PHALACROCLEPTES VERRUCIFORMIS GEN. NOV., SP. NOV., AN UNCILIATED CILIATE FROM THE SABELLID POLYCHAETE SCHIZOBRANCHIA INSIGNIS BUSH

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Of the known ciliates, two separate groups are characterized by the absence of cilia during at least a part of the life history. In the suctorians, the tentaculate and usually predaceous adults are unciliated, but typically they produce one or several ciliated larvae. The larvae, after a short free-living existence, lose their cilia and metamorphose into adults. Scattered argentophilic bodies found in at least some suctorians are thought to be persistent kinetosomes. Studies on the morphogenetic events involved in the production of larvae have shown that kinetosomes proliferate in local areas, then become organized in rows, forming the ciliary field of the prospective larva. In species of *Allantosoma,* which inhabit the digestive tract of horses, no ciliated stage has been reported. Although these organisms become attached to ciliates and probably feed in the same general way as suctorians, they may have evolved along entirely different lines.

Another group of unciliated ciliates belongs to the family Sphenophryidae, referred to the so-called Thigmotricha.[1] The sphenophryids are more or less sessile parasites of the ctenidia of lamellibranch molluscs. The kinetosomes of these bizarre holotrichs are arranged in definite rows, but only one of the several known species (*Lwoffia cilifera* Kozloff) is ciliated throughout its life history. In *Sphenophrya, Gargarius,* and *Pelecyophrya,* portions of the rows of kinetosomes found in the adult are conferred upon the larva, and cilia appear on these portions. The larva is quite similar to certain representatives of the family Ancistrocomidae, and it is presumed that ancistrocomids are ancestral to the sphenophryids.

In the course of studies on the biology of *Ignotocoma sabellarum* Kozloff (1961), an ancistrocomid ciliate living on the sabellid polychaete *Schizobranchia insignis* Bush, I found an entirely different kind of parasite on the pinnules of the prostomial cirri. This organism had no cilia, and its appearance while alive did not provide any good clues to its systematic position. After fixing and staining some specimens, however, I observed that these possessed both a macronucleus and a micronucleus, and that the nuclear events involved in reproduction by fission were like those characteristic of ciliates. Eventually, the occurrence of conjugation was established. There can be no doubt that this organism is an unciliated ciliate. It is perhaps a suctorian or a highly-specialized thigmotrich.

[1] The thigmotrichs, as understood by authors who have attempted to survey the important groups of ciliates, probably do not constitute a completely monophyletic division. Moreover, some of the less highly specialized thigmotrichs are difficult to separate by sharp lines from hymenostomes and peritrichs.

METHODS

The ciliates were studied extensively in the living condition, with the aid of both bright field and phase contrast optical systems. Most of the morphological and cytological studies were based on specimens in smears of the prostomial cirri of *S. insignis* prepared on coverglasses. The fixatives used were those of Bouin, Duboscq and Brasil, Hollande, Schaudinn, Champy, and Flemming (the stronger mixture), as well as a saturated aqueous solution of mercuric chloride containing 5% of acetic acid.

For staining the smears, I tried a variety of methods with material fixed in each of the mixtures listed above. Experimentation was necessary because the micronucleus of the parasite is difficult to stain sharply, especially during certain phases of the process of conjugation. Azocarmine G, acid fuchsin, basic fuchsin (used according to Dippell and Chao's modification of De Lamater's method), iron hematoxylin, alum hematoxylin (progressive staining with a dilute solution), and the Feulgen method all stained the macronucleus intensely, and usually differentiated the micronucleus well enough so that it could be distinguished. As a rule, however, the micronucleus was most suitably demonstrated by iron hematoxylin or the Feulgen nucleal reaction, after fixation in Bouin's fluid, Duboscq and Brasil's fluid, or the mercuric chloride-acetic acid mixture. When the Feulgen method was used, hydrolysis in normal hydrochloric acid at 60° C. was allowed to proceed for 12 to 15 minutes.

Impregnation with silver albumose (Protargol) was tried after fixation in almost all of the fixatives used before staining procedures. The results did not vary a great deal so far as demonstration of certain argentophilic granules was concerned, although cleaner preparations were generally obtained after fixation in the mixtures of Bouin, Duboscq and Brasil, and Hollande. The Chatton and Lwoff method of impregnation with silver nitrate, following brief fixation in Champy's fluid and subsequent treatment with Da Fano's fluid, yielded preparations showing the same granules demonstrated by the Protargol method, but provided no additional information.

