

# Two new subspecies of trypanosomatids (Kinetoplastida: Trypanosomatidae), parasites of bugs (Heteroptera) from Cuba and the United States, with a discussion of trypanosomatids of water striders (Heteroptera: Gerridae)

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*Leptomonas leptoglossi longus* subsp. n. collected in Cuba from *Leptoglossus phyllopus* (Linnaeus) (Heteroptera: Coreidae) and *Blastocrithidia gerridis bozemani* subsp. n. collected in Montana (USA) from *Gerris remigis* Say (Heteroptera: Gerridae) are described.

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## Introduction

The trypanosomatids from insects are poorly investigated compared with those of vertebrates (see Podlipaev, 1990). It is absolutely clear that the majority of insect trypanosomatids are not known till now because only a few insect species and only some regions have been investigated by insect parasitologists (Wallace et al., 1983; Podlipaev, 1990). Isolation of laboratory culture is very important for correct description of new species of trypanosomatids, but if it is impossible to obtain a culture, description based on natural infection of the host may also be justified (Wallace et al., 1983; Podlipaev et al., 1991). If we wait for laboratory cultures in each new case of infection, the fauna of trypanosomatids in large regions may remain unknown. For example, the first reports on trypanosomatids from insects and plants in Central Asia (Podlipaev, 1986, 1988a, 1988b) were done without culture isolation, and it will be hardly possible to obtain cultures from some of these remote areas in near future. Each new insect species and each new region investigated may enrich our poor knowledge of insect trypanosomatids. When culture is absent, the only basis of description are morphological features

and metric characters of the cells that are usually scanty for more or less comprehensive trypanosomatid characterization (Wallace et al., 1983; Podlipaev & Lobanov, 1996). The problem of comparison of old and modern descriptions is very difficult to solve because only two metric characters, the distance between the anterior end of the body and the kinetoplast as well as the kinetoplast index, may be used for the discrimination of species of insect and plant trypanosomatids, but only a few authors measured these parameters (Podlipaev & Lobanov, 1996).

Therefore when we are obliged to describe the trypanosomatid parasite and the culture is absent, it seems to be more accurate to try to link new findings with previous descriptions, if it is possible. Proceeding from the reasons listed above, we describe trypanosomatids in this paper as subspecies, bearing in mind the possibility of revising their taxonomic status if and when the cultures may be isolated.

Insect trypanosomatids of the eastern and southern parts of the USA and southern part of Canada are poorly known and those of Cuba unknown (Laird, 1959; Wallace, 1966, 1979; Lipa, 1968; Podlipaev, 1990).

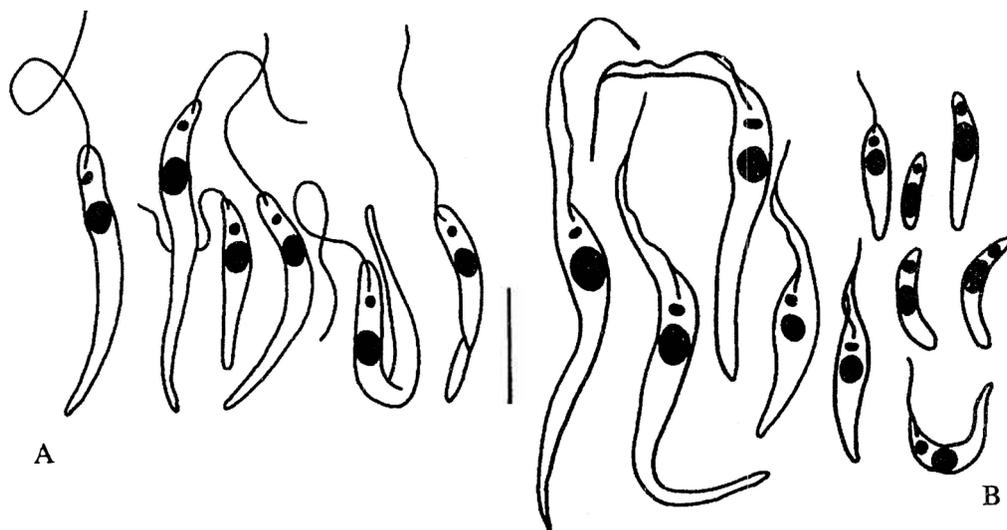


Fig. 1. A, *Leptomonas leptocoris longus* in the insect *Leptoglossus phyllopus*; B, *Blastocrithidia gerridis bozemani* in the insect *Gerris remigis*. Giemsa-Romanowsky staining; camera lucida; scale 10  $\mu$ m.

