

STOMATOGENESIS IN THE PHILASTERINE SCUTICOCILIATE *PHILASTER HIATTI* THOMPSON, 1969¹

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COATS, D. W. & SMALL, E. B. 1976. Stomatogenesis in the philasterine scuticiliate *Philaster hiatti* Thompson, 1969. *Trans. Amer. Micros. Soc.*, 95: 718-725. Clonal cultures of *Philaster hiatti* (Thompson, 1969) were established from specimens collected at Windley Key, Florida. Ciliates were examined after protargol silver impregnation. A detailed account of stomatogenesis is presented, with emphasis on the development of the opisthe haplokinety. Morphological and morphogenetic observations suggest that the haplokinety of *P. hiatti* is composed of three distinct segments.

Small's (1967) creation of the order Scuticociliatida with the three suborders Pleuronematina, Philasterina, and Pseudocohnilembina was based predominantly on the stomatogenic patterns of representative genera. The scarcity of morphogenetic information for the numerous scuticociliate species has impeded acceptance of the order. Recent papers (Didier & Detcheva, 1974; Grolière, 1975; Grolière & Detcheva, 1974; de Puytorac, Didier, Detcheva & Grolière, 1974; de Puytorac, Grolière, Roque & Detcheva, 1974) have contributed significantly to the knowledge of scuticociliate stomatogenesis. These new observations support the recognition of the scuticociliates as a discrete taxon. However, marked variability in morphogenetic patterns makes evaluation of the suborders difficult. Corliss (1975), for example, supports the order but accepts only two of the three suborders included by Small (1967): viz., the Philasterina and the Pleuronematina (adding a different third suborder, discussion of which is beyond the scope of this paper).

One unifying characteristic of scuticociliate stomatogenesis is that the parental haplokinety participates in the development of morphogenetic fields. However, the precise manner of involvement of the haplokinety is not identical for all scuticociliate species. Thus, the haplokinety, its role in stomatogenesis, and its morphogenesis become fundamental aspects of scuticociliate systematics.

The objective of the present study is to offer an analysis of stomatogenesis as it occurs in the scuticociliate *Philaster hiatti*, with particular emphasis on the morphology and morphogenesis of the haplokinety.

MATERIALS AND METHODS

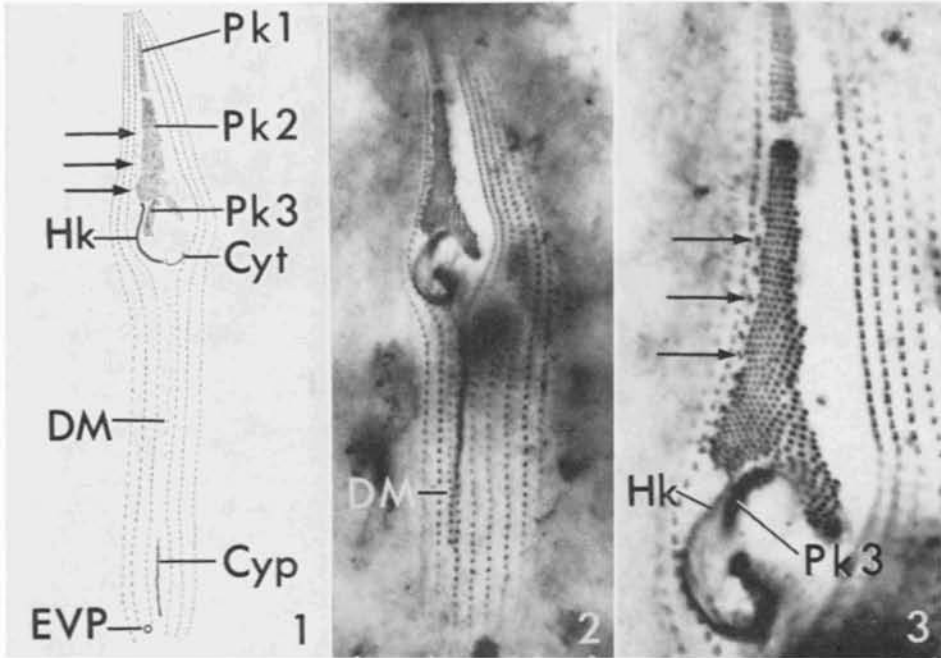
The ciliate studied, *Philaster hiatti*, was collected from a small man-made inlet on the eastern shore of Windley Key, Florida. Cubes of fresh fish tissue housed in peel-a-way tissue capsules² were suspended near shore and ca. 1 ft off the lagoon bottom. The traps were retrieved 1-2 hr later and placed in filtered sea water. Microscopic examination revealed the trap's ciliate population to be predominately of one morphological type, later identified as *P. hiatti*.

