Single, prominent contractile vacuole in posterior-dorsal sector; empties through posterior edge of cell.

Remarks. Host mussels appear to harbor a variable number of *M. pacificae.* It was not uncommon to recover 3–10 ciliates from a mussel collected immediately adjacent to one from which 100 ciliates were collected. We have no evidence to suggest that *M. pacificae* causes deleterious effects on its "host." Apparently, they have a commensal or perhaps even mutualistic relationship with their host. Conjugation was observed with some regularity; its frequency appeared to be enhanced by holding mussels in the laboratory, although conjugation was observed in freshly collected material. Paired ciliates associated with one organism's anterior suture in contact with the anterior oral area of the other ciliate (Fig. 2).

Description—Silver-stained Specimens

Somatic ciliature. Approximately 250 kinetal rows (Figs. 3, 4) originating along a crescent-shaped anterior suture; rows more or less longitudinally oriented terminating in a broad, but less distinct, posterior suture extending across posterior margin of cell; posterior suture offset approximately 5 μm toward right side. Contractile vacuole pores (usually 2–4) at posterior-dorsal margin of cell, in region of posterior suture. Cytoproct not observed.

Buccal ciliature. Shape of oral area resembling outline of elongated teardrop as seen from ventral surface; anterior one-half originates toward left ventral surface of the cell, posterior portion on right ventral surface and within a deep invagination (buccal cavity). Anterior margin of oral area coinciding with ventral-most margin of the anterior suture (arrow, Fig. 4); left and right sides of oral area defined by somatic kineties skn and sk1, respectively (Figs. 5, 8). Oral area extends ⅓–½ of cell length; buccal cavity found in rounded posterior region. Buccal organelles consist of paroral membrane (pm) and three membranelles (m1, m2, m3)[5] (Fig. 8). Paroral membrane originates at anterior suture, parallels right margin of oral area, courses around posterior margin of oral area and curves abruptly into buccal cavity; pm extends to narrow cytopharyngeal region which penetrates cytoplasm of posterior portion of cell. Three membranelles positioned to left of paroral membrane and oriented in longitudinal, staggered array. Membranelle 1 originates at anterior suture and parallels kinety n; m1 composed of two kinetal rows and terminates just an-

[5] For the sake of uniformity, we use here the terms and abbreviations recommended by Corliss (1979) for the buccal organelles of ciliates, but with the minor substitution of lower case for capital letters.

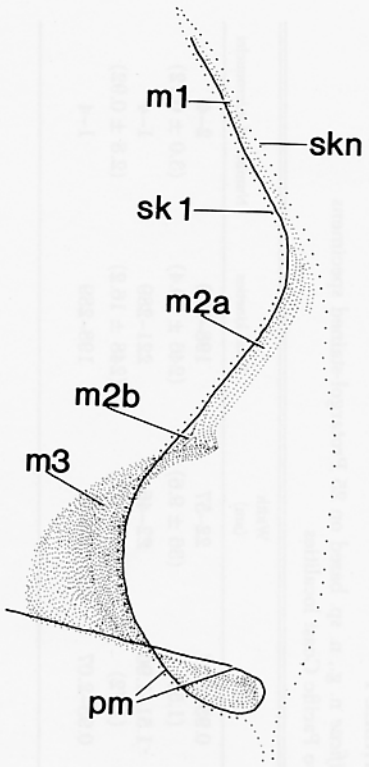


FIG. 8. Buccal structure of *Mytilophilus pacificae* n. g., n. sp. based on Protargol-stained specimens and camera lucida reconstruction from several favorably oriented organisms. sk1, somatic kinety 1; skn, somatic kinety n. Other abbreviations are as indicated in the legends for Figs. 1–7.

terior to buccal cavity (Fig. 5). Membranelle 2 composed of two parts; most prominent (m2a) five kinetal rows wide (Fig. 6), beginning between pm and m1 parallels pm for three-fourths of its length; posterior one-fourth curves dextrally into buccal cavity; posterior terminus of m2a and anterior terminus of m3 on a v-shaped protuberance of buccal cavity (Figs. 6, 7). Just toward pm, and located on protuberance, is m2b which is seen as pleuronematine hairpin-shaped membranelle, or as two or three short kinetal rows (Fig. 6). Membranelle 3, the largest of the membranelles, composed of approximately 30 kinetal rows; C-shaped, and lines anterior, right, and posterior sides of buccal cavity (Figs. 3, 7, 8).