For studying the parasites *in situ* on the pinnules, material fixed in Bouin's fluid and embedded in paraffin was sectioned at 5 μ, and the sections were stained with iron hematoxylin. However, the shrinkage of the ciliates and the pinnules was so marked that the relationship of the parasites to the epithelium could not be demonstrated acceptably. Considerably more instructive preparations were obtained by fixing heavily-infected pinnules in osmium tetroxide buffered to pH 7.5 with *s*-collidine (0.5 ml. of *s*-collidine to 1 ml. of 4% osmium tetroxide), embedding them in Epon epoxy resin according to the procedure of Luft (1961), and cutting sections at 0.5 μ and 1 μ with glass knives.[2] The sections were stained by the methylene blue-azure II mixture recommended by Richardson, Jarett and Finke (1960). Shrinkage appeared to be negligible, and I hope that the relationship of the ciliates to the tissue was preserved rather faithfully.

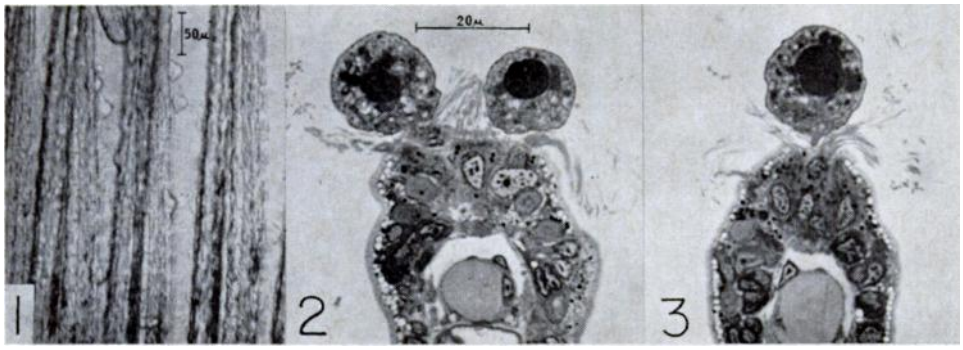
Neutral red was used as a supravital stain for cytoplasmic granules, and lipid inclusions in fresh preparations were stained with Sudan IV in 50% alcohol.

[2] Mr. John Boykin very kindly instructed me at certain points during the procedures of embedding and sectioning. His help is warmly appreciated.

Unsuccessful attempts to demonstrate glycogen were made with Lugol's iodine solution applied to fresh smears, and by fixation in a mixture of nine parts of absolute alcohol to one part of formalin followed by staining by Bauer's method.

Phalacrocleptes verruciformis gen. nov., sp. nov.

This interesting organism was found on *Schizobranchia insignis* collected on floating docks at two localities on San Juan Island, Washington. Of 115 specimens taken at Mitchell Bay (Long. 123° 10′ W.; Lat. 48° 34.2′ N.) and examined at various times within 14 days after the date of collection, 22 were observed to have at least a few ciliates. Of 140 worms collected at Roche Harbor (Long. 123° 05′ W.; Lat. 48° 36.6′ N.) and examined within 24 days after collection, 26 were definitely parasitized. However, as the parasites are sometimes very rare, I could not certify that a particular specimen of *S. insignis* was completely free of the



EXPLANATION OF FIGURES 1–3

Phalacrocleptes verruciformis. Photomicrographs.

FIGURE 1. Living specimens on pinnules of prostomial cirri of *S. insignis.*

FIGURES 2, 3. Transverse sections of specimens attached to pinnules. Buffered osmium tetroxide; Epon sections, 0.5 μ; methylene blue-azure II.

ciliate unless I examined every one of the hundreds of pinnules of the prostomial cirri. All worms from both of the localities were parasitized by *Ignotocoma sabellarum.*

When cirri of a host sabellid are examined under lower magnifications, the ciliates appear as rather clear, wart-like protuberances (Figs. 1, 4). They are restricted to the frontal surfaces of the pinnules. *Ignotocoma sabellarum,* on the other hand, lives attached to the main stems of the cirri and their branches as well as to the pinnules, but it is not common on the frontal surfaces.