Clonal cultures were maintained on bacterized fish tissue. Organisms of log growth cultures were concentrated by centrifugation and inundated with Bouin's fixative. Preserved specimens were heated at 40 C for 48 hr,³ and then

¹ The authors wish to express their gratitude to Miss Lois Reid for her indispensable artistic assistance.

² Tissue capsules were purchased from Peel-a-way Scientific, South El Monte, California.

³ Adequate staining of the ciliates could not be obtained without prior heat treatment.



Abbreviations: AF, anterior field; AF-a, anterior portion of anterior field; AF-p, posterior portion of anterior field; Cyp, cytoproct; Cyt, cytostome; DM, director meridian; EVP, expulsion vesicle pore; Hk, haplokinety, *sensu stricto*; Hka, haplokinetal segment a; Hkb, haplokinetal segment b; Hkc, haplokinetal segment c; PF, posterior field; Pk1, polykinety one; Pk2, polykinety two; Pk3, polykinety three; Pk3a, polykinety three anlage.

FIG. 1. Camera lucida drawing of nondividing *P. hiatti*, ventral aspect. Arrows indicate paired kinetosomes alongside Pk2. Figs. 2, 3. Ventral surface of protargol silver stained *P. hiatti*, same specimen. Organism's right is to observer's left. Fig. 2. Illustrates the oral apparatus and somatic ciliary pattern. $\times 950$. Fig. 3. Higher magnification of buccal structures; note paired kinetosomes (arrows) adjacent to Pk2. $\times 2,300$.

stained by the Bodian protargol silver technique (Tuffrau, 1967). Observations of stomatogenesis were made from protargol stained cells. To assist confirmation of the species, appropriately fixed, nondividing ciliates were stained by the Chatton-Lwoff silver nitrate technique (Corliss, 1953).

RESULTS

Morphology of the Trophont

For orientation, the morphology of the nondividing (trophont) stage of *P. hiatti* must be briefly described. For further details reference is made to Thompson's original description of the species (Thompson, 1969).

As shown in Figures 1-3, the trophont possesses numerous bipolar ciliary meridians; a row of nonciliated kinetosomes lying along the bucco-anal striae, the director meridian; a single cytoproct and expulsion vesicle pore; and a long shallow ventral buccal cavity which deepens posteriorly. The buccal ciliature consists of three polykineties (Pk1, Pk2, and Pk3), a paroral membrane or haplokinety, and a series of nonciliated kinetosomal doublets anterior to the paroral membrane and bordering the right side of Pk2. Polykineties 1 and 2 are located

