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# THE LIFE CYCLE OF *PARACHAENIA MYAE* GEN. NOV., SP. NOV., A CILIATE PARASITIC IN *MYA ARENARIA* LINN. FROM SAN FRANCISCO BAY, CALIFORNIA,

by Charles A. Kofoid and Mildred Bush (Berkeley, U. S. A.).

## Introduction.

A holotrichous ciliate found in the excurrent siphon and the pericardial cavity of the clam, *Mya arenaria* Linn., is here described under the name, *Parachaenia myae* gen. nov., sp. nov. It is found in this host collected from sandy beaches of San Francisco Bay, from near the Oakland airport and from Richmond Beach, and from Tomales Bay. The clams from the beach at Oakland airport are more heavily infected than those from any other locality. The ciliates are evidently specific in *Mya arenaria*, since none was found in *Macoma nasuta, Macoma inquinata, Schizothaerus nuttalli,* nor in the mud-boring *Petricola pholadiformis,* though all of these lamellibranchs were numerous in the same localities with the host species.

## Methods.

One valve was removed and the fluid from the pericardial cavity and the proximal part of the excurrent siphon was pipetted off for study of the living ciliates. Janus green B in aqueous solution used as an *intra vitam* stain facilitated the study of ciliary action and internal structure, as well as staining

mitochondria. Neutral red was used for staining the vacuome. Attempts were made to culture the ciliates outside the host in a hanging drop of a fluid composed of equal parts of Locke's solution and filtered fluid from the clam. The ciliates became almost inactive within three hours and neither division nor conjugation was observed in this medium.

## Fixing and Staining.

Smears were made with the fluid freshly taken from the clam on slides and cover slips. After evaporating the smear down till it was almost dry, it was fixed with Champy's or Susa's fluid, or with Schaudinn's plus five per cent glacial acetic acid, the last two at 60° C. Fair results were obtained by using Mallory's triple stain for fibrils and Borrel's for cytological differentiation. Feulgen's stain gave excellent nuclear pictures and Heidenhain's iron haematoxylin gave good results for study of the morphology.

## Morphology.

*Parachaenia myae* is translucent and pale green in color. As a whole the body is curved banana-shaped with the ventral surface slightly concave and the dorsal surface somewhat more strongly convex than the ventral, with the curvature of each surface increasing gradually posteriorly. The body tapers anteriorly and is bilaterally compressed. The greatest dorsoventral diameter is in the posterior third and is not less than 0.25 of the total length (figs. 1 and 2). The length varies from 40 to 100 $\mu$, the dorso-ventral diameter from 13 to 25 $\mu$, and the transverse diameter is about two-thirds of the dorso-ventral. The ciliates usually come to rest on the side in fixed material. The cilia of *Parachaenia myae,* which is a holotrich, completely cover the body. They are, however, arranged in two distinct groups, one covering the dorso-bilateral region and the other, the ventral surface. The cilia of the dorso-bilateral area are about 20 $\mu$ long at the anterior part of the body, becoming somewhat shorter posteriorly. Those at the dorsal anterior end just above the cytostome are heavier and closer together than the others in the same ciliary line farther back. The dorso-bilateral rows are close together towards the cytostome, some of them uniting before reaching it but gradually diverging posteriorly. Distally they curve ventrally where each may or may not meet one of the opposite side.

The ventral cilia are about one-half the length of the dorso-bilateral cilia. Eight equidistant ventral ciliary lines converge towards the cytostome, some of them uniting before reaching it, then continuing posteriorly, 2 $\mu$ apart, to about three-fourths of the body length, beyond which they either unite with each other, or give place to the dorso-bilateral lines which curve downward across the posterior end.

A circular cytostome is located at the center of the anterior end and opens directly into a cytopharynx which is visible as a long clear area in the living animal, and as a tube in the stained material, leading from the cytostome, nearly to the middle of the body.

The cytopharynx is made up of non-granular protoplasm which usually appears homogeneous in fixed material. In some specimens it appears homogeneous for only one-fourth of its length, then dilates to two or three times its original diameter, and the wall of the dilated part appears then as composed of a reticulated network. The cytopharynx ends in the endoplasm without any definite structure.

