Original Article

OBSERVATIONS ON THE MORPHOLOGY AND LIFE CYCLE OF *Lambornella stegomyiae* (CILIOPHORA: TETRAHYMENIDAE)

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ABSTRACT

The morphology and life-cycle of Lambornella stegomyiae, a facultative parasitic ciliate of larval Aedes albopictus, were described. Parasitic and free-living ciliates multiply by transverse binary fission. Free-living ciliates occasionally undergo conjugation. Infection in larval mosquitoes may be transtadially transmitted to adult stages when larval hosts do not succumb to the infection. Infected adults are fertile. Besides fertile eggs, female mosquitoes deposit ciliates and infected eggs during oviposition. Results suggest that L. stegomyiae may survive through droughts in infected Ae. albopictus eggs and as desiccation resistant cysts.

Keywords: *Lambornella stegomyiae*; morphology; life-cycle; *Aedes albopictus*

INTRODUCTION

Lambornella stegomyiae is a facultative parasitic ciliate of tree -hole and container-breeding mosquitoes. The ciliate was first reported from *Aedes scutellaris* larvae collected in Kuala Lumpur, Malaysia by Lamborn in 1921 and was described by Keilin (1921) [1,2]. It was subsequently reported as *Tetrahymena pyriformis* from Singapore [3], as *T. stegomyiae* from the Philippines [4], as *Glaucoma pyriformis* from North Rhodesia [5,6], as *T. stegomyiae* from South Africa [7], and as *T. stegomyiae* from the Soviet Union [8-10].

The latest description of *L. stegomyiae* was based mainly on specimens collected from South Africa [11]. There is no detail description on *L. stegomyiae* from other parts of the world. The present study provides additional information on the life-cycle of the ciliate collected in Penang, Malaysia, and the morphology of some stages in its development.

MATERIALS AND METHODS

L. stegomyiae used in the present experiment was collected from artificial containers in Penang. Infected *Aedes albopictus* larvae were brought back to the laboratory and prepared for *in vivo* cultures. A laboratory strain of *Ae. albopictus* was used as host to *L. stegomyiae* throughout the experiment. The mosquito was collected in Penang in 1985 and has since been maintained in the laboratory at Universiti Sains Malaysia at 27^{o}C and 80% relative humidity.

In vivo cultures of *L. stegomyiae* were set up in Petri dishes (2 x 9 cm) in the laboratory. A fourth instar mosquito larva infected with ciliates was placed inside a Petri dish with 40 ml of culture medium. This medium was prepared by mixing equal amounts of boiled field water and deionized water. Tetramin^{R} fishfood was used as food for the larva and was added when necessary. Usually the ciliates multiply in the hemocoel of the larva until they fill the whole hemocoel. At the end of their development in the larval hemocoel, the ciliates break out from the mosquito larva. The ciliates are then ready for reinfection. At that stage, the larva was teased to free the rest of the ciliates from the hemocoel and 10 newly hatched first stage mosquito larvae were added into the Petri dish. No food was administered during the first 24 hr after the larvae were exposed to the ciliates. Lack of food retards growth and delays molting of the first instar larvae and thus increases the chance of infection. Stages of development of ciliates were observed fresh in live hosts or fixed in 70% alcohol under a compound microscope (100-1000x).

The silver carbonate method was used to stain the infraciliature and the nuclei of the ciliates [12]. Ciliates that break out from their hosts after completing their parasitic

cycle were first transferred into transparent screw-capped plastic bowls (9 x 9 cm) containing 200 ml culture medium and allowed to live as free living ciliates for 48 hr. The ciliates were then picked, stained and observed under a compound microscope (100-1000x).

Infected mosquito larvae that did not succumb to the infection and pupated were transferred into a bowl in a mosquito cage (30 x 30 x 30 cm) and allowed to emerge. Sugar solution was introduced into the cage as food for the adult mosquitoes. The bowl was removed once all the pupae had emerged. Adult mosquitoes were then blood-fed on white mice. A Petri dish containing 40 ml culture medium was introduced for oviposition. The Petri dish was examined daily for ciliates under a compound microscope (100 - 1000x).