Remarks. No obvious differences were observed between specimens examined from different localities. Morphometric characteristics of 25 Protargol-stained specimens from two different locations are given in Table I. As reported previously (Antipa & Small, 1971), organisms of this general body form undergo a differential shortening, which is reflected by the L/D ratio of living vs. Protargol-stained organisms. Protargol-stained specimens demonstrate dense

TABLE I

Morphometric characteristics of *Mytilophilus pacificae* n. g., n. sp. based on 25 Protargol-stained specimens from each of two Pacific Coast localities

	Length (L) (μm)	Depth (D) (μm)	L/D	Width (μm)	Total kineties	Number of micronuclei
Pigeon Point, California	56–126 (107 ± 17.3)	57–81 (69 ± 8.3)	0.95–2.07 (1.57)	22–57 (36 ± 9.6)	198–270 (246 ± 20.4)	2–4 (3.0 ± 0.72)
Pillar Point, California	86–109 (97 ± 6.8)	47–72 (57 ± 5.4)	1.51–1.90 (1.72)	22–46 (33 ± 8.2)	221–289 (248 ± 16.2)	1–4 (2.8 ± 0.92)
Maximum range of characters observed	56–126	47–81	0.95–2.07	22–57	198–289	1–4

files of basal bodies in the anterior one-third of kinetal rows on the left side of the organism (Fig. 4); this may correspond to the thigmotactic region alluded to above. While a structural differentiation is not apparent by light microscopy, further studies may reveal the nature of the thigmotactic field. None of the specimens of *M. pacificae* examined by us demonstrated post-oral kinetal rows. In our investigation of stomatogenesis, we have determined that m2a and m2b are derived from the same membranellar primordium. The description is based on observations of approximately 200 Chatton-Lwoff and Protargol-stained ciliates from Pigeon Point.

Type locality. The type locality for *Mytilophilus pacificae* n. g., n. sp. is just south of the Pigeon Point lighthouse located approximately midway between Monterey and San Francisco bays (37°11′N, 122°7′W).

Host. All well-established beds of *Mytilus californianus* examined (including Pigeon Point, San Gregorio, Pillar Point, Moss Beach, and Bodega Head, California; Depoe Head and Astoria, Oregon) were infested with *Mytilophilus.* The coastal distance between the southernmost (Pigeon Point) and the northernmost (Astoria) points exceeds 1,100 km. Systematic investigation either south of Pigeon Point or north of Astoria has not been conducted to date.

Type specimens. The holotype-specimen material, USNM Slide No. 33318, and paratypes (USNM No. 33319) have been deposited in the ciliate type-collection at the Smithsonian Institution, Washington, D.C., U.S.A. Additional paratype slides are in the personal collection of G.A.A.

Discussion

Mytilophilus pacificae is a dominant thigmotactic scuticociliate found in *Mytilus californianus* in the eastern Pacific basin. We have no evidence to suggest that *M. pacificae* causes any deleterious effects on its host. Indeed, *M. pacificae* appears to inhabit the same ecological niche as *Peniculistoma mytili* (De Morgan) Jankowski found in the bay mussel *Mytilus edulis* (see Beers, 1959; Fenchel, 1965; Kidder, 1933) and *Conchophthirus* found in fresh-water unionids (Raabe, 1933). The general size and body form of these three genera are strikingly similar, and all three are found primarily on the foot and mantle surface of their respective hosts. The three genera have a thigmotactic region within the left anterior portion of the somatic ciliature. However, neither *Mytilophilus* (reported here) nor *Peniculistoma* (Antipa, unpublished observations) appear to have the degree of structural differentiation reported for *Conchophthirus* (see Antipa, 1971).

Based on general morphology and buccal structure described herein, clearly *M. pacificae* is a pleuronematine scuticociliate. *M. pacificae* is distinct from all other pleuronematines because of the dominance of the third membranelle, a characteristic that has not been described for any other organism in the group (Small & Lynn, 1985). Based on the suite of characteristics *M. pacificae* shares with *Peniculistoma mytili* (De Morgan, 1925), the monospecific familial type species, we place the genus *Mytilophilus* provisionally within the family Peniculistomatidae Fenchel, 1965. This determination was also arrived at by Corliss (1979). Although the buccal apparatus of *M. pacificae* is quite distinct

from that described for *P. mytili* (see Fenchel, 1965), actual qualitative differences can be deduced only through comparative stomatogenesis.

Although the description of new species may be regarded as a time-consuming chore with little recognition (Corliss, 1962, 1974), we do not believe that such a position creates a healthy atmosphere for research that seeks to further our understanding of ciliate relationships. *Mytilophilus* may have been overlooked for years because of its general resemblance to *Peniculistoma*. Yet, further investigation of the *Mytilophilus–Peniculistoma* relationship, and ones like it, will provide new ideas concerning speciation within the Ciliophora and aid in the resolution of the problematic issue of structural stability which appears to exist within the group (Williams, 1984a,b). Since it is populations of species that evolve and have evolved and not higher taxa nor necessarily existing, described taxa, our attention to ecological relationships among sibling species will be critical to our appreciation of ciliophoran evolution. As a consequence, we should look to detailed investigations of ciliate look-alikes for the data base required for interpretations of structural stability, and, ultimately, for our understanding of the course of ciliate evolution.

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