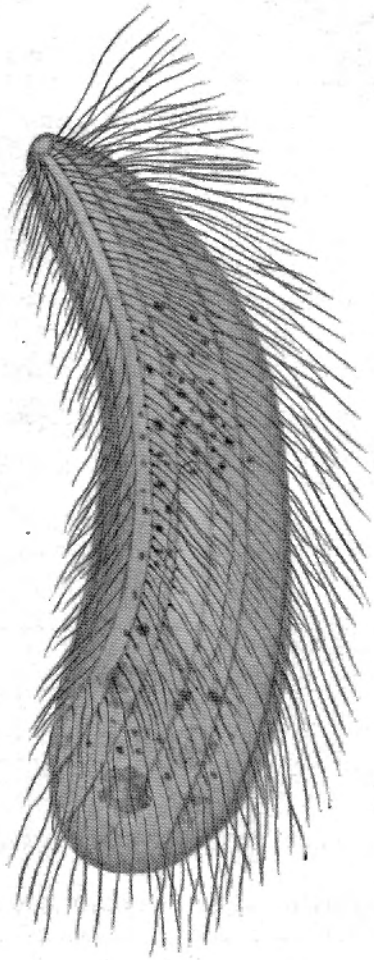
The large macronucleus appears as an elongated ovoidal or slightly irregular structure near the center of the body; its length is nearly 0.25 of the total length of the body, and its diameter is 0.33 of its length. It stains a light rose pink in Janus green B. It also stains deeply in all nuclear stains, numerous irregular chromatin blobs staining more deeply in the nucleoplasm (fig. 2). The micronucleus is relatively large, ovoidal in form, with chromatin particles scattered irregularly in its substance. It lies close to the ventral side of the macronucleus, as shown in figure 2 (1).

A thin transparent, elastic *pellicle* covers the surface of the body. The ectoplasm is a thin clear layer. The endoplasm in the anterior part is finely granular, containing only small vacuoles, while the posterior part contains many larger vacuoles usually filled with food material (fig. 2).

Janus green B, used intra-vitally, stains rounded or irregular mitochondria which are most numerous anterior and ventral to the nucleus, with some larger ones among the posterior food vacuoles (fig. 1).

(1) All figures drawn with camera lucida, × 2480. Abbreviations for methods of preparations: F. Feulgen's stain; H., Heidenhain's iron haematoxylin; J., Janus green B *intra vitam;* Sch., Schaudinn's fluid; Su., Susa's fluid.

The vacuome, a term used to designate the sum total of the inclusions which stain readily with neutral red, consists of small rounded granules. These are most numerous around the nucleus, with other granules scattered irregularly in the posterior part. Other inclusions appearing as globular yellowish



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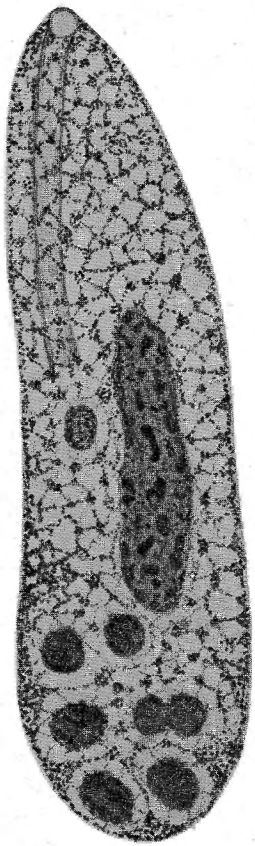

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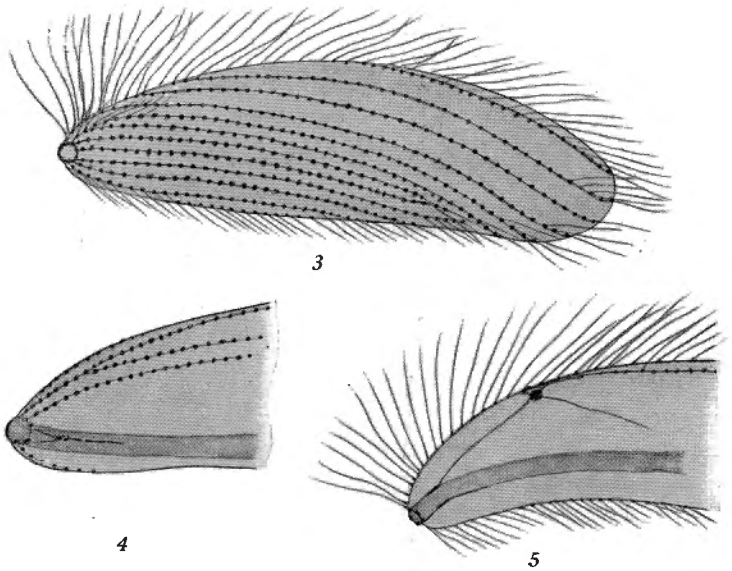
*Parachaenia myae* gen. nov., sp. nov., × 2/3.