RESULTS

Life-cycle and sexuality

The development of *L. stegomyiae* was observed in 30 *Ae. albopictus* larvae in 3 Petri dishes, each containing 10 larvae. Observations were made at 4 hr intervals. Formation of cuticular cysts was first observed 4 hr after the larvae were exposed to ciliates (Fig.1). The largest number of cuticular cysts was observed at 12 hr after exposure. The number of free living ciliates in the Petri dishes decreased with time and can hardly be found at 16 hr. Once attached to the cuticle, the ciliates within the cuticular cysts went on to penetrate into the hemocoel of the mosquito larvae. The first ciliate was observed in the hemocoel 12 hr after exposure. By 16 hr most ciliates had penetrated into the hemocoel and they were seen as small rounded forms slowly rotating in about the same spot. The empty cysts were left attached to the outside of the larval cuticle. Between 16 to 92 hr after exposure, the ciliates transformed into large, sluggish but actively dividing ameboid forms. Multiplication was by binary fission. From 88 hr, the ameboid forms began to retransform back into rounded forms. The locomotion of these rounded forms was more active than the ameboid forms. Multiplication was sustained at a rapid rate and by 188 hr, the ciliates filled the entire hemocoel of the host. At 136 hr, the ciliates began to transform into swift swimming pear-shaped ciliates. At 212 hr, the pear-shaped ciliates were seen escaping from dead mosquito larvae into the surrounding medium. Ciliates were also seen escaping from live mosquito larvae. Some punctured larvae lived for several hours before dying.

Ciliates that broke out from the mosquito larvae continued to multiply asexually and sexually in the culture medium. Asexual reproduction was by transverse binary fission with the division plane cutting across the kineties (Fig.3). The fission began with the division of the micronucleus followed by the division of the macronucleus. The division was accomplished by a transverse constriction. Sexual reproduction was achieved through conjugation (Fig. 4). Pairs of ciliates were seen to remain fused anterior-laterally for hours.

***Morphology of ciliates* (n=25)**

The general shape of the free living ciliates from *in vivo* cultures (fixed in 70% alcohol) was pear-shaped (Fig. 5). The buccal overture was round or oval and located ventrally in the anterior fourth of the body. The preoral suture was located to the left of the buccal overture. Mean body length 70.3 µm (range 48.6-89.5); mean body width 50.5 µm (range 29.5-65.7); macronucleus and micronucleus single; mean width of macronucleus 25.6 µ m (range 18.1-34.3); mean width of micronucleus 4.7 µm (range 3.8-6.7); median number of kineties 38 (range 31-43); median number of postoral meridians 4 (range 2-5).

Infection in adult mosquitoes

Six adult male and 6 adult female mosquitoes emerged from pupae that did not succumb to the infection. The