Some trypanosomatids are causative agents of plant diseases of economic importance (Dollet, 1984), and the origin of new insect-trypanosomatid system and the danger of the new plant diseases may be predicted (Podlipaev, 1996). Therefore phytophagous bugs (especially Coreidae, Pentatomidae and Lygaeidae) attract exceptional interest as possible vectors of trypanosomatids of plants (Camargo & Wallace, 1994).

Giemsa stained slides were investigated. One hundred cells were measured on each slide. Drawings were prepared with camera lucida. Holotypes are kept in the Zoological Institute, St.Petersburg.

#### *Leptomonas leptoglossi longus* subsp. n.

*Holotype*. Slide with many protozoans, Cuba, San Antonio de los Baños, 12.XI.1986, ex gut of *Leptoglossus phyllopus*, leg. A.F. Emeljanov & I.M. Kerzhner.

*Host*. *Leptoglossus phyllopus* (Linnaeus) (Heteroptera: Coreidae).

*Description*. Promastigotes (Fig. 1A), often with extended posterior part of the body. Nucleus close to anterior quarter of the body, kinetoplast about midway between anterior part of body and nucleus. Measurements are presented in Table.

*Comparison*. *Leptomonas leptoglossi longus* differs from *L. leptoglossi leptoglossi* Hanson

& McGhee, 1961 (USA) in the 1.5 times longer body and nucleus situated closer to the anterior end of the body.

*Discussion*. Trypanosomatids were found in 34 species of the family Coreidae, but the majority of flagellates were not described and not named (see Podlipaev, 1990; Camargo & Wallace, 1994).

In *Anoplocnemis* sp., *Cletus varius*, *Coreus marginatus* (Lipa, 1966 – as *Mesocerus marginatus*) and *Leptocoris trivittatus*, some *Blastocrithidia* species were recognized (Hindle & Lewis, 1912; McCulloch, 1917; Short, 1923; Lipa, 1966; Podlipaev, 1988a).

Several *Leptomonas* species were noted in Coreidae bugs. *L. tortum* was described from *Camptopus lateralis* in France (Poisson, 1930) and *L. tortum kirgizorum* from the same species of bugs in Central Asia (Podlipaev, 1988b). *L. capsularis* was found in *Cletus ochraceus* from South Africa (Gibbs, 1951); *L. mirperi* in *Mirperus jaculus*; *L. serinethae* in *Serinetha fraterna* (all from Congo, Catanga) (Rodhain et al., 1913). All of them differ clearly from *L. leptoglossi longus* in the size and shape.

Trypanosomatids inhabit two species of the genus *Leptoglossus*. *Leptomonas* sp. was noted in *Leptoglossus membranaceus* in Uganda (Robertson, 1912) but the author did not name the flagellate. In *Leptoglossus phyllopus* from Texas (USA), *Leptomonas*

**Table.** Body sizes of *Leptomonas leptoglossi longus* subsp. n. and *Blastocrithidia gerridis bozemani* subsp. n. ( $\mu\text{m}$ , the mean value  $\pm$  standard deviation, in brackets – maximum and minimum)

Measurements	<i>Leptomonas leptoglossi longus</i>	<i>Blastocrithidia gerridis bozemani</i> (epimastigotes)
Body length	20.69 $\pm$ 1.20 (29.8-12.4)	30.87 $\pm$ 0.47 (45.4-15.7)
Body width	2.13 $\pm$ 0.09 (3.1-1.5)	2.15 $\pm$ 0.36 (2.0-1.2)
Nucleus	1.93 $\pm$ 0.09 (2.9-1.1)	3.05 $\pm$ 0.11 (7.2-1.8)
Kinetoplast	0.80 $\pm$ 0.03 (1.2-0.6)	1.70 $\pm$ 0.18 (2.6-0.6)
From anterior end of body to nucleus (A-N)	5.88 $\pm$ 0.31 (8.7-3.4)	9.75 $\pm$ 0.39 (19.5-3.7)
From posterior end of body to nucleus (P-N)	12.44 $\pm$ 0.88 (17.9-6.2)	18.25 $\pm$ 0.54 (23.5-11.3)
From anterior end of body to kinetoplast (A-K)	2.90 $\pm$ 0.13 (4.3-1.6)	7.92 $\pm$ 0.43 (17.4-2.4)
From kinetoplast to nucleus (K-N)	3.03 $\pm$ 0.24 (5.21-0.96)	1.82 $\pm$ 0.13 (2.5-1.1)
Nuclear index (A-N/P-N)	0.48 $\pm$ 0.02 (0.7-0.3)	0.49 $\pm$ 0.02 (0.84-0.24)
Kinetoplast index (A-K/K-N)	1.09 $\pm$ 0.10 (2.0-0.5)	3.13 $\pm$ 0.24 (7.64-1.67)