Fig. 1. — Surface view, from life, showing distribution of cilia; dark particles are mitochondria. J.

Fig. 2. — Internal morphology showing cytopharynx, macronucleus, micronucleus, and food vacuoles. Sch., H.

plastids, somewhat larger than the mitochondria or vacuome granules, are distributed through the endoplasm. The appearance of these and the fact that they are blackened by osmic




*Parachaenia myae* gen. nov., sp. nov., × 1/2.

Fig. 3. — Peripheral structure of neuromotor system, showing circumoral ring, basal granules of cilia, and longitudinal fibrils. Sch., H.

Fig. 4. — Internal neuromotor fibrillar structure, showing ventral cytopharyngeal fibrils. Su., H.

Fig. 5. — Internal neuromotor fibrillar structures, showing dorsal cytopharyngeal fiber and a dorsal body. Su., H.

acid and dissolved by turpentine indicate that they are lipoid droplets.

*Parachaenia myae* swims rapidly with a zigzag forward movement, swaying, without rotation, from side to side at the same time. The anterior part of the body performs seemingly exploratory movements, jerking up and down, sidewise, or twisting. In these movements the long heavy cilia at the dorsal anterior end initiate the ciliary wave with a strong beat, then the cilia farther back beat, and the wave moves posteriorly with decreasing force. The cilia of the dorso-lateral area beat with a back-

ward-upward-forward motion while those of the ventral area move forward and back so that the two areas are divided along a ventro-lateral line in both structure and action (fig. 1). As the ciliate swims about in this fashion, it may hesitate and rotate rapidly clockwise, then reverse to a counter clockwise direction with equal rapidity. Occasionally it stops and, with a vigorous ciliary activity, produces a current in the medium, which carries food particles to the mouth.

A few instances of food taking were observed. Debris containing bacteria enters the mouth and moves along the cytopharynx, forming little globules which continue back and aggregate in the large food vacuoles which distend the posterior part of the body. Stained specimens show some vacuoles containing broken-up nuclear material similar to that of the epithelial cells which are removed when the fluid is taken from the clam.

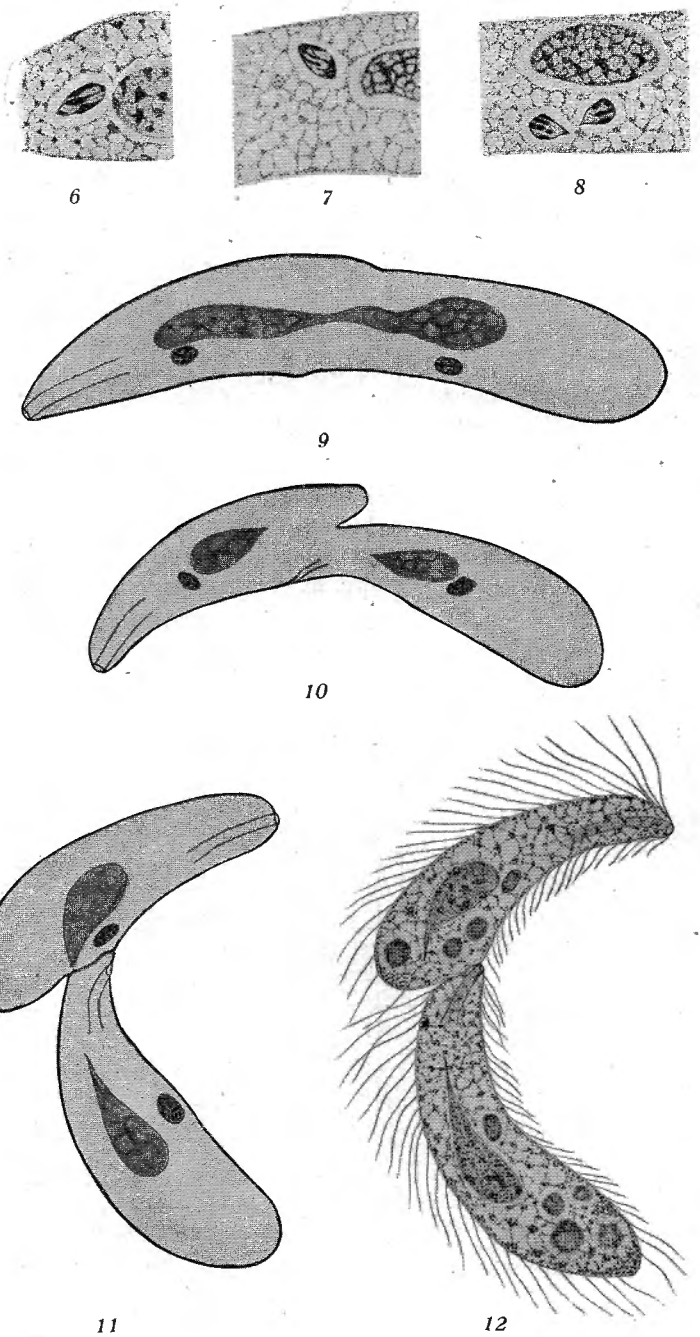
In specimens stained in Heidenhain's iron haematoxylin, the neuromotor system is shown as a ring around the cytostome, from which the longitudinal ciliary lines extend posteriorly in the dorso-bilateral and ventral groups. Each line contains a series of large basal granules, each of which gives rise to a single cilium. These cilia are connected by a heavy longitudinal fibril (fig. 3) of the longitudinal ciliary line.