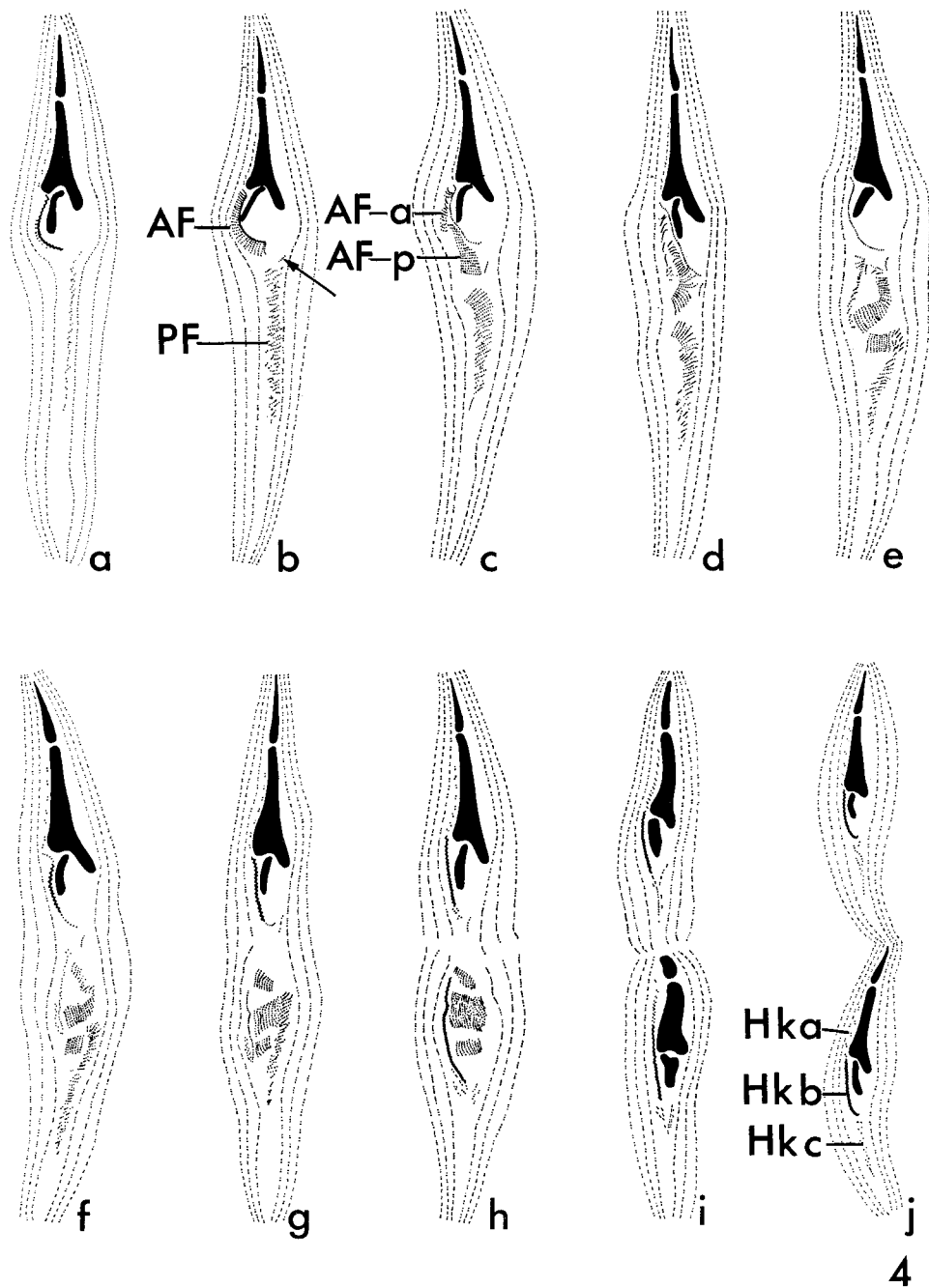


FIG. 4. Semi-schematic drawings of the stomatogenic stages of *P. hiatti*.

anterior to a depression leading to the cytostome. Pk3 and the paroral membrane are positioned at a deeper level on the right ledge of the buccal indentation.

Formation of Opisthe Buccal Organelles

The first indication of stomatogenesis in *P. hiatti* is the appearance of new kinetosomes to the left of each kinetosome of the director meridian (Figs. 4a, 5). This right-to-left proliferation appears to begin and occur more rapidly at the anterior end of the director meridian. Kinetosomal replication continues, producing a triangular field located mid-ventrally between the posterior tip of the haplokinety and the cytoproct (Fig. 4b). This field will be designated as the posterior field (PF), since a second field, which appears simultaneously, occupies a more anterior position. The PF is composed of numerous short rows of kinetosomes. The most anterior row of the PF (arrow, Fig. 4b) lies to the left of the remaining rows and, as will be discussed later, acts independently during stomatogenesis.

Accompanying PF formation, kinetosomes are proliferated to the right of the haplokinety, giving rise to an anteriorly located field (AF, Figs. 4b, 6). Early in stomatogenesis the AF borders the haplokinety; it is narrow at its anterior end, and gradually broadens toward the posterior tip of the haplokinety. Like the PF, the forming AF consists of short nonciliated rows of kinetosomes.

While still elaborating kinetosomes, the AF begins a posterior migration and separates transversely into two unequal portions. It is convenient to label the smaller anterior segment as AF-a and the larger posterior kinetosomal mass as AF-p (Figs. 4c, 7).

