

- Philasterina trouvé dans la cavité générale du polychète *Nereis diversicolor* O. F. Müller. *Protistologica*, 10: 101–111.
- SMALL, E. B. 1967. The Scuticociliatida, a new order of the class Ciliatea (phylum Protozoa, subphylum Ciliophora). *Trans. Amer. Micros. Soc.*, 86: 345–370.
- THOMPSON, J. C., JR. 1969. *Philaster hiatti* n. sp., a holotrichous ciliate from Hawaii. *J. Protozool.*, 16: 81–83.
- THOMPSON, J. C., JR. & MOEWUS, L. 1964. *Miamiensis avidus* n. g., n. sp., a marine facultative parasite in the ciliate order Hymenostomatida. *J. Protozool.*, 11: 378–381.
- TUFFRAU, M. 1967. Perfectionnements et pratique de la technique d'imprégnation au protargol des infusoires ciliés. *Protistologica*, 3: 91–98.

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

JOHN O. CORLISS and D. WAYNE COATS

Department of Zoology, University of Maryland, College Park, Maryland 20742

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

¹ Acknowledgment is gratefully made for the support of National Science Foundation grant No. 76-19272 awarded to the senior author. We wish also to express our thanks to Dr. Truman B. Clark for his encouragement and his indispensable aid through generously supplying us not only with fixed ciliates of our new species but also with the photomicrographs comprising our Figures 5–10. Finally, the artistic assistance of Miss Lois Reid on Figures 1–4 must be mentioned with gratitude.

of the all-parasitic hypostome order Apostomatida which have an encysted stage that can dissolve its way through the epicuticle of certain crabs and shrimp (see Bradbury et al., 1974). The only other species possibly possessing such an ability is represented by certain oligohymenophoran ciliates of the order Hymenostomatida, mosquito-parasitizing organisms still of rather uncertain taxonomic status, first known from papers by Lamborn (1921) and Keilin (1921). But the "cysts" described from poorly fixed material of the organism called *Lambornella stegomyiae* by Keilin were either totally ignored or not accepted as playing any role in entry of the parasite into the body cavity from the outside (e.g., see Kirby, 1941; Steinhaus, 1947; Wenyon, 1926), even following Muspratt's (1945, 1947) alleged rediscovery and further description of the curious culicophilic organisms (e.g., see Grassnick & Rowley, 1973; Kellen et al., 1961; Lipa, 1963; Sanders, 1972). Corliss (1960)—later nicely supported by McLaughlin (1971)—did accept the cyst and Muspratt's view that it played a major part in the life cycle of the ciliate; he removed Keilin's organism to the genus *Tetrahymena*. However, he offered no new data to substantiate Muspratt's hypothesis that the endoparasite made its entry into the haemocoel (body cavity) through the cuticle.

Therefore—in light of the briefly presented historical facts above—the very recent demonstration by Clark & Brandl (1976) of both cyst-formation on and invasion through the cuticle of larval *Aedes sierrensis* by a hymenostome ciliate has been a most exciting and significant revelation, confirming possession of such a penetration ability in a group of usually free-living, free-swimming organisms and strongly suggesting that the deductions of the early workers were correct. Remaining is the knotty taxonomic problem of proper identification of the several "endoparasitic" species involved in all such studies: this is the main objective of the present paper. We wish also to bring up to date a review of the status of ciliatosis in mosquitoes (the first since Corliss, 1960, a paper mainly concerned, however, with other topics), because the potential of such culturable organisms in the biological control of mosquitoes of biomedical interest is worthy of wider recognition.

MATERIALS AND METHODS

Our material—aside from data in the published literature, not to be ignored—was principally of five kinds or from five sources:

(1) Silver-impregnated material which we have made of fixed ciliates from the host treehole-breeding mosquito *Aedes sierrensis* (larval instars), organisms kindly supplied on several occasions by Dr. Truman B. Clark (then in the USDA-ARS WIAMA Laboratory in Fresno). We used the Chatton-Lwoff silver technique (Corliss, 1953). Unfortunately, attempts by Dr. Clark to send living material from California were unsuccessful, all ciliates arriving in dead or moribund condition.

(2) Silver-impregnated material of "*Tetrahymena stegomyiae*" from the collection of the senior author; and the type-specimen slide of this species kindly loaned (back) to us from the International Collection of Ciliate Type-Specimens by the curator, Dr. Klaus Ruetzler, United States National Museum. The "*T. stegomyiae*" specimens came originally from Dr. Muspratt (in the year 1963), taken from larvae of *Aedes* species and fixed in de Fano's fluid by Muspratt, and impregnated with silver by the senior author soon after their arrival from South Africa.

(3) Slides (of poor quality, unfortunately) made from material sent (fixed) from Dr. J. A. Reid (in the late 1950's), Kuala Lumpur, Malaysia, taken from the body cavity of both larval and adult *Armigeres* mosquitoes.

(4) Photomicrographs and electronmicrographs belonging to Dr. Clark (many unpublished and all generously put at our disposal), representing further "proof" of the closeness of the association of his ciliate and their mosquito larvae, both in their treehole habitat and in the laboratory.

(5) Finally, for comparative taxonomic purposes, cytological preparations of numerous other tetrahymenine ciliates (genera *Colpidium*, *Glaucoma*, *Tetrahymena*, etc.: both symbiotic and free-living forms) from the University of Maryland Reference Slide Collection.

OBSERVATIONS AND TAXONOMIC CONCLUSIONS

Microscopical observations of all of the strains and specimens available to us (see above), plus data gleaned from the all-too-scanty pathogenic ciliate-mosquito literature, have led us to the following general conclusions, some more tentative in nature (as indicated in subsequent sections) than others.

General Remarks

1. Muspratt's ciliates and very likely Keilin's organism deserve to be replaced in the (resurrected) genus *Lambornella* as *L. stegomyiae* Keilin, 1921, as originally named and described. Probably the species very recently reported by Dzerzhinsky et al. (1976) belongs here, too. A redescription and an explanation of why the ciliate should not be called *Tetrahymena stegomyiae* (Keilin, 1921) Corliss, 1960 are offered in the following sections of this paper.

2. Clark's ciliates (Clark & Brandl, 1976) and quite likely those of other California workers—Kellen et al. (1961) and Sanders (1972)—all from larvae of the same host, appear to represent at least a separate species, which we are here naming *Lambornella clarki* n. sp.²

3. The experimental organism employed by Grassmick & Rowley (1973) in their continuing investigations is a micronucleate *Tetrahymena pyriformis*, syn-gen 1, according to their identification (and there are no cuticular cysts, etc., etc.). On the other hand, a number of "accidentally" or "facultatively" parasitic ciliates found in nature (according to observations of the senior author and others), in mosquitoes and other hosts, are amiconucleate strains of *T. pyriformis*, as undoubtedly are (were) some (but *not* all!) of the ciliates called "*Glaucoma pyriformis*" in certain of the older published accounts.