Internally, a fibril from the mid-dorsal point of the cytostomal ring extends along the dorsal wall of the cytopharynx about one-fifth of its length to a slight thickening on the cytopharyngeal surface, and is then diverted towards the dorsal body surface where it joins a relatively large granule which is closely associated with the mid-dorsal ciliary fibril. From this granule, the fibril extends posteriorly and downward towards the cytopharynx (fig. 5).

From points of the cytostomal ring on the ventral side, two fibrils are given off which soon unite and continue as a slender thread along the ventral surface of the cytopharynx (fig. 4).

*Parachaenia myae* gen. nov., sp. nov. in binary fission, × 1/2.

Fig. 6. — Micronucleus dividing, metaphase. Sch., H.
Fig. 7. — Micronucleus dividing, anaphase. Sch., H.
Fig. 8. — Micronuclear division completed. Sch., H.
Fig. 9. — Plasmotomy beginning, micronuclei migrating to ends of macronucleus. Su., H.
Fig. 10. — Plasmotomy in advanced stage. Su., H.
Fig. 11. — Plasmotomy almost completed. Su., H.
Fig. 12. — Binary fission almost completed, showing structure. Sch., H.




Figs. 6-12. — *Parachaenia myae* gen. nov., sp. nov. in binary fission, × 1/2.

## Binary Fission.

Size is apparently not a determining factor in binary fission, since nearly equal numbers of long and short individuals are found in various stages of division.

The micronucleus divides with the formation of an indistinct spindle. The chromatin material is assembled in 6-8 blobs, presumably chromosomes, which move from the equator to form polar masses. A heavy chromatin thread connecting the polar masses, perhaps a lagging chromosome, persists for some time but is finally broken in the middle as the nuclei separate (figs. 6-8).

The daughter micronuclei move toward the ends of the macronucleus which has begun to divide by thinning out in the middle. It eventually divides into two approximately equal parts. Meanwhile, as observed in a lateral view of the animal, a hump on the dorsal side and a slight protrusion on the ventral side have formed, marking the oblique line of division (figs. 9, 10). The posterior individual forms a new cytostome and cytopharynx and its anterior dorso-lateral cilia become elongated as division proceeds. The anterior individual adjusts the posterior region to a rounded form (figs. 11, 12). Finally, with violent jerking, the two are separated and rest for a few moments before swimming about.

*Parachaenia myae* gen. nov., sp. nov. in conjugation, × 7/16.

Fig. 13. — Aboral fusion of conjugants beginning. SCH., F.

Fig. 14. — Fusion of conjugants almost completed in arc-formation. SCH., F.