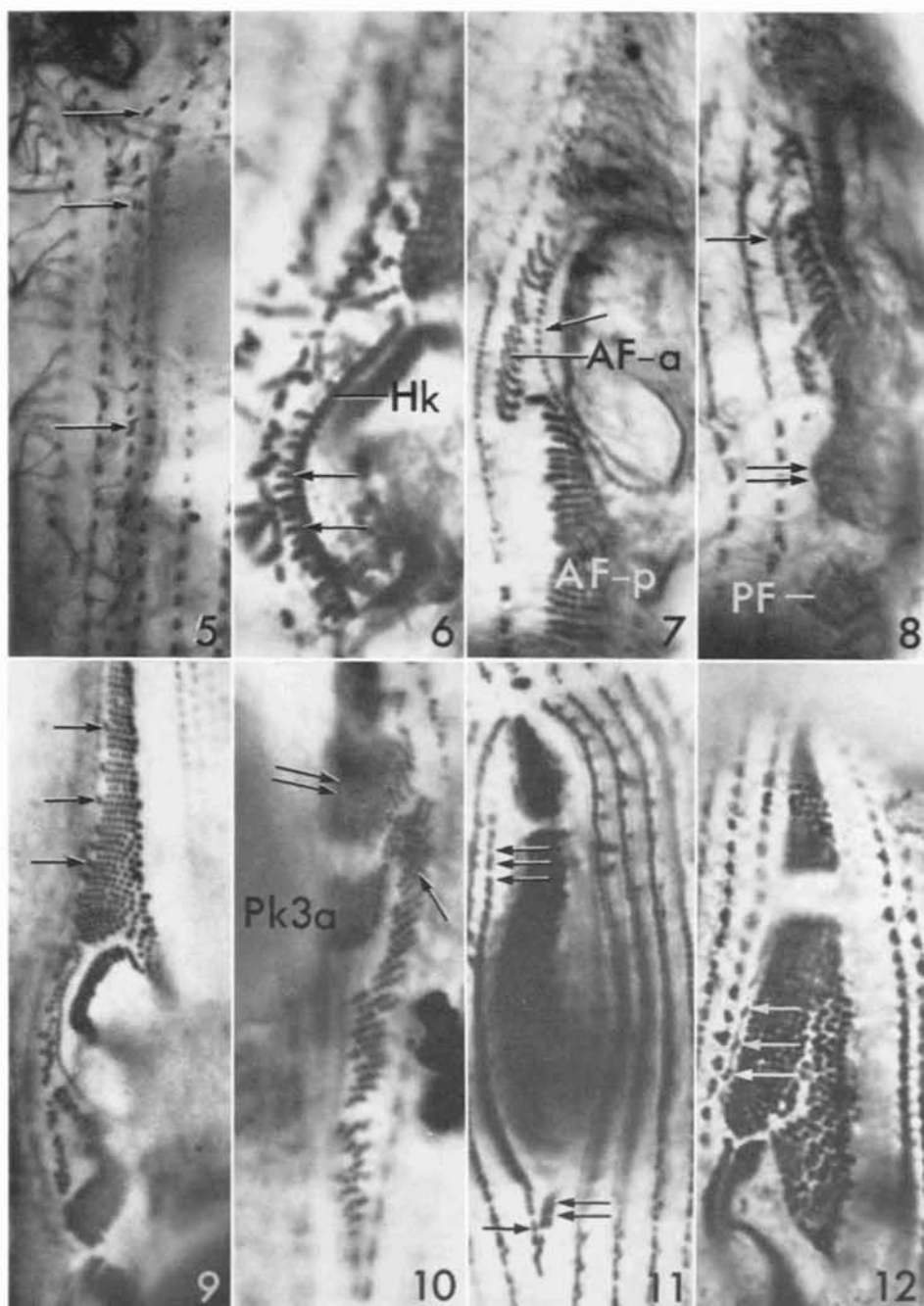
As the two units of the anterior developmental field (AF-a and AF-p) migrate away from the parental buccal area, the head of the PF is displaced posteriorly and rotated to the right (Fig. 4c-e, 8). As mentioned previously, the PF possesses one independent row of kinetosomes. This row is not included in the migration of the PF, but remains approximately in its original position (Figs. 4c-f).

With continued posterior movement, the AF-p arches to the right taking on a J-configuration, and the kinetosomes of AF-a are drawn out, forming a column to the right of the J-shaped AF-p (Figs. 4d-e, 9). Kinetosomal elaboration may continue in the AF-a, as it extends further and develops a scutico-field (Figs. 4e-g). The AF-a gives rise to a partially differentiated opisthe haplokinety which has a zig-zag configuration along its length, except in the terminal scutico-field (Fig. 4h). In this developmental stage, a noticeable indentation exists in the anterior third of the maturing opisthe haplokinety. Those kinetosomes anterior to this indentation separate from the rest of the haplokinety and produce the paired kinetosomes observed to the right of Pk2 in the trophic organism (Figs. 4i-j, 11).