4. Some hymenostome ciliates in mosquitoes (and other hosts) may well be members of still *different* genera, possibly new taxa: for example, the frustratingly enigmatic forms reported by Corliss (1961b) from larval *and* adult *Armigeres* from Malaysia.

5. Some cases have been and are undoubtedly *mixed* infections, and/or with contamination of subsequent cultured populations. The most common "contaminant" is *Tetrahymena pyriformis* (amiconucleate), because it grows so well and so rapidly in practically any nutrient medium. [Two repeatedly verified mixed infections, incidentally (though the hosts are not mosquitoes), are occurrences of *T. limacis* and *T. rostrata* in the same slug and of *T. chironomi* and *T. pyriformis* (amiconucleate) in the same larval chironomid (see references in Corliss, 1973a).]

² Named in honor of the discoverer of the true nature of their cuticular or "invasion" cyst, Dr. Truman B. Clark, now with the USDA, ARS, PPI at Beltsville, Md. In his paper (Clark & Brandl, 1976), incidentally, the senior author of the present work was responsible both for suggesting that Clark conservatively consider his organism to be *Lambornella stegomyiae* (rather than a separate species) and for supplying an alleged title of a "Corliss & Coats" paper, which title became altered considerably by the time that the manuscript was finally approved for its appearance here.

Generic Diagnosis of Lambornella Keilin, 1921

Of particular concern to us in this brief paper are a fresh characterization of the resurrected genus *Lambornella* and new descriptions of the type-species (by monotypy), *L. stegomyiae*, and of the second included species, *L. clarki* n. sp.

Lambornella (syn. *Tetrahymena* pro parte) may be succinctly described as follows:

Body of medium size (often 70–80 μm in length), elongate to pyriform, sometimes somewhat spindle-shaped with tapered anterior and posterior ends, rounded and with very pliable pellicle when well-fed; kineties converge anteriorly onto preoral suture which may be skewed to organism's left; uniformly and densely ciliated, with range in number of rows 28–52, but different modal numbers (30 and 46) for the two included species; similarly, 3–9 postoral meridians (POM's), usually 3–4 in one species, 7 in the other; buccal overture nearly as wide as long, broadly pyriform in outline, often ca. $13 \times 11 \mu\text{m}$, leading to buccal cavity containing typical tetrahymenal ciliary apparatus (tripartite AZM and single UM), with infraciliary bases of membranelles showing variation in location and in conformation; stomatogenesis parakinetal, with oral replacement in proter common³; contractile vacuole pores (CVP's) generally two (range 1–5) in number, on right-ventral-posterior surface of body, involving kineties 6–10, but exact location and juxtaposition (one to the other) not identical in the two species; cytoproct (CYP) near posterior end of body, midventral, at end of stomatogenic kinary number 1; polar basal body- (PBB-) complex known in only one species; macronucleus compact, ovoid to spherical, centrally located; micronucleus prominent, rounded, not far from macronucleus; a cuticular or invasion cyst (range in diameter, 22–60 μm), transparent but multiwalled, regularly forms on cuticle of host (larval culicine mosquitoes, particularly treehole-breeding species), with subsequent invasion by ciliate through cuticle into underlying tissues or haemocoel (with rapid multiplication in latter); apparently world-wide in distribution, with infection fatal for host; whether obligately or facultatively an endoparasite not yet determined, but organism able to live and divide free of host.

Redescription of L. stegomyiae and Description of L. clarki n. sp.

Keilin's (1921) original description of *Lambornella stegomyiae* was scant, and the subsequent contributions to its diagnosis by Muspratt (1945, 1947) and Corliss (1960)—Muspratt calling it "*Glaucoma pyriformis*" and Corliss "*Tetrahymena stegomyiae*"—added rather little to our understanding of this curious ciliate. Unfortunately, both the very recent account by Clark & Brandl (1976) and the limited data presented in the present paper on the second species, *L. clarki* n. sp., still do not provide us with as much as we might desire to know about members of the genus, especially with respect to the degree of possible dependency of the organisms on their host, the amount of morphological (and physiological) variability realizable at all stages in their full life history and under differing conditions (including controlled laboratory cultivation), and details in the morphogenetics of their fission and stomatogenesis.⁴

³ The term "parakinetal" was coined by Corliss (1973b) in a preliminary revision of modes of stomatogenesis in ciliates. Oral replacement of the proter's mouthparts during fission is not particularly common in tetrahymenine species, though it has been considered "the rule" in the *Tetrahymena bergeri* (= a strain of *T. rostrata*?) described by Roque et al. (1971). We have noted the phenomenon (via stained slides) only in *Lambornella clarki* n. sp. in the present study.

⁴ In fact, were it not for the pressing need to call attention to the taxonomic resurrection of *Lambornella* as a genus separate from *Tetrahymena* (and to revive serious interest in the potential of such ciliates in mosquito control) and were it not also for the fact that our par-

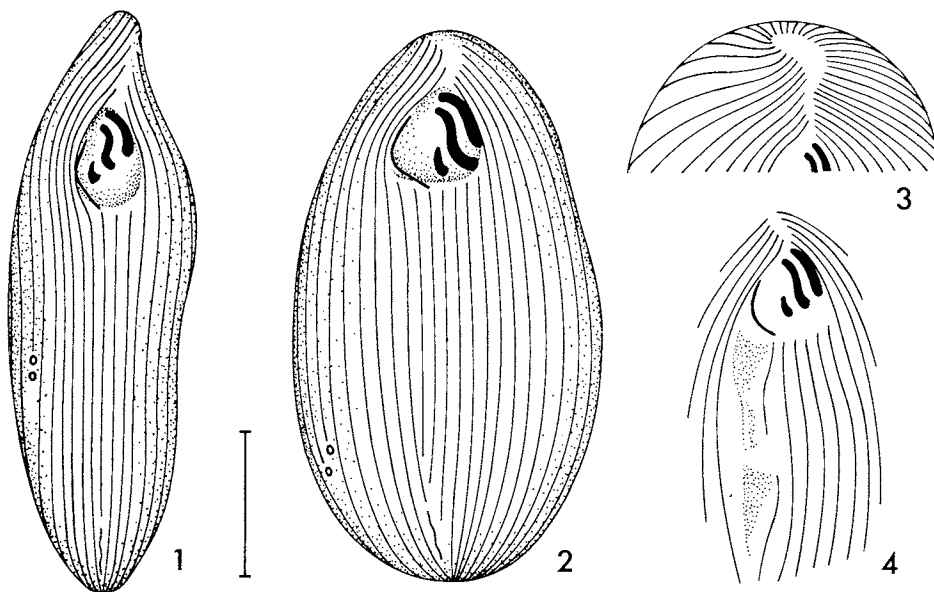


FIG. 1. Drawing of *Lambornella stegomyiae*, in ventral view, from specimens silver-impregnated according to the Chatton-Lwoff technique and originally derived from one lot of da Fano-fixed material sent by J. Muspratt from South Africa in 1963 to senior author. Note features of diagnostic importance: shape of body, number of ciliary rows and their anterior convergence onto preoral suture, number of postoral kineties, number and position of contractile vacuole pores, location of cytoproct, and conformation of infraciliary bases of ciliary membranelles in broad buccal cavity. Scale = 20 μ m.

