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Herpetomonas megaseliae sp. n. (Protozoa: Trypanosomatidae) from *Megaselia scalaris* (Loew, 1866) Schmitz, 1929 (Diptera: Phoridae)

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HERPETOMONAS MEGASELIAE SP. N. (PROTOZOA: TRYPANOSOMATIDAE) FROM *MEGASELIA SCALARIS* (LOEW, 1866) SCHMITZ, 1929 (DIPTERA: PHORIDAE)*

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ABSTRACT: A new intestinal trypanosomatid was found parasitizing *Megaselia scalaris*. Forms with posterior kinetoplasts occurred in both natural infections and cloned cultures, but not in experimental infections. Average size and morphological variation were reduced in experimental infections with cloned stocks, compared to natural infections. A growth comparison was made between *Herpetomonas muscarum* and *H. megaseliae*. Differences in day of peak population, maximum numbers, and total time of survival were observed in culture. Attempts to infect *Drosophila melanogaster* with *H. megaseliae* were unsuccessful.

The primary characteristic used to separate *Herpetomonas* from other genera of insect trypanosomatids is a posterior kinetoplast in up to 40% of culture forms (Rogers and Wallace, 1971). An intestinal trypanosomatid which exhibited a high percentage of posterior kinetoplasts in culture was discovered in *Megaselia scalaris* (Loew, 1866) Schmitz, 1929 (see Borgmeier, 1965, for synonyms). The organism was isolated and a study was made of its morphology in culture and in the insect host. In addition, a comparison with *Herpetomonas muscarum* was made of growth in culture. Morphological and host differences between the newly isolated trypanosomatid and published descriptions indicated that the parasite was an undescribed species of the genus *Herpetomonas*. The isolate was cloned and the description below is based on cloned material in culture and in experimental infections.

MATERIALS AND METHODS

Four culture media were used in the study: (1) Mansour's Medium (Dollahon and Janovy, 1971); (2) 1% proteose–peptone–glucose (PPG); (3) Wallace's Medium (Wallace and Clark, 1959); and (4) A medium consisting of 5 ml Locke's Solution (Tobie et al., 1950) into which one guinea pig fecal pellet was placed (LPG). The LPG was then autoclaved.

The parasites were isolated from the larvae of *M. scalaris* into Mansour's Medium with 700 mg dihydrostreptomycin and 700 units procaine penicillin-G (Penstrep) per ml. The isolate was cloned 4 times, twice by serial dilution and twice by isolation of a single organism with a capillary pipet. Each clone was made from a stock which had been previously cloned. Our present stock has thus been successively cloned 4 times. A combination of 4,000 units of procaine penicillin-G and 5 mg dihydrostreptomycin (Penstrep) per ml was used in the first transfer after the fourth cloning. A week-old culture in Wallace's Medium, a descendant of the final clone, was used to study the morphology and to describe the trypanosomatid in culture. Cultures were maintained at 25 C.

H. muscarum, isolated from *Phaenicia sericata*, was obtained from Dr. F. G. Wallace, University of Minnesota. It was received in Wallace's Medium and subsequently maintained in our laboratory in Mansour's or Wallace's Medium.

For growth studies, 20 ml of LPG, PPG, or Mansour's Medium was added aseptically to sidearm flasks. A flask of each medium was inoculated with 0.25 ml of a *H. muscarum* culture of the new trypanosomatid. Hemocytometer counts were made on the 3rd, 5th, 7th, and 10th days after inoculation. Zero time populations were calculated from hemocytometer counts of the inoculum.

Colonies of uninfected host flies, *M. scalaris* (identification confirmed by Dr. William Robinson, Virginia Polytechnic Institute), were initiated with eggs gathered from guinea pig litter pans. Eggs were washed once in a 1:10,000 zephiran–chloride solution and once in distilled water. Washed eggs were placed in 8-oz cotton-gauze-stoppered French-quartered bottles containing 50 ml of a *Drosophila* medium (Strickburger, 1967) which had been modified by the substitution of 250 ml of autoclaved, ground Purina Guinea Pig Chow for 38 ml dried yeast. Uninfected fly colonies were periodically checked for infection. Colonies of infected flies were initiated with larvae from guinea pig litter pans. *Drosophila melanogaster*, Oregon-R strain, were obtained from Dr. D. D. Miller, University of Nebraska, and were maintained in the same fly medium. The *Drosophila* were not found to be naturally infected with trypanosomatid parasites.