In most individuals of *S. insignis* observed to be parasitized by *P. verruciformis,* relatively few ciliates were present. Sometimes I noted only one or two specimens on widely scattered pinnules. In other instances, a number of specimens were found on several adjacent pinnules of certain cirri. When heavy and more or less general infestations were observed, perhaps half of the many pinnules on each cirrus had at least one or two parasites, and up to 12 specimens were observed on some pinnules. As a rule, the ciliates were more abundant on the pinnules arising

from the distal half of a particular cirrus than from the proximal half; in certain cases, however, the reverse was true, and in many hosts the parasites were rather evenly distributed.

The contact of the ciliates with the pinnules is evidently rather tight. When entire pinnules are dropped into a fixative, the ciliates rarely fall off; in fresh preparations, they remain attached until they are moribund. Even when smears are prepared, some of the parasites may remain *in situ*.

The shape of *P. verruciformis* varies considerably. When several living specimens from a single pinnule or from different pinnules are compared, no two of them may be quite alike. However, although the ciliates are capable of slow changes in general shape or in the shape of certain portions of the body, I have never observed specimens to move actively from one part of a pinnule to another.

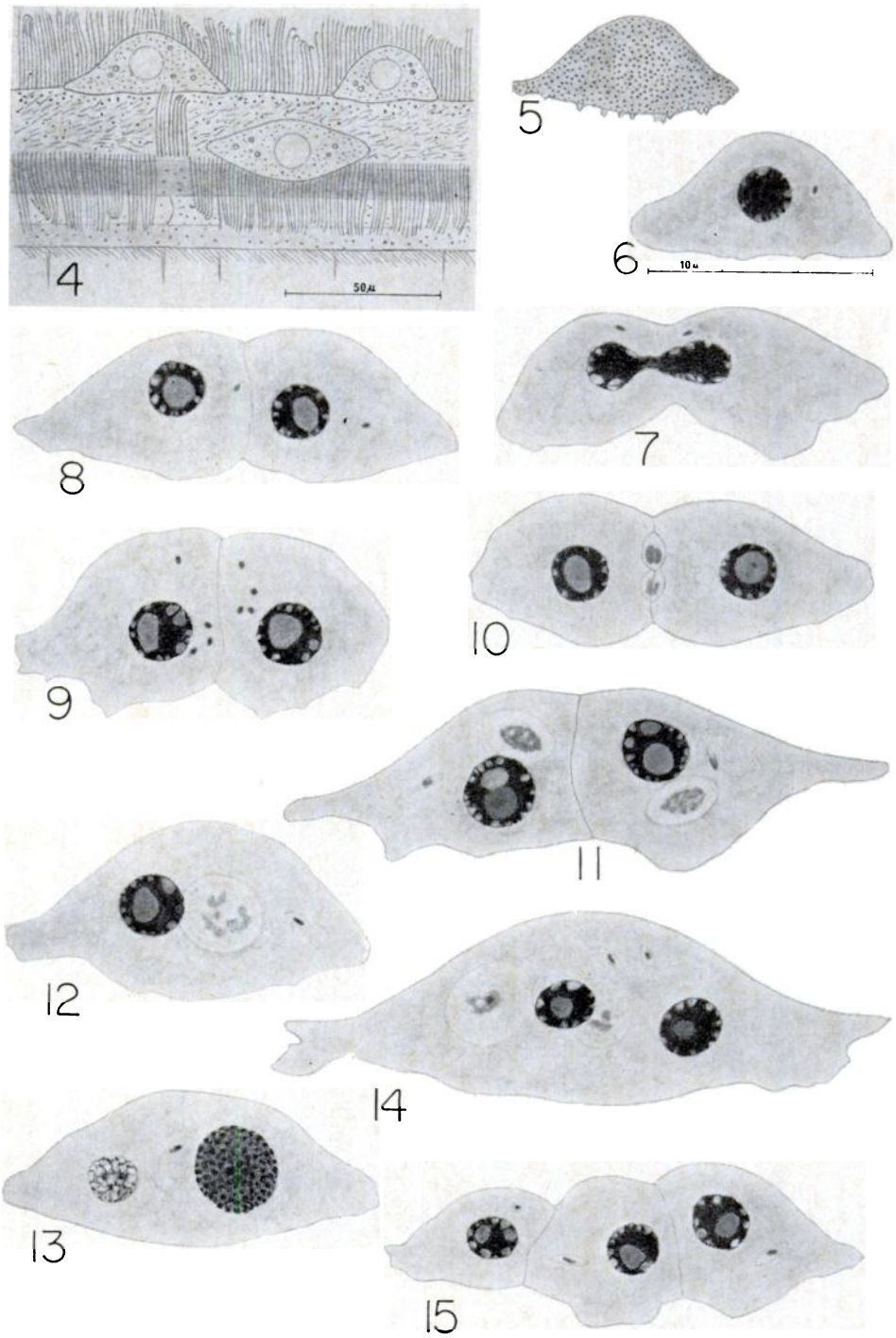
When viewed from the side (Fig. 4), the outline of the body ranges from one which is semicircular to one which is humped in the center but less convex near the extremities. The size (length × thickness) of specimens observed from this aspect ranges from about 27 × 13 μ to about 50 × 17 μ. When the ciliates are viewed with the superior or inferior surface uppermost, the outline varies from broadly oval to fusiform; the width is one-third to two-thirds the length.