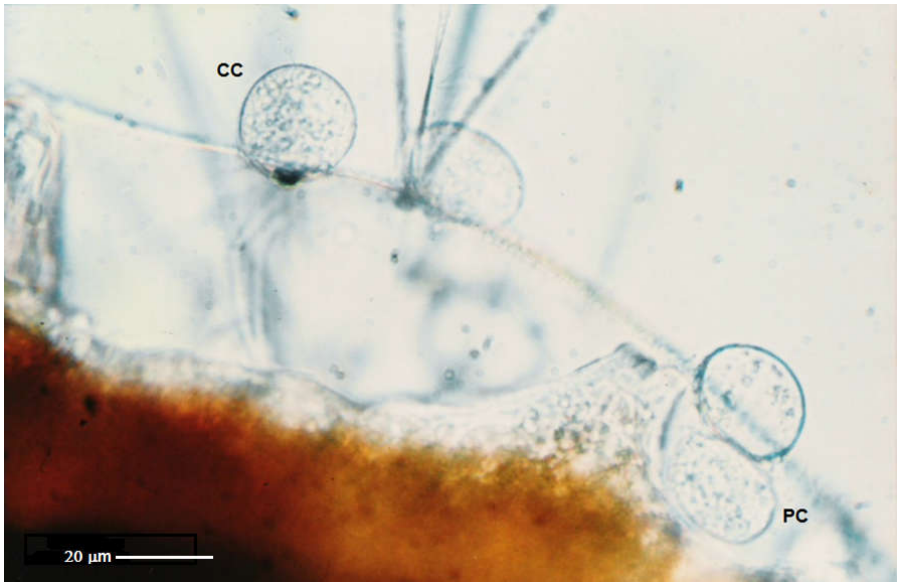

Figure 1: Cuticular cysts (CC) of *Lambornella stegomyiae* on cuticle of first-instar larva of *Aedes albopictus*. Note the newly penetrated ciliate (PC).

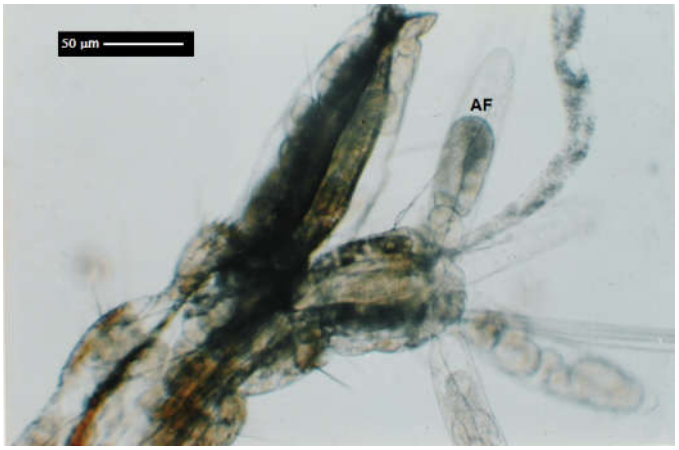

Figure 2: Ameboid forms (AF) of *Lambornella stegomyiae* in the anal papilla of *Aedes albopictus* larva.

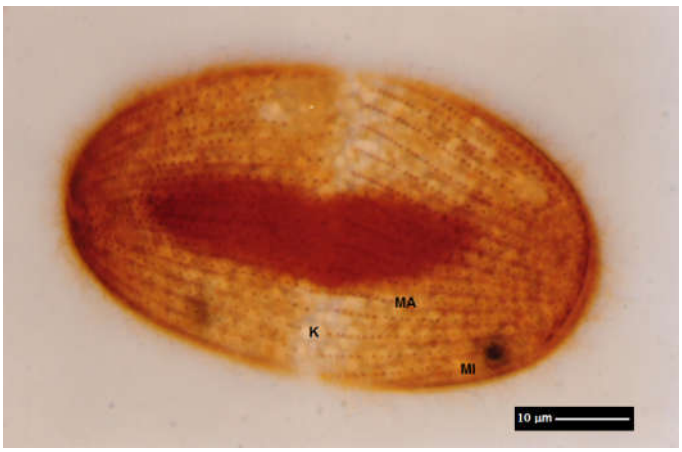

Figure 3: Silver carbonate preparation of a *Lambornella stegomyiae* trophont undergoing binary fission. Fission began with the division of the micronucleus (MI) and followed by the macronucleus (MA). The fission plane cuts transversely across the kineties (K).