FIGS. 2-4. Drawings of *L. clarki* n. sp., using specimens silver-impregnated by the junior author and Mr. Paul Kile from material originally fixed in Champy's (and subsequently stored in da Fano's) and sent to us by T. B. Clark from California in 1975. Fig. 2. The organism in ventral view, drawn to same scale as in Figure 1. Compare and contrast "silverline" (cortico-type) characteristics with those listed above for *L. stegomyiae* (Fig. 1). Fig. 3. Anterior polar view revealing pattern of convergence of somatic kineties (numbers 2-40 visible here) at this end of organism. Fig. 4. Early division stage of ciliate showing parakinetal stomatogenesis in the presumptive opisthe and oral replacement in the proter. Fields of "erratic" kinetosomes seem, in both cases, to have been derived from portions of stomatogenetic kinety number 1 (which, in this specimen, has thereby lost its own identity).

1. *Lambornella stegomyiae* Keilin, 1921 may be briefly characterized as follows (and see Fig. 1), keeping in mind that the generic traits treated above need not be repeated here:

Body $78 \times 22 \mu$ m in (mean) size and somewhat spindle-shaped, with anterior end more strongly tapered than posterior; modal number of kineties ca. 30, with 3-5 POM's, and with preoral suture to (organism's) left of midline; typically two CVP's (range 1-3), usually in meridian number 8 or 9 with one immediately above the other, at a subequatorial level generally above lower third of body; buccal overture little longer than wide, with bases of ciliary membranelles arranged predominantly on anterior back wall of buccal cavity; caudal cilium never described nor PBB-complex detected in material studied to date; diameter of macronucleus ca. 15μ m, of micronucleus ca. 4μ m; hemispherical

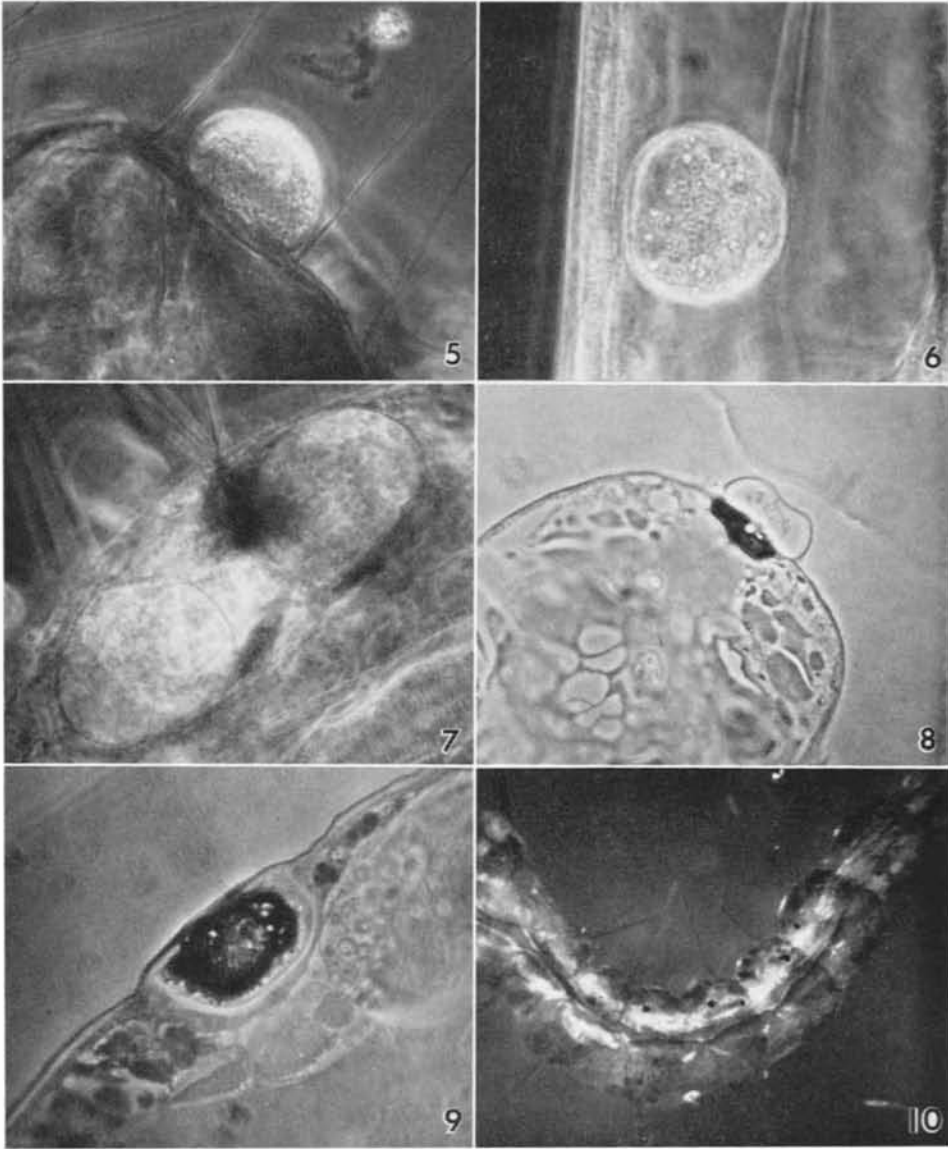
ticular source of the oak treehole *L. clarki* n. sp. has, literally, dried up (delaying study of additional material indefinitely), we should have been strongly tempted to postpone publication of this paper until we could, ourselves, tell a more satisfactorily complete story.