The character of the inferior surface is perhaps the most variable feature of this ciliate. In some specimens, it is so markedly flattened that the body is approximately semicircular in transverse section; in others it is more or less rounded (Fig. 2) or ridge-like (Fig. 3) in transverse section. The plasticity of the inferior surface is apparent not only in gross changes of shape which it may undergo, but also in the production, withdrawal, and alteration of protuberances and marginal sinuosities. Portions of the body which are not close to the epithelium of the pinnule are sometimes slightly wrinkled, but lack definite protuberances.

The inferior surface also characteristically exhibits a number of very delicate cylindrical or conical projections, which are probably used for obtaining food from the epithelial cells of the pinnule. These projections are difficult to see in living specimens, probably because they tend to be withdrawn. Evidently they are not well preserved by most of the fixatives I tried on smear preparations; Champy's fluid and Flemming's fluid were the only ones which gave moderately acceptable results as far as these projections were concerned. However, in specimens impregnated with Protargol after fixation in the mixtures of Bouin, Hollande, and Duboscq and Brasil, they are often very clear, even if obviously shrunken (Fig. 5). They show up best in material in which the impregnation is at least moderately heavy. The projections are quite evident in thin Epon sections of ciliates fixed *in situ*. They can definitely be seen to contact the surfaces of epithelial cells, but I have never seen them penetrating the cells. The pellicle surrounding these projections, especially at their distal portions, appears to be thinner than that surrounding the body of the ciliate as a whole.

The cytoplasm of living specimens contains abundant small granules up to about 0.7 μ in diameter. These are usually more or less evenly distributed, but sometimes they are concentrated in clusters; they exhibit no obvious Brownian movement. The granules are refractile and very distinct when examined with a phase contrast system, and they are stained deep red by neutral red.

In smear preparations which have been fixed and stained, the granules which



FIGS. 4–15.

are conspicuous in living ciliates are not often evident. They are visible in thin Epon sections stained by methylene blue and azure II, but are not very distinct because the cytoplasm is usually stained almost as deeply. Protargol impregnates a large number of small cytoplasmic granules (Fig. 5), and those which are close to the pellicle appear much like kinetosomes. What seem to be the same granules are often distinctly blackened by the Chatton and Lwoff method of impregnation with silver nitrate. At no time during fission or conjugation do the granules near the surface of the body form any particular pattern or show a tendency to become more heavily concentrated in certain regions; they simply remain more or less evenly distributed. In any case, it seems unlikely that any of them can be homologized with kinetosomes.

The macronucleus is evident in living ciliates as a spherical and almost homogeneous body. None of the fixatives which I used for smear preparations preserved the macronucleus very well. Most of them caused the chromatin to be clumped in coarse masses (Fig. 6); the numerous small vacuoles commonly noted in peripheral areas of the macronucleus in fixed specimens, especially those fixed in the mercuric chloride-acetic acid mixture, appear to be artifacts. In thin sections of material fixed in buffered osmium tetroxide and embedded in Epon (Figs. 2, 3), the macronucleus shows what appears to be a more natural form: a smooth outline and nearly homogeneous contents.