Insects were infected with culture forms con-

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centrated by centrifugation. Routinely, 2 culture tubes each containing large populations of late log phase organisms (1 week after loop transfer) in 5 ml Mansour's Medium were centrifuged and both pellets resuspended in a total of 3 to 4 ml fresh Mansour's Medium. The fluid was added to the fly bottles and gathered in small depressions in the fly medium. Larvae were observed to feed readily in these areas. No attempts were made to regulate or determine infective doses, although flagellate suspensions prepared as above generally contained at least 10^7 flagellates per ml. Fly bottles contained up to several hundred larvae in all stages of development, as well as adults and pupae.

Morphological studies were based on Giemsa-stained smears of insect gut contents and smears of the culture forms which had been centrifuged, resuspended in rabbit serum, and stained with Giemsa. Illustrations of stained material were made with the aid of a camera lucida. In the following description, measurements are in microns. Range is followed by average in parentheses.

Herpetomonas megaseliae sp. n.
(Figs. 1–12)

Parasite in culture (Figs. 1–6): $N = 30$. Seven-day culture in Wallace's Medium. Body short, wide, with short to long flagellum. Usually truncate anteriorly, but occasionally rounded or pointed. Posterior end rounded or pointed. Some biflagellate. Length 8 to 22 (13); maximum width 4 to 5 (4); flagellum 8 to 30 (14). Nucleus subcircular, 1 to 5 (3) wide, 1 to 4 (3) long. Kinetoplast anterior to nucleus, 67%; lateral, 11%; posterior, 22%.

Parasite in experimental infection (Figs. 7–12): $N = 30$. Eighteen days after inoculation of fly colony. In larval intestine, body long, narrow with long flagellum. Most truncate or rounded anteriorly. Posterior pointed. Length 8 to 26 (16); maximum width 1 to 3 (2); flagellum 19 to 44 (28). Nucleus oval, 1 to 2 (1) wide and 1 to 7 (4) long. Kinetoplast 100% anterior. Infection light in pupae and adults.

Host: *Megaselia scalaris* (Loew, 1866) Schmitz, 1929 (Diptera: Phoridae).

Site of infection: Throughout intestinal tract of larvae and adults. Undetermined in pupae.

Type locality: Lincoln, Lancaster County, Nebraska.

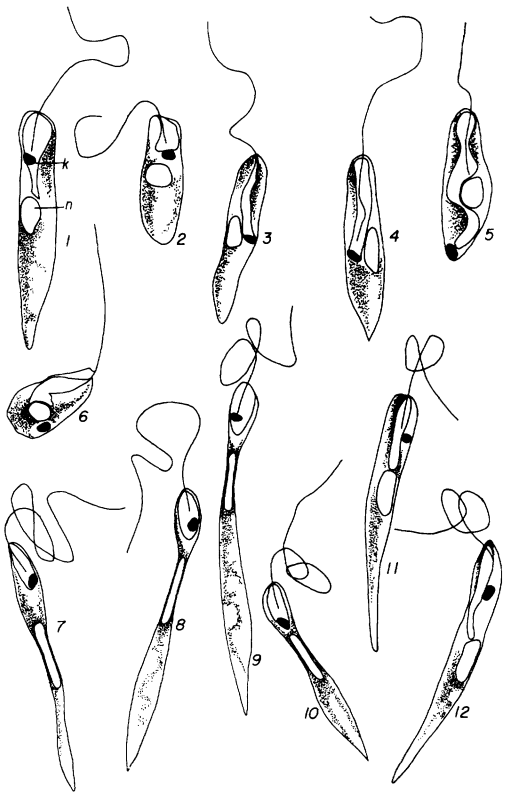
Type specimens: USNM Prot. Collection No. 63088.

Paratype specimens: USNM Prot. Collection No. 63089. Additional specimens deposited in the Harold W. Manter Laboratory, Division of Parasitology, University of Nebraska State Museum.

Remarks

The parasite probably occurs in mixed infections in the natural host. All developmental stages of the insect were infected, but natural infections tended to be heaviest in the larvae. In the natural infection in larvae only a few rounded or tuncate forms were seen. Most were long and narrow. Occasionally biflagellate forms or forms with a thickened flagellum were observed. The parasites (random selections of all forms $N = 30$) in natural infections varied from 18 to 48 (29) in length, 1 to 6 (3) in maximum width, with flagellum 14 to 42 (27), and an oval nucleus 2 to 8 (5) by 1 to 4 (2). Kinetoplast position with respect to the nucleus varied from anterior (96%) to lateral (2%) to posterior (2%).

Uninfected *M. scalaris* were successfully infected experimentally with *H. megaseliae* at each attempt. Experimental infections were



FIGURES 1–12. *Herpetomonas megaseliae*. **1–6**. Representative forms from 7-day-old culture in Wallace's Medium. Trypanosomatids centrifuged, resuspended in rabbit serum, smeared and stained with Giemsa. **7–12**. Representative forms in larvae of experimentally infected *Megaselia scalaris* (18 days after introduction). Trypanosomatids were mixed in lysed rabbit blood, smeared and stained with Giemsa. n = nucleus, k = kinetoplast.