TABLE I
Ciliated protozoa found in association with culicine mosquitoes in nature

Specific name originally used	Reference	Host; location of infection; origin and geographical source of material; comments	Suggested systematic status
None	Lamborn (1921)	<i>Aedes</i> (<i>Stegomyia</i>) <i>scutellaris</i> [= <i>A. (S.) albopictus</i> ?]; in body cavity, anal gills, and (one case) on cuticle of larvae; from earthenware pot, Kuala Lumpur, Malaysia, S. E. Asia; infection fatal	<i>Lambornella stegomyiae</i>
<i>Lambornella stegomyiae</i>	Keilin (1921)	As above; considered to be <i>Glaucoma pyriformis</i> by most subsequent workers	As above
None	MacArthur (1922)	<i>Culiseta</i> (<i>Culiseta</i>) <i>annulata</i> ; in body cavity, especially head region, of larvae; from field dyke near Blackpool, England, G. B.; said to differ from Keilin's <i>Lambornella</i> ; infection fatal	<i>Tetrahymena pyriformis</i> ?
<i>Turchiniella culicis</i>	Grassé & de Boissezon (1929) and de Boissezon (1930)	<i>Culex</i> (<i>Culex</i>) <i>pipiens</i> (?); in haemocoel of single adult female; from Villeneuve (Haute-Garonne), France; infection considered not fatal, but larvae suggested as "normal" host	<i>T. pyriformis</i> ? or?
<i>Glaucoma pyriformis</i>	Wenyon (1926)	MacArthur's (1922) material: see above; description still inadequate	<i>T. pyriformis</i> ?
<i>G. pyriformis</i>	Muspratt (1945, 1947)	Treehole-breeding <i>Aedes</i> (<i>Aedimorphus</i>) <i>haworthi</i> , <i>A. (A.) marshalli</i> , <i>A. (Finlaya) fulgens</i> , <i>A. (Stegomyia) aegypti</i> , <i>A. (S.) calceatus</i> , <i>A. (S.) metallicus</i> , <i>Culex</i> (<i>Culex</i>) <i>decens</i> , <i>C. (Culiciomyia) nebulosus</i> ; generally restricted to body cavity, anal papillae, and cuticle of larval stages, but occasionally in adult; from Livingstone, Northern Rhodesia, Africa; <i>A. fulgens</i> believed most suitable host; principal tree with rot holes attractive to breeding mosquitoes was <i>Ricinodendron rautanenii</i> ; ciliate considered likely identical with Keilin's organism; role of cuticular cyst stressed; infection fatal	<i>L. stegomyiae</i>
<i>Tetrahymena</i> sp. and <i>T. pyriformis</i>	Corliss (1954, 1960)	<i>Wyeomyia</i> (<i>Wyeomyia</i>) <i>smithii</i> ; in body cavity of larvae; from pitcher plants, Bethany Bog, Connecticut, U.S.A.; ciliates all amiconucleate	<i>T. pyriformis</i>
<i>T. pyriformis</i>	Laird (1959)	<i>Aedes</i> (<i>Stegomyia</i>) <i>albopictus</i> , <i>Culex</i> (<i>Culex</i>) <i>fuscocephalus</i> , <i>C. (C.) gelidus</i> , <i>C. (C.) taeniorhynchus trisiamensis</i> [= <i>C. (C.) t. summorosus</i>]; in body cavity of larvae; from Singapore, S. E. Asia; original identification now doubtful	<i>L. stegomyiae</i> ?
<i>T. stegomyiae</i>	Corliss (1960)	Treehole-breeding <i>Aedes</i> (<i>Stegomyia</i>) <i>metallicus</i> and <i>A. (S.) aegypti</i> ; in body cavity and on cuticle of larvae; from mideastern Transvaal, South Africa (courtesy of J. Muspratt)	<i>L. stegomyiae</i>

Table I, continued

Specific name originally used	Reference	Host; location of infection; origin and geographical source of material; comments	Suggested systematic status
<i>T. sp.</i> (?)	Corliss (1960, 1961b)	Bamboo-breeding <i>Armigeres (Leicostera) dolichocephalus</i> , <i>A. (L.) dentatus</i> , <i>A. (L.) digitatus</i> ; in body cavities, anal gills, etc. of both larvae and adults; from bamboo forests near Kuala Lumpur, Malaysia, S. E. Asia (courtesy of J. A. Reid); insufficient data for confident identification	<i>T. sp.</i> ? or?
<i>G. pyriformis</i>	Kellen et al. (1961)	Treehole-breeding <i>Aedes (Ochlerotatus) sierrensis</i> ; in body cavity and anal papillae of single larvae; from oak tree (<i>Quercus agrifolia</i>) in Marin County, California, U.S.A.; no cysts reported	<i>L. clarki</i>
<i>T. sp.</i>	Sanders (1972)	Treehole-breeding <i>Aedes (O.) sierrensis</i> ; in body cavity and anal papillae of number of larvae (sometimes along with a mermithid worm); infection fatal; from trees (unnamed) near Novato, Marin County, California, U.S.A.; no cysts reported	As above
<i>T. stegomyiae</i>	Dzerzhinsky et al. (1976)	Treehole-breeding <i>Aedes (Stegomyia) aegypti</i> ; in body cavity of larvae (sometimes with a eugregarine sporozoon, <i>Lankesteria culicis</i>); infection fatal	<i>L. stegomyiae</i>
<i>L. stegomyiae</i>	Clark & Brandl (1976)	Treehole-breeding <i>Aedes (O.) sierrensis</i> ; in body cavity, anal papillae, etc., and on cuticle (in cysts) and between cuticle and epidermis, etc. enroute to body cavity; infection fatal; no infection in midge <i>Culicoides cavaticus</i> from same treehole; from two oak trees near Kings River in Fresno County, California, U.S.A.; stressed were mode of entry, melanized spots, and potential in biological control	<i>L. clarki</i>
<i>L. stegomyiae</i> and <i>L. clarki</i> n. sp.	Corliss & Coats (present paper)	As above; Keilin's genus <i>Lambornella</i> resurrected for <i>L. stegomyiae</i> , but material from T. B. Clark placed in separate, new species, here named <i>L. clarki</i> n. sp.; known ciliatoses in all culicine mosquitoes reviewed	<i>L. stegomyiae</i> and <i>L. clarki</i>