The small micronucleus is usually ellipsoidal or fusiform. It is rarely distinguishable in living specimens, even when a phase contrast optical system is used. The position of the micronucleus varies, but it is more likely to be found in the upper half of the ciliate. As has been pointed out previously, it is not often stained intensely by any method I have tried.

As far as I have been able to determine, there is no contractile vacuole. No inclusions which can be identified as constituents of the epithelial cells of the host have been recognized within the cytoplasm. There are, however, a number of scattered lipid inclusions; the largest of these reach a diameter of about 3.5 μ. In thin Epon sections (Figs. 2, 3), there are characteristically a number of masses

EXPLANATION OF FIGURES 4–15

Phalacrocleptes verruciformis. All figures except Figure 4 were prepared with the aid of a camera lucida to the scale accompanying Figure 6, and unless otherwise noted are based on specimens fixed in mercuric chloride-acetic acid and stained by the Feulgen nucleal reaction and orange G. (The character of the macronuclei is not well preserved.)

FIGURE 4. Living specimens on frontal surface of pinnule of *S. insignis.*

FIGURE 5. Projections of inferior surface, and argentophilic granules. Bouin's fixative; Protargol.

FIGURE 6. Typical trophic individual.

FIGURE 7. Binary fission.

FIGURE 8. Conjugation, prior to first division of micronuclei.

FIGURE 9. First two micronuclear divisions completed.

FIGURE 10. Synkaryons in region of contact of conjugants.

FIGURE 11. Old macronuclei, new macronuclei, and micronuclei in conjugants.

FIGURE 12. Exconjugant.

FIGURE 13. Exconjugant with old macronucleus undergoing resorption and large new macronucleus.

FIGURE 14. Individual with two old macronuclei, two new macronuclei, and two micronuclei; probably the result of complete fusion of conjugants.

FIGURE 15. Conjugation of three individuals.

within the cytoplasm which have a rather vague outline and which are stained deep blue by methylene blue-azure II. Most of these masses are close to the macronucleus, and some appear to be in actual contact with it. Their significance is not clear.

Binary fission in this ciliate follows the conventional pattern. Division of the micronucleus is completed as the macronucleus elongates. Constriction of the macronucleus into two more or less equal masses evidently takes place not long before separation of the daughter ciliates is completed (Fig. 7). Extrusion of macronuclear chromatin during division has not been observed.

Stages of conjugation are very abundant in my preparations. However, the micronuclei often stain so weakly that it is impossible for one to be sure that all of the micronuclei—including those undergoing resorption—have been accounted for. The behavior of the micronuclei is particularly difficult to follow at certain stages when they are almost Feulgen-negative. I have never been able to distinguish what I could be sure were chromosomes. My interpretation of the sequence of nuclear changes involved in what I believe to be typical conjugation may therefore not be completely correct.

When two specimens which are close together on the same side or opposite sides of the field of frontal cilia become joined in conjugation, they remain tightly affixed to the pinnule. The lobations of the margins of the inferior surface may persist; they are, in fact, very prominent in many conjugants. In fixed and stained preparations, the macronucleus of recently-united individuals (Fig. 8) characteristically exhibits at least one large vacuole-like clear area in addition to the numerous smaller peripheral vacuoles found in the macronuclei of non-conjugating individuals.

The micronucleus of each conjugant divides twice. Three of the four micronuclei produced by these divisions (Fig. 9) are resorbed. The other micronucleus divides to form the two gametic nuclei. This division typically takes place very close to the region of contact between the conjugants. The synkaryons seem actually to be formed between the conjugants (Fig. 10), and then to re-enter the cytoplasm; they are surprisingly poor in Feulgen-positive material.

I have never observed stages in the division of the synkaryon. However, it appears likely that the micronucleus and young macronucleus found in exconjugants (Fig. 12) and members of many conjugating pairs (Fig. 11) are ordinarily produced by just one division.

At first, much of the young macronucleus is occupied by an irregular, almost Feulgen-negative mass (Fig. 11). After the macronucleus reaches maximum size, the material peripheral to this mass gradually becomes richer in Feulgen-positive material. In time, large granules of very strongly Feulgen-positive chromatin fill the macronucleus (Fig. 13), and what was once a sizeable central mass appears to be represented only by one or more small remnants. In time, the young macronucleus becomes slightly smaller than its maximum size, and begins to resemble the macronucleus of trophic individuals.