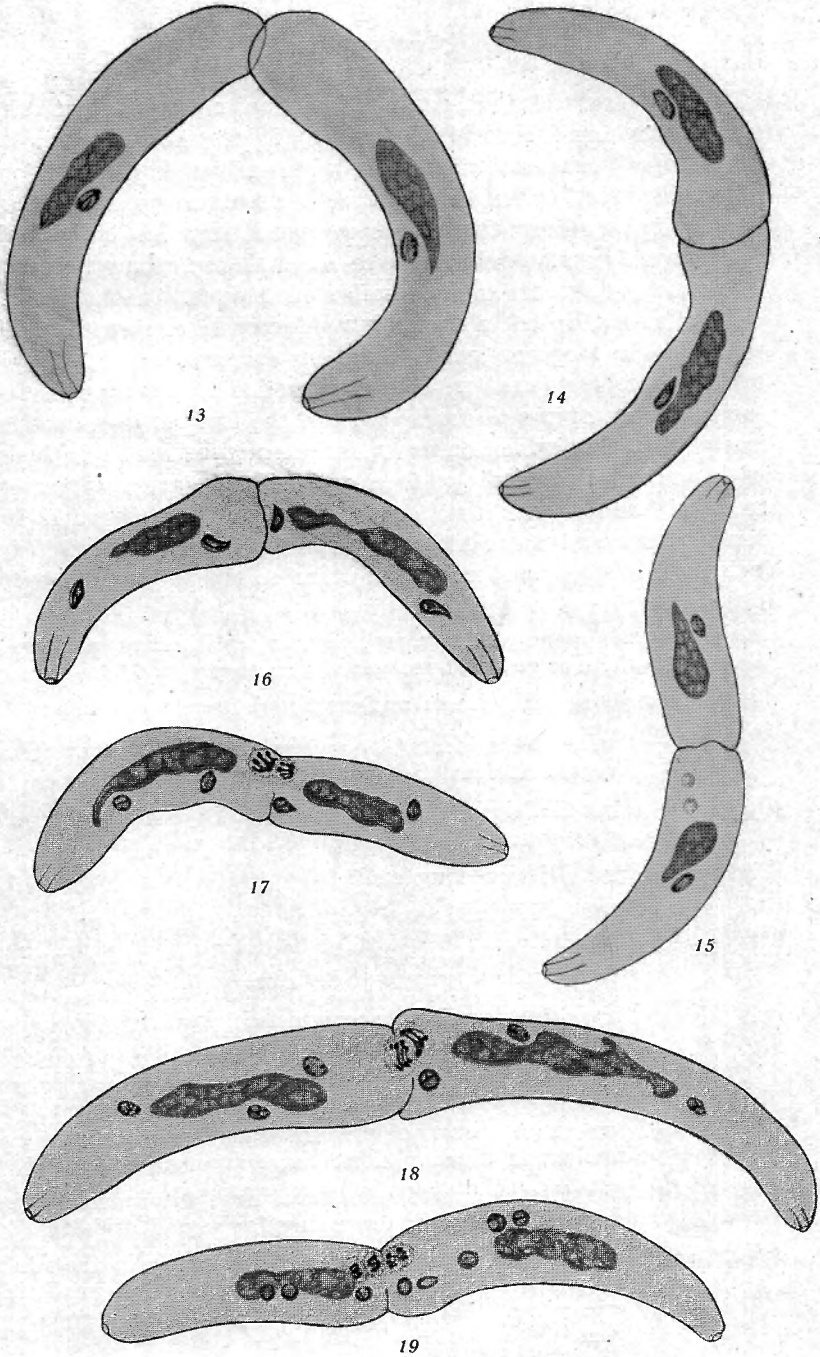
Fig. 15. — Fusion of conjugants in sigmoid curve. SU., F.

Fig. 16. — First maturation division completed, micronuclei migrating. SU., F. One Cyte I nucleus in each conjugant migrating aborally to fusion region, and one migrating anteriorly.

Fig. 17. — Second maturation (reducing) division of the anterior nucleus completed, but lagging in the posterior nucleus, in each conjugant. SCH., F. Post-gametic divisions of posterior gamete in progress at fusion plane.

Fig. 18. — Both second maturation divisions completed. Post-gametic equation divisions of aborally located Cytes II at fusion margin approaching completion. Macronuclei beginning to degenerate in the longer conjugant. Three Cytes II degenerating.

Fig. 19. — A later stage with one post-gametic division completed, forming resident and migrant pronuclei, the other in late anaphase.




Figs. 13-19. — *Parachaenia myae* gen. nov., sp. nov. in conjugation, × 7/16.