FIGS. 5-9. Photomicrographs, kindly supplied by Dr. Truman B. Clark, of *Lambornella clarki* n. sp. (Figs. 5-7, phase contrast of live material.) Fig. 5. Newly encysted individual settled on cuticle of thorax of second instar larvae of host, *Aedes sierrensis*. $\times 440$. Fig. 6. Ciliate immediately after penetration of cuticle of second instar larva of mosquito host. $\times 550$. Fig. 7. Products of division of an individual ciliate apparently trapped between new and old cuticle in molting mosquito larva. $\times 550$. Fig. 8. Phase contrast of sectioned unstained but fixed material revealing empty cyst on outside of host cuticle and melanized body, underneath, of the ciliate, which never succeeded in penetrating through the epidermis into the haemocoel. $\times 280$. Fig. 9. Phase contrast of section through unstained but fixed material showing dead and melanized ciliate just underneath cuticle of host (empty cyst, normally on outside, lost from this particular section). $\times 550$. Fig. 10. Photograph of fourth instar larva of mosquito host *A. sierrensis* showing black (melanized) spots indicating loci of unsuccessful invasion attempts by would-be ciliate endoparasite *L. clarki* n. sp. $\times 20$.

cuticular cysts 30–40 μm (Muspratt reported range 22–30 μm) in diameter and ca. 20 μm in height when on mosquito; hosts include larval instars of tropical treehole-breeding species of several subgenera (including *Stegomyia*) belonging to culicine genera *Aedes* and *Culex* (see Table I) from Malaysia, Rhodesia, and South Africa.

Neotype-specimen material (slide of silver-impregnated specimens from ciliates fixed by J. Muspratt in South Africa, deposited by J. O. Corliss in March 1971): USNM No. 24117.

2. *Lambornella clarki* n. sp. may be briefly described, as a second species in the genus, as follows (and see Figs. 2–10), using principally our own data (and that of Clark & Brandl, 1976) but concluding that the organisms noted without much description by Kellen et al. (1961) and by Sanders (1972) are very likely conspecific with our strain(s):

Body $71 \times 45 \mu\text{m}$ in (mean) size and generally broadly pyriform in shape, though occasionally tailed; modal number of kineties 46, with range 44–52; 6–9 POM's, with 7 most common number; preoral suture directly in body axis; usually 2–3 CVP's (range 1–5), involving meridians 6–7, 7–8, or 8–9, not one above other nor parallel, typically in lower fourth of body; buccal overture with width as great as length, especially evident in large rounded specimens, with bases of buccal membranelles more or less parallel to one another, generally at slight angle to body axis, and mostly left of midline; caudal cilium itself never noted in living material, but PBB-complex clearly present in number of silver-impregnated specimens; diameter of macronucleus ca. 18 μm , of micronucleus ca. 5 μm ; hemispherical cuticular "invasion" cyst 40–60 μm in diameter and 25–40 μm in height when on mosquito; numerous black spots (sites of melanization of damaged host tissue and/or of killed ciliates in act of invasion) between cuticle and epidermis, particularly prominent in post-second instar larvae (of *Aedes sierrensis* from oak treeholes), characteristic of infected (or "attacked") hosts; found in host from two nonadjacent counties of central California.

Holotype-specimen material (slide of silver-impregnated specimens from ciliates fixed by T. B. Clark in California, deposited by us in September 1976): USNM No. 24490.

Suprageneric Allocation of These Ciliates

We do not hesitate to include *Lambornella*, along with *Colpidium*, *Deltopylum*, *Stegochilum*, and *Tetrahymena*, in the family Tetrahymenidae Corliss, 1952 of the hymenostome suborder Tetrahymenina Fauré-Fremiet in Corliss, 1956. While many of its characteristics (see preceding section) are particularly reminiscent of *Tetrahymena*, others resemble *Colpidium* or *Deltopylum*, a few (e.g., multiple number of postoral meridians) are *Glaucoma*-like (family Glaucomidae), and some are completely unique (for an outstanding example, the cuticular cyst⁵). In short, the constellation of characters defining *Lambornella* both support its own generic integrity and suggest its assignment to the same family as that containing the well-known *Tetrahymena* species.

In reaching the above conclusions, we have restudied "reference material" from a variety of tetrahymenine (and other) hymenostome groups. The most pertinent papers of recent years concerned with the overall systematics of genera closest to *Lambornella* are those of Corliss (1970, 1971b, 1973a) and Czapik (1968). Of entomophilic endoparasitic ciliates described to date, the species

⁵ Such a cyst is unlike practically any of the other kinds of cysts known for ciliates and other protozoa (Corliss & Esser, 1974), particularly in the role it plays in entry of the organism into the body cavity of its host.

all appear to belong to either *Lambornella* or *Tetrahymena*⁶ (see Discussion and Table I).

It should be noted that the familial diagnosis for the Tetrahymenidae has to be broadened to include *Lambornella*. An emendation is necessitated, however, solely with respect to the number of POM's: instead of "one to three" post-oral kineties that characteristic now must be described as "one to three (but as many as nine in *Lambornella*)."

Nomenclatural Considerations

Closely tied to taxonomic conclusions are, inevitably, nomenclatural decisions. But there are no difficult problems to solve in the present situation. Corliss & Dougherty (1967), in their lengthy petition to the International Commission on Zoological Nomenclature concerned with conservation of the well-known but relatively "youthful" generic name *Tetrahymena* Furgason, 1940 and thus involving more than a dozen older names of which *Lambornella* Keilin, 1921 was just one, requested simply that *Lambornella* be conditionally suppressed, removing it as a threat to *Tetrahymena* but allowing it to remain potentially available if a separate genus were ever needed for its type. The ICZN (1970), in the formal language of its Opinion 915 on the petition, ruled that *Tetrahymena* is to be given precedence over the generic name *Lambornella* [and over others, not relevant here] ". . . by any zoologist who considers the type-species of these genera to belong to the same genus-group taxon."

Whereas Corliss (e.g., 1960, 1970, 1973a)—and others, following his lead—have for some years considered *Tetrahymena pyriformis* and *Lambornella stegomyiae*, type-species of their respective genera, to be congeneric, we have presented evidence in the present paper for a reversal of that conclusion. Since this is perfectly allowable under the provisions of the Opinion quoted above, no ruling is being contravened nor is any further ruling required.

Furthermore, the transfer (back) to *Lambornella* from *Tetrahymena* of the species *L. stegomyiae* does not affect the existence nor the status of the type-specimen material (USNM No. 24117) housed in the International Collection of Ciliate Type-Specimens in the Smithsonian Institution (see Corliss, 1971a, 1972a). For the new, second species in the genus, we are depositing another slide, USNM No. 24490, as mentioned in a preceding section of this paper.

DISCUSSION

There is no doubt but that both the most outstanding and the most significant unique characteristic of species of the resurrected genus *Lambornella* is their cuticular cyst from which the organism, if successful, invades the haemocoel of its larval culicine host. Careful confirmation of Muspratt's (1945) early hypothesis that this was the case—by Clark & Brandl (1976)—allows us to fully accept this important and unusual ability among ciliates and to reflect it in the taxonomic placement of the organisms possessing it. When such additional features as manifestation of multiple (more than two) postoral kineties and predilection for certain culicine mosquitoes as host are considered, the need to (re)separate the genus from the much better-known *Tetrahymena*—some of the species of which also associate facultatively with mosquitoes (see Table I)—seems to us fully justified.