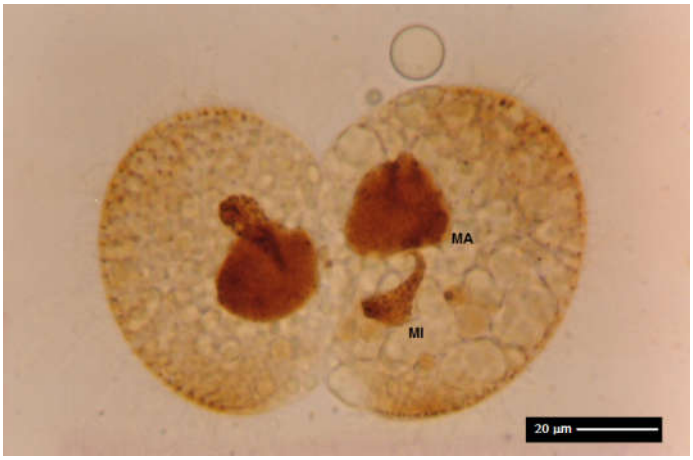

Figure 4: Silver carbonate preparation of a pair of conjugating *Lambornella stegomyiae*. Note the macronuclei (MA) and the dividing micronuclei (MI).

Figure 5: Silver carbonate preparation of *Lambornella stegomyiae* trophont showing the undulating membrane (UM), the tripartite adoral zone of membranelles (AZM), preoral suture (PS), macronucleus (MA) and micronucleus (MI).

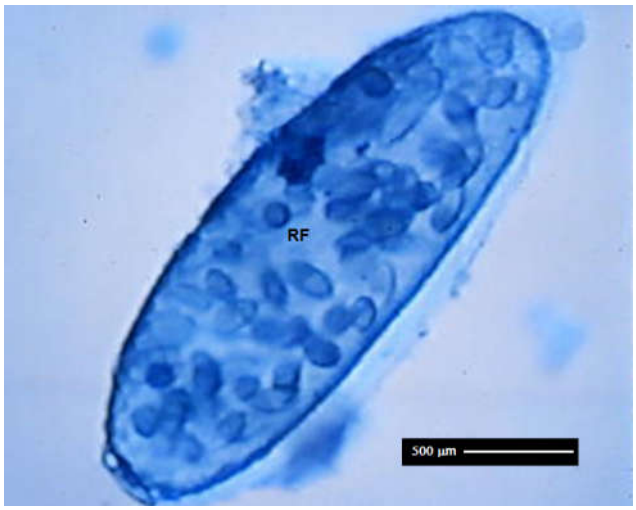

Figure 6: Rounded forms (RF) of *Lambornella stegomyiae* in an *Aedes albopictus* egg.

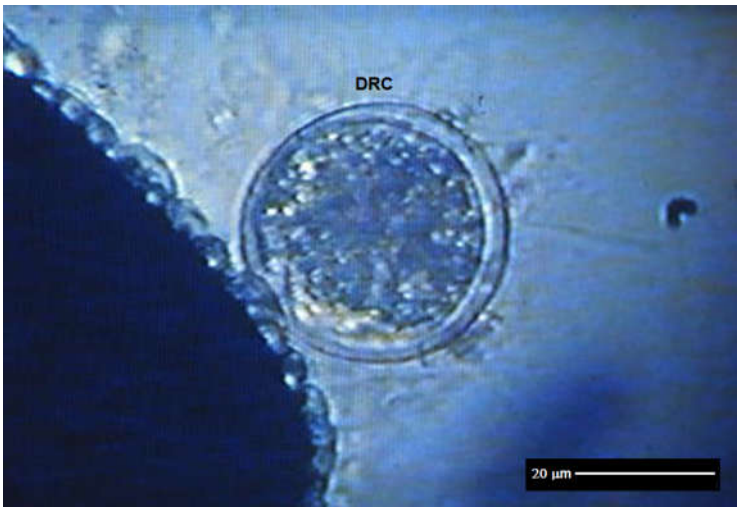

Figure 7: Desiccation resistant cyst (DRC) of *Lambornella stegomyiae* next to an *Aedes albopictus* egg.

infected adult female mosquitoes laid fertile eggs in the Petri dish placed in the mosquito cage. When the eggs were dried and hatched, the larvae were free from ciliates. Two eggs were infected with ciliates (Fig. 6). Both were almost completely filled with rounded and pear-shaped ciliates. No cuticular cysts were found on the egg shells. The eggs were ruptured for closer examination of the ciliates. Some uninfected eggs in the Petri dish had cuticular cysts. However, none of the ciliates from the cysts penetrated into the eggs. When the eggs hatched, the ciliates remained in the cysts on the egg shells. Examination of the culture medium in the Petri dish showed the presence of free living ciliates. Infection was attained when first stage mosquito larvae were exposed to the ciliates. Two desiccation resistant cysts were found at the bottom of the Petri dish (Fig. 7). The cysts were spherical with a diameter of 40 μm. The single ciliate in the cyst could be seen sluggishly rotating within the transparent cyst wall.