## Conjugation.

It was observed that conjugation was not prevalent when there were many individuals present in the host. It was not necessarily prevalent when there were only a few, but it was noticeable that when there were several conjugating pairs present in any host, there were comparatively few individuals. No reason for this could be given since all the clams were kept in flowing sea water under similar conditions.

Relative size is not a determining factor in conjugation. The pair may be large or small, equal or unequal in size. Usually one is somewhat smaller. The conjugation is peculiar in that fusion occurs *at the posterior ends* of the two conjugants. Normally, i. e. that which occurs most often, the two begin fusion with ventral sides facing; then as fusion progresses, the two tend to form an arc or almost a straight line (figs. 13-19). Sometimes they become attached with the dorsal surface of one facing the ventral surface of the other so that a sigmoid curve

*Parachaenia myae* gen. nov., sp. nov. in fertilization, nuclear multiplication and reorganization stages, × 7/16.

All fixed in Schaudinn's and stained in Feulgen's stain.

Fig. 20. — Fusion of resident (♀) and migrant (♂) pronuclei in zygote nucleus. Conjugants separating.

Fig. 21. — First division of zygote nucleus. Degeneration of old macronuclei progressing.

Fig. 22. — First division completed.

Fig. 23. — Second divisions completed.

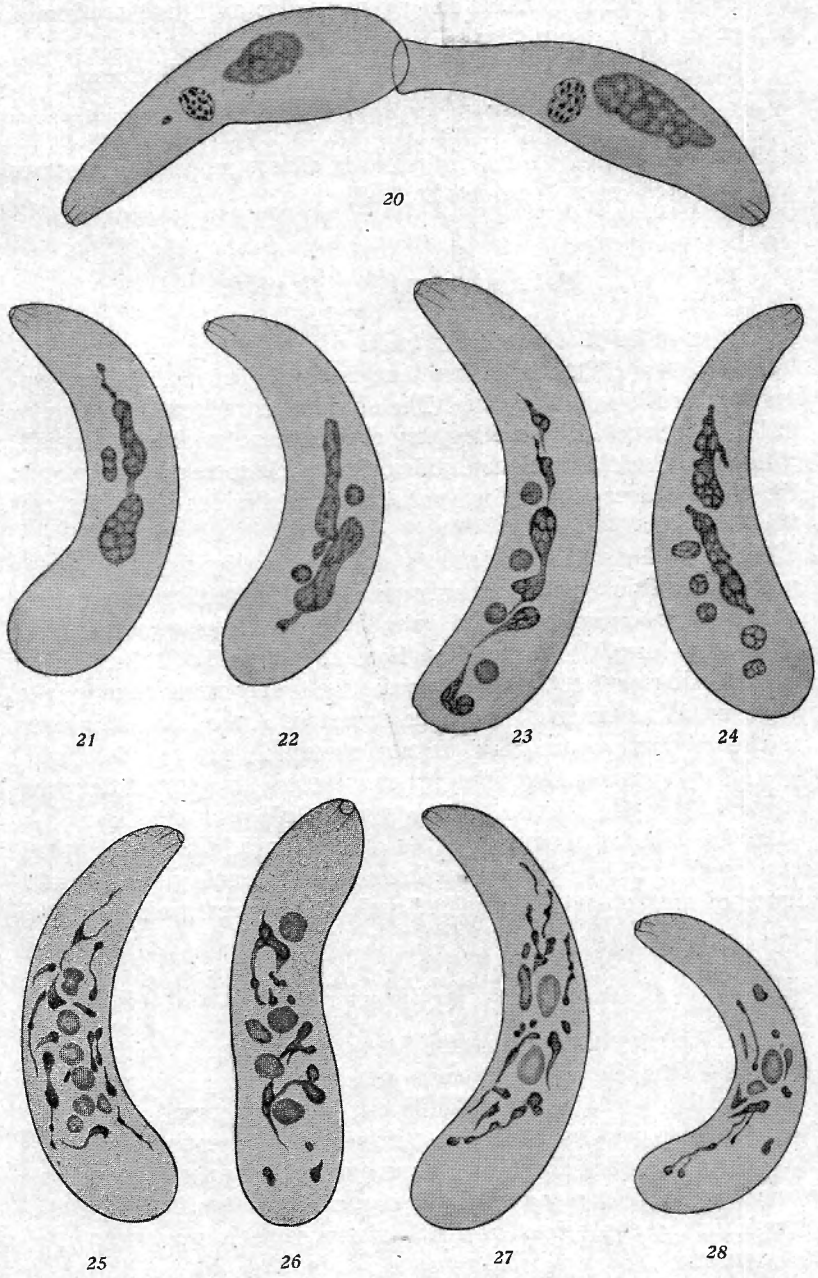
Fig. 24. — Third divisions, one completed, resulting in five nuclei, three still in process of division.