Because the Keilin-Clark organisms have both been confused with or identified as *Tetrahymena pyriformis*, even as recently as four years ago (Sanders,

⁶ The material kindly sent some years ago by J. A. Reid to the senior author (see Corliss, 1960, 1961b), from the bamboo-breeding *Armigeres dolichocephalus* from Kuala Lumpur (see Table I), still resists certain taxonomic identification: it is possible that it represents a species of an undescribed genus, although Corliss has tentatively decided to call it *T. sp.*

1972), thus handicapping the further investigation so badly needed on their full life cycles, physiology of cuticle-dissolution, etc., it is worthwhile to review the evidence to date on all ciliate-mosquito interrelationships (see Table I), stressing particularly infectivity, pathogenicity, and mode of entry into the host.

Infectivity and Host Resistance

Exact information on extent of infectivity is sparse in the older literature: Keilin (1921) reported five of eight larvae of *Aedes* (*Stegomyia*) *scutellaris* [= *A. (S.) albopictus*?], from Lamborn's (1921) material, as having ciliates in the body cavity and anal gills. Muspratt (1945, 1947) found "many" infected larvae in nature and in laboratory experimentation, particularly with *Aedes* (*Stegomyia*) *aegypti*, but offered no data on negative cases. Out of several hundred fourth instar larvae of *Aedes* (*Ochlerotatus*) *sierrensis*, Kellen et al. (1961) found only one larva with ciliates in the haemocoel. In the extensive collections from 18 treeholes made by Sanders (1972), ciliates were found in the same mosquitoes from only one treehole, but in 77 of the 320 larvae examined from that habitat.

Clark & Brandl (1976) do not give figures on percentage of infected larvae in their collections of *Aedes sierrensis* from oak treeholes. But among the third and fourth instar larvae which comprised one collection half of the larvae which bore "black spots" (melanized areas) had populations of living ciliates in their haemocoels. Later, in laboratory experimentation, the investigators seem to have obtained high rates of infectivity, but no exact data are given on this subject. The relationship of the host's reaction via melanization of the invading ciliate and the observations that the thickened cuticle of later larval instar stages affects (i.e., reduces) penetration were not pursued in detail. Such factors in host resistance deserve future in-depth investigation.

The recent laboratory-controlled observations of Grassmick & Rowley (1973) are very interesting: they used larvae of *Culex* (*Culex*) *tarsalis* and *Aedes* (*Stegomyia*) *aegypti* with a cultured "sexual" (micronucleate) strain of *Tetrahymena pyriformis* and found, briefly, that infection and larval mortality were far higher in the *Culex* than in the *Aedes* host. But further discussion of the important and sophisticated work of this team of investigators is mostly beyond the scope of the present paper, since their study employed *only* laboratory-reared organisms (of both host and endoparasite) and the ciliate involved is a true *T. pyriformis*, not a species of *Lambornella*. Grassmick and Rowley have set an example, however, for the kind of research which ought, now, to be carried out also with members of the genus (re)described here by us.

Degree of Pathogenicity

As in the case of extent of infectivity, discussed briefly above, precise data are generally not available for the pathogenic effect of ciliate "attacks" on mosquito larvae. "Infection fatal" is the usual conclusion drawn for the instances in which the body cavity of a larval mosquito is found filled with thousands of "endoparasitic" ciliates; but often the exact fate of such a host (in nature, especially) is *not* known. And the scattered reports in the literature (e.g., Corliss, 1961b; Grassé & de Boissezon, 1929) of the appearance of ciliates in *adult* mosquitoes raises the unanswered question of how they got there.

Mode of Entry into Host

Corliss (1960) discussed four possible routes of entry—through the mouth, through the cuticle, through artificial breaks or wounds in the body wall, and through "natural breaks or weaknesses" during molting—but careful experimental

evidence has seldom been available in past years in support of any of these modes with respect to implicated species of the family Tetrahymenidae. Observations, though sometimes only inferential in nature, have suggested entrance through the mouth for *Tetrahymena limacis* in slugs (see Brooks, 1968), and through abrasions of the integument for *T. chironomi* in midge larvae (see Corliss, 1960) and for at least some strains of *T. corlissi* in guppies and various vertebrate larvae (see Corliss, 1973a; Hoffman et al., 1975). Brooks (1968) has demonstrated beautifully, for *T. rostrata*, that entrance into its slug host can be directly through a little-known dorsal integumentary pouch.

For *Lambornella*, Muspratt (1945, 1947) surmised and Clark & Brandl (1976) have definitely proven that the cuticular cyst is an "invasion" site for entrance of the ciliate into the haemocoel by means of dissolution of the cuticle, provided that host mechanisms of some sort do not result in melanization of the invader before penetration of the underlying epidermis has been effected. But many details of the process still need to be worked out under controlled laboratory conditions. It is interesting to note that Keilin (1921) observed cysts on only one of the eight larvae at his disposal.

Keilin (1921) and many others, incidentally, influenced by the fact that so many "contamination-type" parasites—bacterial, fungal, protozoan, and metazoan—enter their hapless hosts *per os* (through the mouth), have jumped to the conclusion that tetrahymenine ciliates must use the same place and mode of entry. It is thus worthy of remention (see above) that apparently only *Tetrahymena limacis*, among the kinds of ciliates under discussion here, successfully employs that route into the host's body. Furthermore, both Clark & Brandl (1976) and Grassmick & Rowley (1973) have pointed out that, in cultures of the two organisms, ciliates are deliberately taken in through the mouth of the larval mosquito to be digested as food.

Facultative or Obligate Parasitism?

Are the *Lambornella* species associated with larval culicine mosquitoes to be considered as facultative or obligate endoparasites? Without "proof" either way, most investigators have suggested "facultative," based on the apparent ability of the ciliates to thrive free of their hosts in nature as well as in the laboratory. Clark & Brandl (1976) support such a view, pointing out that their ciliate (now *L. clarki* n. sp.) would undoubtedly not survive for long if its relationship with *Aedes sierrensis* were obligatory, since it must avoid being eaten, must find young instars of the host, must attach successfully at "safe" sites on the cuticle, must not get trapped between cuticle and epidermis, and, finally, must escape from the carcass of the host it has killed.