The PF and AF-p each split into two parts (Figs. 4f, 10). The anterior portion of the AF-p forms opisthe Pk1 anlage. The detached head of the PF develops into the anlage of opisthe Pk3. The remaining kinetosomal masses, one from the AF-p and one from the PF, fuse and the kinetosomes integrate into opisthe Pk2 anlage (Figs. 4g-j). Some kinetosomes at the posterior end of the PF do not move into the presumptive opisthe Pk2. These kinetosomes join with those of the opisthe scutico-field to produce the opisthe director meridian (Figs. 4i-j, 11).

Fate of Parental Buccal Structures

Early in stomatogenesis the parental cytostome dedifferentiates and the cyto-stomal depression is lost. These structures reform very late, possibly after the



FIGS. 5-11. Division stages of *P. hiatti*. Protargol silver impregnation. Fig. 5. Early replication of kinetosomes (arrows) along the director meridian. $\times 1,250$. Fig. 6. Multiplication of kinetosomes (arrows) to the right of the haplokinety, *sensu stricto*. $\times 2,250$. Fig. 7. Division stage equivalent to Figure 4c. As the two portions of the anterior field move posteriorly, the paroral membrane infraciliature (arrow) is reduced to a single line of kinetosomes.

completion of cytokinesis. As the posterior region of the buccal cavity flattens, Pk3 and the haplokinety rise to the surface and stretch posteriad. Pk1 and PK2 undergo no noticeable migration, and all three polykineties retain ciliation throughout stomatogenesis. The kinetosomal pairs on the right of Pk2 are always present.

After the anterior fields migrate away from the parental buccal area, the proter haplokinety remains as a single line of kinetosomes (Figs. 4c-e, 7, 9). The parental haplokinety begins redifferentiation just prior to opisthe haplokinety differentiation (Figs. 4f-g). A few kinetosomes at the posterior tip of the proter haplokinety combine with the independent kinetosomal row of the PF to form the proter director meridian (Figs. 4i-j).

DISCUSSION

Stomatogenesis in *P. hiatti* commences with the production of two morphogenetically active kinetosomal fields. The first field (PF) forms by the multiplication of the director meridional kinetosomes and develops into the anlagen of opisthe Pk3, Pk2 in part, and contributes kinetosomes to both proter and opisthe director meridians. The second field (AF), derived from the parental paroral membrane, gives rise to the anlagen of opisthe Pk1, Pk2 in part, and haplokinety. The haplokinety anlage (AF-p) differentiates into the opisthe paroral membrane, several paired kinetosomes bordering the right side of Pk2, and donates kinetosomes (via a scutico-field) to the opisthe director meridian.

The stomatogenesis of *P. hiatti* is nearly identical to that reported for *Philaster digitiformis* (Grolière, 1975). In *P. digitiformis*, Pk2 is reported to form only from the field developed from the scutico-vestige. However, Grolière's illustrations of *P. digitiformis* stomatogenesis permit the interpretation that Pk2 in this species, like the Pk2 of *P. hiatti*, is formed in part from the field issued from the parental paroral membrane and in part from the field produced from the director meridian.

As shown in Figure 12, *P. digitiformis* possesses paired kinetosomes anterior to the paroral membrane in the same location as those of *P. hiatti*. These kinetosomes have not been previously reported; however, some illustrations of *Philaster* sp. stomatogenesis (Small, 1967) depict structures which may represent the kinetosomes in question. Presumably, the development of these kinetosomes would be the same in both *P. digitiformis* and *P. hiatti*.

The paired kinetosomes anterior to the haplokinety and adjacent to polykinety 2 pose an interesting question. Are these kinetosomes a unique buccal structure or are they part of an oral membranelle? Since these kinetosomes segregate late in stomatogenesis from what is interpreted as the haplokinety

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× 3,000. Fig. 8. With posteriad migration of the AF-a (single arrow) and the AF-p, the leading end of the AF-p (double arrows) and the anterior portion of the PF rotate to the right. Stomatogenic phase comparable to that of Figure 4d. × 2,800. Fig. 9. Time in development between those shown in Figures 4d and 4e. Paired kinetosomes (arrows) bordering parental Pk2 remain throughout stomatogenesis. × 2,100. Fig. 10. Division stage depicted in Figure 4f. The separated tail of the PF (single arrow) and the separated head of the AF-p later fuse to form Pk2 anlage. × 3,250. Fig. 11. Late in stomatogenesis kinetosomes from the opisthe scutico-field (single arrow) and from the posterior tip of the PF (double arrows) begin formation of opisthe director meridian. Paired kinetosomes have separated from the anterior end of the developing opisthe haplokinety (triple arrows). Stage equivalent to Figure 4i. × 2,300. Fig. 12. Chatton-Lwoff silver nitrate stain of nondividing *Philaster digitiformis*. Paired kinetosomes (arrows) are located adjacent to Pk2. × 2,600. [Specimen photographed from slide on loan to the junior author from Dr. J. Dragesco.]

anlage, we believe that these kinetosomes represent a morphologically distinct segment of the haplokinety. Ultrastructural examination (Coats, personal observation) shows that these kinetosomes lack cilia and associated microtubules.