DISCUSSION

Three distinct forms of *L. stegomyiae viz*., spherical, ameboid and pear-shaped have been observed during the development of the ciliate in the hemocoel of larval *Ae. albopictus*. Different forms of *L. stegomyiae* in larval mosquitoes had earlier been reported by Muspratt (1945). He observed two types of ciliates - a rapidly multiplying form and a larger slowly multiplying form in *Aedes* and *Culex* mosquitoes in Livingstone, Northern Rhodesia. He later concluded that the 2 types of ciliates were actually different forms of the same ciliate *Glaucoma pyriformis* (probably *L. stegomyiae*) but in mosquito hosts of differing susceptibility [6]. While different forms may be attributable to differing host susceptibility, we found that morphological transformations always occur during the development of the ciliate in its natural host.

L. stegomyiae multiplies by binary fission during its parasitic and free living existence. Conjugations were observed only among free living ciliates. As conjugations were rare and occurred with low frequency, only a few pairs of conjugants were observed and only one pair was successfully stained. The fate of the exconjugants were not known. All conjugating pairs were seen attached anterior-laterally (the anterior ends of the ciliates were more strongly tapered than the posterior). There is reason to suppose that the ciliates adhere in a typical ciliate fashion at the oral region.

Lamborn (1921) described the shape of *L. stegomyiae* from Kuala Lumpur as pear-shaped [1]. Keilin (1921) examined Lamborn's collection preserved in formalin and described the shape of the ciliates and as generally elongately oval but occasionally pear-shaped [2]. Corliss and Coats (1976) examined preserved samples of *L. stegomyiae* from South Africa and Kuala Lumpur and reported that the ciliates were somewhat spindle-shaped with mean body measurements of 78 x 22 μm [11]. The shape of *L. stegomyiae* within any single sample may vary from pear-shaped to spindle-shaped. We observed some variation in the shape of the ciliates in our samples but they were generally pear-shaped. It is possible that Corliss and Coats based their description mainly on *L. stegomyiae* from South Africa which might have been spindle-shaped. Furthermore, *L. stegomyiae* described by Corliss and Coats had fewer number of kineties (30) than those described in the present study (38) although they are of about the same length.

Although the oviposition behavior of the infected adult mosquitoes was not followed, there is reason to believe that ciliates were deposited by adult female mosquitoes during oviposition. Egerter *et al.* (1986) reported that females of *Ae. sierrensis* infected with *L. clarki* were parasitically castrated [13]. The females exhibited oviposition behavior by which ciliates were actively dispersed. However, *Ae. albopictus* females infected with *L. stegomyiae* in the present study were not castrated and were able to lay fertile eggs.

Ciliates survive occasional drought seasons in desiccation resistant cysts and in mosquito eggs. The eggs must have been infected while they were inside the mosquito and before the egg shells were deposited as no cuticular cysts were found on them. Once deposited, *Ae. albopictus* eggs were impenetrable to the ciliates. Surviving in mosquito eggs has advantages over surviving in desiccation resistant cysts. The ciliates multiply in mosquito eggs like they normally do in larval mosquitoes. The infected eggs hatch simultaneously with embryonated eggs and immediately infect the first stage mosquito larvae. Infection and multiplication in mosquito eggs is important for the survival of the ciliates. Tropical mosquitoes like *Ae. albopictus* develop rapidly during their larval stages. The larval instars may only take about a week. Thus parasitic ciliates like *L. stegomyiae* must find a mechanism by which they can quickly infect the mosquito larvae so that they can complete their life-cycle before the larvae pupate.

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