Corliss (1972b, 1973a), however, has listed his "*Tetrahymena stegomyiae*," along with *T. limacis*, as obligately parasitic forms—in contrast to his three other categories of facultatively free-living (with *T. chironomi* and *T. corlissi*), facultatively parasitic (*T. pyriformis* and *T. rostrata*), and obligately free-living forms (*T. setifera* and the interrelated *T. patula*, *T. vorax*, *T. paravorax*)—tentatively concluding that these two species are found in nature as endoparasites associated with specific hosts, even though they can be cultured free of the host; irrelevant are the facts that *T. limacis* is only mildly pathogenic and *T. (= Lambornella) stegomyiae* apparently causes a fatal infection.

Much more work needs to be done to settle the point.

Other Symbiotic Tetrahymenine Ciliates

In preceding sections, comparisons have been made between the situation obtaining for *Lambornella* species and other ciliates, with the emphasis on mos-

quito associations (and see particularly Table I). Comprehensive reviews are available in Corliss (1960, 1961a,b, 1965, 1972b, 1973a) and Czapik (1968); and Grassmick & Rowley (1973) present an extremely brief but valuable historical note on the subject.

Within the suborder Tetrahymenina, except for the family Curimostomatidae (rather rare, small, mouthless forms all obligate endosymbionts in snails, limpets, clams, and turbellarians), the only named genus (other than *Lambornella*) with species that exhibit some degree or other of the "parasitic habit" is *Tetrahymena*, (also) of the family Tetrahymenidae. No members of the third bonafide family comprising the suborder, the recently erected Glaucomidae Corliss, 1971, live symbiotic lives to any significant degree (Corliss, 1971b).

Infectivity rates, degrees of pathogenicity, and modes of entry—not to mention taxonomic review—of the "nonmosquito" symbiotic tetrahymenid species are treated, though seldom in depth, in such works as Barthelmes (1960), Brooks (1968), Corliss (1960, 1972b, 1973a), Hoffman et al. (1975), Kozloff (1956), Lynn (1975), Michelson (1971), Seaman et al. (1972), Stout (1954), and Thompson (1958), all important from a broad comparative point of view.

Ciliate-mosquito relationships are exclusively summarized in Table I.

LITERATURE CITED

- BARTHELMES, D. 1960. *Tetrahymena parasitica* (Penard 1922) Corliss 1952 als Parasit in Larven vom *Chironomus plumosus*-Typ. *Z. Fisch. Hilfswiss.*, 9: 273-280.
- BOISSEZON, P. DE. 1930. Contribution à l'étude de la biologie et de l'histophysiologie de *Culex pipiens* L. *Arch. Zool. Exp. Gén.*, 70: 281-431.
- BRADBURY, P. C., CLAMP, J. C. & LYON, J. T., III. 1974. *Terebrospira chattoni* sp. n., a parasite of the endocuticle of the shrimp *Palaemonetes pugio* Holthuis. *J. Protozool.*, 21: 678-686.
- BROOKS, W. M. 1968. Tetrahymenid ciliates as parasites of the gray garden slug. *Hilgardia*, 39: 205-276.
- CLARK, T. B. & BRANDL, D. G. 1976. Observations on the infection of *Aedes sierrensis* by a tetrahymenine ciliate. *J. Invert. Path.*, 23: 341-349.
- CORLISS, J. O. 1953. Silver impregnation of ciliated protozoa by the Chatton-Lwoff technic. *Stain Tech.*, 28: 97-100.
1954. The literature on *Tetrahymena*: its history, growth, and recent trends. *J. Protozool.*, 1: 156-169.
1960. *Tetrahymena chironomi* sp. nov., a ciliate from midge larvae, and the current status of facultative parasitism in the genus *Tetrahymena*. *Parasitology*, 50: 111-153.
- 1961a. *The Ciliated Protozoa: Characterization, Classification, and Guide to the Literature*. Pergamon Press, London and New York. 310 pp.
- 1961b. Natural infection of tropical mosquitoes by ciliated protozoa of the genus *Tetrahymena*. *Trans. Roy. Soc. Trop. Med. Hyg.*, 55: 149-152.
1965. *Tetrahymena*, a ciliate genus of unusual importance in modern biological research. *Acta Protozool.*, 3: 1-20.
1970. The comparative systematics of species comprising the hymenostome ciliate genus *Tetrahymena*. *J. Protozool.*, 17: 198-209.
- 1971a. Type-specimens for the ten species of *Tetrahymena* (Protozoa: Ciliophora). *Trans. Amer. Micros. Soc.*, 90: 243-252.
- 1971b. Establishment of a new family (Glaucomidae n. fam.) in the holotrich hymenostome ciliate suborder Tetrahymenina, and description of a new genus (*Epenardia* n. g.) and a new species (*Glaucoma dragescui* n. sp.) contained therein. *Trans. Amer. Micros. Soc.*, 90: 344-362.
- 1972a. Current status of the International Collection of Ciliate Type-Specimens and guidelines for future contributors. *Trans. Amer. Micros. Soc.*, 91: 221-235.