The degeneration of the old macronucleus evidently takes place rather slowly. It often does not appear to change a great deal between the start of conjugation and the time the development of the new macronucleus has been almost completed. Eventually, however, the chromatin of the old macronucleus becomes severely disrupted (Fig. 13), and then very much condensed into a nearly homogeneous and

intensely Feulgen-positive mass. A few small granules of chromatin are sometimes observed around the main mass of the degenerating macronucleus.

Certain stages which I have observed rather frequently do not fit into the pattern I have described. Some of them perhaps represent stages in alternate pathways of development of exconjugants, and others are probably anomalies. Individuals with two micronuclei, two old macronuclei, and two new macronuclei (Fig. 14) are moderately common. It seems likely that these result from complete fusion of conjugants. In some exconjugants, or in one or both of two conjugants which are still paired, there are two young macronuclei, one of which may be more advanced than the other. This suggests that the first micronuclear division following division of the synkaryon may also give rise to a macronucleus. The presence of two micronuclei and four young macronuclei in addition to two old macronuclei suggests that this has taken place in both of two conjugants which have fused completely. Conjugation between three individuals (Fig. 15) is observed occasionally, and I have also seen what is probably union of one individual with the product of a complete fusion of two others.

Three syntype slides of *P. verruciformis,* prepared from material collected at Mitchell Bay, San Juan Island, Washington, have been deposited in the United States National Museum (USNM Nos. 23751–23753).

The characteristics of the new genus *Phalacrocleptes* may be summarized as follows: Outline of the body, as viewed from one side, ranging from semicircular to slightly elongated and humped in the center; as viewed from the superior or inferior aspect, oval to fusiform. Inferior surface, in contact with the epithelium of the host, rounded, ridge-like, or somewhat flattened, generally with protuberances or marginal sinuosities; the character of the inferior surface and protuberances subject to considerable alteration. Delicate cylindrical or conical projections arising from the inferior surface contact the epithelium of the pinnules of the host and probably serve as feeding organelles. Macronucleus and micronucleus present. Cilia absent. Kinetosomes believed to be absent. No contractile vacuole. Nuclear changes during reproduction by binary fission proceeding as in typical ciliates. Conjugation in general similar to that of other ciliates. Type species: *Phalacrocleptes verruciformis* Kozloff, inhabiting the pinnules of the prostomial cirri of the sabellid polychaete *Schizobranchia insignis* Bush.

As this organism cannot at the present time be conscientiously assigned to an existing family, I propose it be referred to a new family, the Phalacrocleptidae. The distinguishing characteristics of this family are those of the type genus, *Phalacrocleptes.*

Discussion

Although the parasite which I have described apparently has no cilia at any time, and probably has no kinetosomes, the behavior of its nuclei during fission and conjugation clearly indicates that it is a ciliate. Finding a lower taxon into which it can be fitted with some conviction is more difficult.

Perhaps this curious organism is a suctorian which does not produce a ciliated larva. However, there is a remote possibility that it is a relative of the ancistrocomids parasitizing sabellids. Aside from the fact that it occurs on a type of polychaete known to be populated by representatives of this already quite highly

specialized group, there is no other evidence to influence my speculations concerning its possible origin.

If *P. verruciformis* is derived from an ancistrocomid parasitizing sabellids, its superficial similarity to the unciliated sessile stage of *Sphenophrya, Pelecyophrya,* and *Gargarius* cannot very well be taken as an indication that it is closely related to the sphenophryids. All known sphenophryids are parasites of the ctenidia of pelecypods, and are probably derived from a group of ancistrocomids associated with these mollusks.

Summary

Phalacrocleptes verruciformis lives on the frontal surfaces of the prostomial pinnules of *Schizobranchia insignis* (Sabellidae). Delicate projections extended from its changeable inferior surface contact the epithelial cells of the host and probably serve as feeding organelles. Although it has no cilia at any stage in its life history, and probably no kinetosomes, *P. verruciformis* has both a macronucleus and micronucleus, and reproduces by binary fission in much the same way as most other ciliates; moreover, the sequence of nuclear changes involved in its conjugation fits the pattern for ciliates in general. The systematic position of this organism within the group of ciliates is uncertain. It is perhaps a suctorian which does not produce ciliated larvae. There is also a possibility, however, that it is related to the ancistrocomid thigmotrichs which parasitize the prostomial pinnules of sabellids.

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