TABLE I. *Growth of* Herpetomonas muscarum *and* H. megaseliae *in three media. Numbers represent organisms per ml (average of two counts per day). Day zero numbers calculated from hemocytometer counts of inoculum.*

	Day				
	0	3	5	7	10
PPG					
H. muscarum	22,500	175,000	225,000	300,000	0
H. megaseliae	3,500	75,000	75,000	675,000	750,000
LPG					
H. muscarum	22,500	3,400,000	2,450,000	3,200,000	2,975,000
H. megaseliae	3,500	50,000	250,000	1,800,000	2,675,000
Mansour's Medium					
H. muscarum	22,500	5,750,000	16,000,000	13,500,000	8,750,000
H. megaseliae	3,500	3,000,000	52,750,000	31,250,000	48,250,000

maintained in the colony and through subsequent transfers of the flies to fresh media. Experimental infections tended to be lighter than natural infections in all host stages.

Attempts to infect *D. melanogaster* larvae with *H. megaseliae* were not successful. Parasites were active in the anterior portion of the larval gut only up to 48 hr.

Growth in culture: The growth of *H. megaseliae* differed considerably from that of *H. muscarum* in the three different media (Table I). In two media, *H. megaseliae* reached higher numbers than *H. muscarum* and continued growing after the latter organisms were declining. Peak populations in PPG declined with succeeding transfers in that medium. Longest term survival was in LPG. *H. megaseliae* survived 200 days in a flask containing 20 ml of LPG; *H. muscarum* survived 84 days.

Culture morphology: The organisms in culture bore some resemblance to those pictured in the original description of *Crithidia luciliae*. Strickland (1911) presented illustrations of parasites from the "oesophageal diverticulum," from the "midgut and small intestine," and "hindgut and rectum." Wallace and Clark (1959) stated that Strickland was observing a mixed infection, and separated Strickland's description into *H. muscarum* ("forms in the midgut") and *C. luciliae* ("oesophageal diverticulum and rectum"). Three of the 15 flagellates in Strickland's plate VIII (figs. 7–22, "forms from the hindgut and rectum") have posterior kinetoplasts. However, forms with a kinetoplast posterior to the nucleus were excluded from *C. luciliae* for both infections and culture by Wallace and Clark (1959). In our stocks of *C. luciliae* from the American Type Culture Collection, in 7-day-old cultures in Wallace's Medium, we have not observed forms with posterior kinetoplasts. Researchers using these original descriptions of insect trypanosomatids (Strickland, 1911), as amended by Wallace and Clark (1959), should be aware of this difference between what Strickland illustrated as representative of "hindgut and rectum" parasites and the present American Type Culture Collection stocks of *C. luciliae*.

DISCUSSION

H. megaseliae, dimensionally and in the percentage of culture forms with posterior kinetoplasts, is within the range reported for *H. muscarum* (Rogers and Wallace, 1971). However, growth in the three media indicates that the two are quite different physiologically.

In *H. megaseliae*, the presence of a truncate anterior end, a wide reservoir, and few biflagellate forms in both culture and experimental infections are not generally characteristic of previously described *Herpetomonas*. These characteristics tend to resemble those of *Crithidia*, but Rogers and Wallace (1971) have shown in their study that tendencies toward these characteristics occur in opisthomastigote forms of *H. muscarum*. However, the distinctive generic character is the presence of a postnuclear kinetoplast, which is restricted to *Herpetomonas*.

It is perhaps characteristic of the problems of trypanosomatid taxonomists that a shift in the nature of a taxonomic character can occur between two environments. The proportion of individuals of *H. megaseliae* with posterior kinetoplasts was observed at 22% in culture, but that proportion was reduced to zero when the cloned stocks were introduced into uninfected flies (see taxonomic section above). Thus, in the present study, the *Herpetomonas* generic character disappeared when the new species was reintroduced into its natural host. A comparison of the natural and experimental infections indicated that the natural infections may have been a mixture of the new species and another undescribed species. Average size and percentage of posterior kinetoplasts in the natural hosts were both reduced by the cloning process.

The culture media used were selected for several reasons. Dollahon and Janovy (1971) observed trypanosomatids in the urine and feces in guinea pig litter pans. LPG was used in an attempt to approximate these conditions. The long survival of both *H. megaseliae* and *H. muscarum* indicate that similar natural conditions may facilitate distribution of these parasites. Since *H. muscarum* was most recently redescribed from Wallace's (Wallace and Clark, 1959), *H. megaseliae* was described from the same medium. PPG was used as a simplified medium for comparative growth. Mansour's is the medium in which trypanosomatids are normally maintained in our laboratory.

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