Fig. 25. — Third divisions, three completed, resulting in seven nuclei, 4 large macronuclei, 2 small micronuclei, one micronucleus still in process of division. Eight nucleate stage nearly completed.

Fig. 26. — Four large macronuclei; one small surviving micronucleus in division. Three have degenerated.

Fig. 27. — After first binary fission, two large macronuclei; one small micronucleus preparing for second division.

Fig. 28. — Second binary fission completed, resulting in the adult stage with one large macronucleus, and one small micronucleus, the old macronucleus almost completely gone.




Figs. 20-28. — *Parachaenia myae* gen. nov., sp. nov. in fertilization, nuclear multiplication and reorganization stages, × 7/16.

is formed (fig. 15). Such conjugants twist as if attempting to adjust to the arc position.

Conjugating pairs do not move about rapidly, but remain in one spot circling slowly. There seems to be some contest as to which shall direct movement. If one is larger, that one leads and the other is carried about with its ciliary movement reversed and this acts as a drag on the leader.

## Maturation and Fertilization.

As fusion of the two individuals proceeds, the first maturation division of the micronucleus in each occurs. The micronucleus swells and elongates. The chromatin collects at the poles in blobs connected by the lagging chromosome or chromatin rod, which parts as the nucleus divides. One nucleus moves backward; the other moves forward or remains near the center of the cell (fig. 16).

The second division follows, making four micronuclei in each conjugant. One of these moves back to the fusion membrane, where it enlarges, forms a spindle with 4 chromosomes and divides to form the pronuclei (figs. 18, 19). The other three nuclei disintegrate. As this proceeds, the fusion membrane breaks down until no trace of it can be detected. Actual crossing over of the migrant pronuclei was not observed, but as the conjugants begin separating, the zygote nucleus is formed in each individual by the fission of the two haploid pronuclei.

At the time of separation, the animals gradually pull apart with one individual, and sometimes both, becoming slender and distorted as the posterior wall of each is being restored (fig. 20).

## Growth and Adolescence.

After the conjugants separate the macronucleus of each begins to degenerate. Generally it constricts into two parts and then into smaller parts, or it extends out in long strands carrying blobs of chromatin material throughout the cytoplasm. The blobs become vacuolated and eventually disappear as reconstruction of the cleavage nuclei proceeds (figs. 20-28). This completes the equivalent of death of the somatic nucleus of the conjugant.