- 1972b. *Tetrahymena* and some thoughts on the evolutionary origin of endoparasitism. *Trans. Amer. Micros. Soc.*, 91: 566-573.
- 1973a. History, taxonomy, ecology, and evolution of species of *Tetrahymena*. In Elliott, A. M., ed., *Biology of Tetrahymena*, Dowden, Hutchinson & Ross, Stroudsburg, Pa., pp. 1-55.
- 1973b. Evolutionary trends in patterns of stomatogenesis in the ciliate Protozoa. (Abstr.) *J. Protozool.*, 20: 506.
- CORLISS, J. O. & DOUGHERTY, E. C. 1967. An appeal for stabilization of certain names in the protozoan family Tetrahymenidae (subphylum Ciliophora, order Hymenostomatida), with special reference to the generic name *Tetrahymena* Furgason, 1940. *Bull. Zool. Nomencl.*, 24: 155-185.
- CORLISS, J. O. & ESSER, S. C. 1974. Comments on the role of the cyst in the life cycle and survival of free-living protozoa. *Trans. Amer. Micros. Soc.*, 93: 578-593.
- CZAPIK, A. 1968. La famille Tetrahymenidae et son importance dans la systématique et l'évolution des ciliés. *Acta Protozool.*, 5: 315-358.
- DZERZHINSKY, V. A., NAM, E. A. & DUBITSKY, A. M. 1976. The finding of *Lankesteria culicis* and *Tetrahymena stegomyiae* in larvae of *Aedes aegypti*. *Parazitologia*, 10: 381-382. (in Russian with English summary)
- GRASSÉ, P.-P. & BOISSEZON, P. DE 1929. *Turchiniella culicis* n. g., n. sp. infusoire parasite de l'hémocoèle d'un *Culex* adulte. *Bull. Soc. Zool. Fr.*, 54: 187-191.
- GRASSMICK, R. A. & ROWLEY, W. A. 1973. Larval mortality of *Culex tarsalis* and *Aedes aegypti* when reared with different concentrations of *Tetrahymena pyriformis*. *J. Invert. Path.*, 22: 86-93.
- HOFFMAN, G. L., LANDOLT, M., CAMPER, J. E., COATS, D. W., STOOKEY, J. L. & BUREK, J. D. 1975. A disease of freshwater fishes caused by *Tetrahymena corlissi* Thompson, 1955, and a key for identification of holotrich ciliates of freshwater fishes. *J. Parasit.*, 61: 217-223.
- INTERNATIONAL COMMISSION ON ZOOLOGICAL NOMENCLATURE. 1970. [Opinion 915] *Tetrahymena* Furgason, 1940 (Ciliophora, Hymenostomatida): preserved under the plenary powers and related matters. *Bull. Zool. Nomencl.*, 27: 33-38.
- KEILIN, D. 1921. On a new ciliate: *Lambornella stegomyiae* n. g., n. sp., parasitic in the body-cavity of the larvae of *Stegomyia scutellaris* Walker (Diptera, Nematocera, Culicidae). *Parasitology*, 13: 216-224.
- KELLEN, W. R., WILLS, W. & LINDEGREN, J. E. 1961. Ciliatosis in *Aedes sierrensis* (Ludlow). *J. Insect Path.*, 3: 335-338.
- KIRBY, H. 1941. Relationships between certain protozoa and other animals. In Calkins, G. N. & Summers, F. M., eds., *Protozoa in Biological Research*, Columbia University Press, New York, pp. 890-1008.
- KOZLOFF, E. N. 1956. Experimental infection of the gray garden slug, *Deroceras reticulatum* (Müller), by the holotrichous ciliate *Tetrahymena pyriformis* (Ehrenberg). *J. Protozool.*, 3: 17-19.
- LAIRD, M. 1959. Parasites of Singapore mosquitoes, with particular reference to the significance of larval epibionts as an index of habitat pollution. *Ecology*, 40: 206-221.
- LAMBORN, W. A. 1921. A protozoon pathogenic to mosquito larvae. *Parasitology*, 13: 213-215.
- LIPA, J. J. 1963. Infections caused by Protozoa other than Sporozoa. In Steinhaus, E. A., ed., *Insect Pathology*, Vol. 2, Academic Press, New York and London, pp. 335-361.
- LYNN, D. L. 1975. The life cycle of the histophagous ciliate *Tetrahymena corlissi* Thompson, 1955. *J. Protozool.*, 22: 188-195.
- MACARTHUR, W. P. 1922. A holotrichous ciliate pathogenic to *Theobaldia annulata* Schrank. *J. R. Army Med. Cps.*, 38: 83-92.
- MCCLAUGHLIN, R. E. 1971. Use of protozoans for microbial control of insects. In Burges, H. D. & Hussey, N. W., eds., *Microbial Control of Insects and Mites*, Academic Press, New York and London, pp. 151-172.

- MICHELSON, E. H. 1971. Distribution and pathogenicity of *Tetrahymena limacis* in the slug *Deroceras reticulatum*. *Parasitology*, 62: 125–131.
- MUSPRATT, J. 1945. Observations on the larvae of tree-hole breeding Culicini (Diptera: Culicidae) and two of their parasites. *J. Ent. Soc. S. Afr.*, 8: 13–20.
1947. Notes on a ciliate protozoan, probably *Glaucoma pyriformis*, parasitic in culicine mosquito larvae. *Parasitology*, 38: 107–110.
- ROQUE, M., PUYTORAC, P. DE & SAVOIE, A. 1971. Caractéristiques morphologiques et biologiques de *Tetrahymena bergeri* sp. nov., cilié hyménostome tétrahyménien. *Protistologica*, 6(year 1970): 343–351.
- SANDERS, R. D. 1972. Microbial mortality factors in *Aedes sierrensis* populations. *Proc. Calif. Mosquito Control Assoc.*, 40: 66–68.
- SEAMAN, G. R., TOSNEY, T., BERGLUND, R. & GOLDBERG, G. 1972. Infectivity and recovery of *Tetrahymena pyriformis* strain S from adult female cockroaches (*Periplaneta americana*). *J. Protozool.*, 19: 644–647.
- STEINHAUS, E. A. 1947. *Insect Microbiology*. Comstock, Ithaca. 763 pp.
- STOUT, J. D. 1954. The ecology, life history and parasitism of *Tetrahymena* [*Paraglaucoma*] *rostrata* (Kahl) Corliss. *J. Protozool.*, 1: 211–215.
- THOMPSON, J. C., JR. 1958. Experimental infections of various animals with strains of the genus *Tetrahymena*. *J. Protozool.*, 5: 203–205.
- WENYON, C. M. 1926. *Protozoology—A Manual for Medical Men, Veterinarians and Zoologists*. Vols. 1 and 2. Baillière, Tindall & Cox, London. 1563 pp. (reprinted in 1965 by Hafner Press)

A PROPOSED SUBPHYLETIC DIVISION OF THE PHYLUM CILIOPHORA DOFLEIN, 1901¹

EUGENE B. SMALL

Department of Zoology, University of Maryland,
College Park, Maryland 20742

SMALL, E. B. 1976. A proposed subphyletic division of the phylum Ciliophora Doflein, 1901. *Trans. Amer. Micros. Soc.*, 95: 739–751. A proposal is made for separation of the Ciliophora into two new subphyla, the Rhabdophora n. subphylum and the Cyrtophora n. subphylum, based on the constancy of two major criteria, one morphological and the other morphogenetic. Both criteria are related to the cytostome-cytopharyngeal complex of ciliates. Comparisons are made with recently published revisions of the classification of the phylum to illustrate how the new subphyla would affect the systematic ordering of included higher taxa. Due to our disturbingly sparse critical comparative ultrastructural knowledge of the suctoria, their systematic status is the least certain, whereas other major groups appear to be placed relatively easily within the new subphyla proposed in the present work.

The past few years have seen the appearance of several revisions of the systematic organization of the ciliated protozoa, especially at the level of the suprafamilial groups. Corliss' (1961) first edition of *The Ciliated Protozoa* has been followed more recently by two long papers (1974a, 1975) which signal significant changes in his views since 1961 on both the number and characteriza-

¹The author wishes to express his thanks to the ciliatologists with whom over the years he has discussed the ideas embodied in this paper. Particularly to be mentioned are Denis Lynn, Gavin P. Riordan, the author's graduate students, and Maryland colleague J. O. Corliss.