The kinetosomes of the opisthe scutico-field separate from the differentiating paroral membrane, migrate posteriad, and come to rest along the bucco-anal striae. It is tempting to view the director meridian as another segment of the haplokinety, but this is not easily justified, since some kinetosomes of the director meridian are derived from a polykinety anlagen. Yet, that the kinetosomes of the director meridian originate from a presumptive oral structure is clear. Thus, in *Philaster hiatti*, the haplokinety consists of an A-segment (Hka) composed of widely spaced, nonciliated paired kinetosomes; a B-segment (Hkb, the paroral membrane) possessing the typical zig-zag haplokinety configuration; and possibly a C-segment (Hkc, the scutico-vestige of Small, 1967) included within the director meridian.

Not all philasterine scuticociliates exhibit as clear a haplokinetal segmentation as does *Philaster*. However, several genera, *Cohnilembus* (Didier & Detcheva, 1974), *Miamiensis* (Thompson & Moewus, 1964), *Paralembus* (Grolière, 1975), and *Uronema* (de Puytorac, Grolière, Roque & Detcheva, 1974), show an indentation in the anterior portion of the haplokinety similar to that seen during haplokinetal development in *Philaster hiatti*. Such indentations may represent a demarcation between the Hka and Hkb segments. Three other genera, *Plagiopyliella* (Lynn & Berger, 1972), *Thyrophylax* (Lynn & Berger, 1973), and *Potomacus* (Brownlee et al., 1975), each have a morphologically distinct Hka segment. All philasterine scuticociliates have a scutico-vestige (Small, 1967) and thus, presumably, a haplokinetal segment C.

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A NEW CUTICULAR CYST-PRODUCING TETRAHYMENID CILIAE, *LAMBORNELLA CLARKI* N. SP., AND THE CURRENT STATUS OF CILIATOSIS IN CULICINE MOSQUITOES¹

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CORLISS, J. O. & COATS, D. W. 1976. A new cuticular cyst-producing tetrahymenid ciliate, *Lambornella clarki* n. sp., and the current status of ciliatosis in culicine mosquitoes. *Trans. Amer. Micros. Soc.*, 95: 725–739. On the basis of data gathered through study of properly fixed and silver-impregnated material, a ciliate from the body cavity of larvae of the treehole-breeding mosquito *Aedes sierrensis*, from California, is considered to be a new species, *Lambornella clarki* n. sp., congeneric with *L. stegomyiae* Keilin, 1921, an organism treated for the past 15–16 years as a member of the ubiquitous, and sometimes entomophilic, genus *Tetrahymena*. The single most important differentiating characteristic is the cuticular “invasion” cyst formed by species of *Lambornella*. By means of it, as T. B. Clark has very recently demonstrated, the host's cuticle is penetrated and the ciliate is (often) able to reach the haemocoel of the larval mosquito, where it multiplies and causes the death of the host. Possession of a larger number of postoral kineties than known in *Tetrahymena* is another major character justifying separation of the two genera both of which, however, are to be considered members of the family Tetrahymenidae. Topics reviewed for all known cases of ciliatosis in culicine mosquitoes include infectivity and host resistance, degree of pathogenicity, mode of entry into host, and facultative vs. obligate parasitism. Comparisons are made of the situation for *Lambornella* with those obtaining for other tetrahymenine ciliates (notably *Tetrahymena* species), and taxonomic conclusions are offered concerning organisms described—often inadequately—in the past literature. The potential importance of such ciliates in possible biological control of mosquitoes of biomedical interest underlies the need for further comparative investigation of their morphology, bionomics, and taxonomic status, and especially for controlled laboratory experimentation.

Although many species of ciliated protozoa are “symphorionts” on the exoskeleton or integument of “host” species belonging to numerous invertebrate groups, very few are believed to be able to penetrate the host cuticle and invade the underlying tissues or the haemocoel. Best-known examples are three members

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