Fusion of the pronuclei occurs shortly before separation of

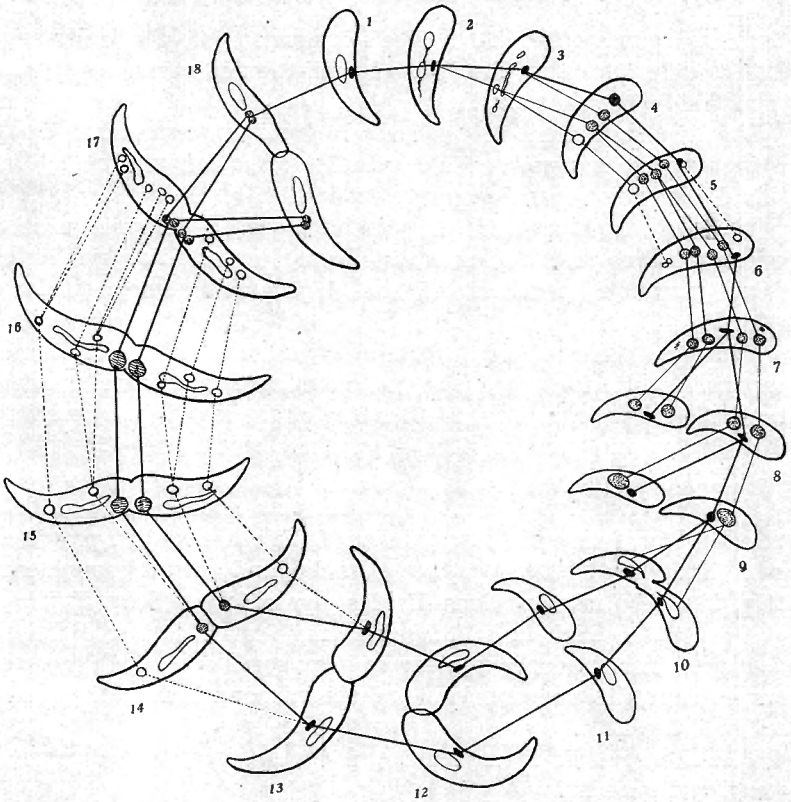



Fig. 29. — Life Cycle of *Parachaenia myae* gen. nov., sp. nov.

1. Diploid zygote, with degenerating macronucleus (death).
2-9. Cleavage, adolescence, differentiation, and adult maturity.
2-6. Cleavage to the eight nucleate stage.
5-6. Differentiation of sex (four micronuclei) and somatic (four macronuclei) nuclei.
7. Adolescence. Three of the four sex nuclei degenerate, leaving one micronucleus.
8-9. Adult maturity. Distribution of four macronuclei by two divisions, resulting in four adults, each capable of binary fission or conjugation.
10-11. Asexual reproduction of adult by binary fission.
12. Conjugation by posterior apposition.
13-15. Meiosis. — 14. First meiotic division. — 15. Second (reducing) meiotic division resulting in haploid gametes, each with four chromosomes.
16. Post-gametic (equation) division (asexual multiplication in gamete stage).
17. Resident and migrant haploid pronuclei formed by an equation division of one gamete. Three gamete micronuclei degenerating.
18. Fertilization, fusion of migrant and resident pronuclei. Macronuclei of conjugants degenerating (death).

the conjugants (fig. 20). The chromatin of the resulting amphinucleus becomes distributed until the new nucleus appears remarkably homogeneous.

The first metagamic or « cleavage » division occurs after separation of the conjugants. No spindle formation was noted in this division nor in subsequent divisions (fig. 21).

The two resulting nuclei appear homogeneous in structure in nuclear stains and do not stain deeply, contrasting with the dark vacuolate particles of the degenerating macronucleus (fig. 22).

The two nuclei divide, forming four (fig. 23), and these in turn divide, forming eight nuclei, four of which are smaller than the other four (figs. 24-25). One of the small ones and the four larger ones persist. The other three small ones disappear (fig. 26).

The surviving small nucleus is the new micronucleus which then divides, followed by a division of the ciliate which provides each individual with two large nuclei and one micronucleus (fig. 27). The micronucleus divides, again followed by division of the ciliate. Each individual resulting from this last division now possesses one large nucleus and one small one which become respectively the macronucleus and the micronucleus of the adult animal (fig. 28).

The life cycle is interpreted and summarized in the accompanying figure 29.

## Parachaenia gen. nov.

*Diagnosis.* — Small holotrichous ciliates, compressed bilaterally, ventral surface slightly concave, dorsal surface more strongly convex; cilia long, differentiated in two areas, a ventral area consisting of close-set rows extending 0.75 of the length of the body, a dorso-bilateral area consisting of 7 rows close together at the cytostome and more widely separated posteriorly; cytostome circular or slightly oval; cytopharynx long, narrow; no contractile vacuole. Type species: *Parachaenia myae* found in the pericardial cavity and the siphon of *Mya arenaria* Linn. from San Francisco Bay, California.

## Parachaenia myae sp. nov.

*Diagnosis.* — Length 40-100 $\mu$; seven rows of long cilia in the dorso-bilateral area, eight rows of shorter cilia in the ventral area; cytopharynx 0.5 the length of the body.

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## SUMMARY.

1. *Parachaenia mya* gen. nov., sp. nov., found in the pericardial cavity and excurrent siphon of *Mya arenaria* Linn., in the San Francisco Bay region, is described.
2. The cilia are arranged in two definite areas, a dorso-bilateral area of long cilia and a ventral area of shorter cilia, the two areas being divided along a ventro-lateral line in both structure and action. A simple integrated neuromotor system is described.
3. A long, tube-like cytopharynx is present.
4. Conjugation is peculiar in that the individuals become fused at their posterior ends. It conforms in nuclear pattern to the life cycle of *Paramaecium putrinum* as described by Doflein in his *Lehrbuch* (1909).

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