sp. was noted, but neither a description nor a name were published (Packchianian, 1957). From the same species of bugs collected in the vicinity of Athens (Georgia, USA), *Leptomonas leptoglossi* was described (Hanson & McGhee, 1961).

#### ***Blastocrithidia gerridis bozemani* subsp. n.**

*Holotype.* Slide with many protozoans, USA, Montana, Bozeman, 9.VII.1992, ex gut of *Gerris remigis*, leg. S.A. Podlipaev.

*Host.* *Gerris remigis* Say (Heteroptera: Gerridae), found in three of the six examined specimens.

*Description.* Slender epimastigotes, cyst-like amastigotes and single cells with weakly developed undulating membrane (Fig. 1B). Nucleus in anterior half of body; distance between kinetoplast and anterior end of body 2-3 times that between kinetoplast and nucleus, but kinetoplast not adjacent to sur-

face of nucleus. Measurements are presented in Table.

*Comparison.* *Blastocrithidia gerridis bozemani* differs from *B. gerridis gerridis* (Patton, 1908), as re-described by Wallace et al., (1965), in the presence of cyst-like cells, shorter body, and kinetoplast more distant from nucleus.

*Discussion.* From ten species of *Gerris*, one *Crithidia*, two *Leptomonas* and three *Blastocrithidia* species are known (Wallace, 1966; Podlipaev, 1990).

*Blastocrithidia gerridis* is listed from nine *Gerris* species all over the world including the USA and Canada (Wallace, 1966; Podlipaev, 1990).

*B. veliae* is noted from *Velia "currens"* (actually, *V. caprai* or *V. saulii*) in Ireland and *Gerris comatus* in USA (Dunkerly, 1913; Wallace et al., 1965). The possibility must not be ruled out that *B. veliae* is a part of the life cycle of another *Blastocrithidia* (see be-

low). We did not find in our new subspecies epimastigotes with thick rounded body typical of *B. veliae*, therefore *B. gerridis bozemani* differs from *B. veliae* very clearly.

*B. gerricola* was described from *Gerris lacustris* collected in NW Russia. It differs from *B. gerridis* in the size and morphology and, additionally, from *B. gerridis bozemani* in the absence of cyst-like cells (Podlipaev, 1985).

In water striders, we observed all cell morphotypes noted by Patton (1908) and Vivanti (1917), except for cyst-like rounded amastigotes. In addition, we observed in our material slightly curved amastigotes similar to the cyst-like amastigotes of *Blastocrithidia* sp. (Frolov et al., 1997).

Trypanosomatid infection of water striders of the family Gerridae has been a complicated problem for a long time. Patton (1908) described *Blastocrithidia gerridis* (as *Crithidia*) from *Gerris fossarum* in India. He has illustrated some morphological types of cells in the host, including typical epimastigotes, epimastigote-like cells with undulating membrane running around rounded body, almost promastigote-like cells with poorly developed undulating membrane, and small cyst-like nonflagellate forms (Patton, 1908). Following Patton, these morphotypes were considered as the stages of the life cycle of the parasite by some authors (Porter, 1909; Vivanti, 1917).

Dunkerly (1913) in Ireland found a very similar set of cell morphotypes in *Velia "currens"* infected by trypanosomatids and described *Blastocrithidia veliae* (as *Leptomonas (Crithidia)*) based on the presence of cells with thick rounded body, which he considered not as a stage of life cycle but as protozoans belonging to a new species. Wallace et al. (1960, 1965) considered that several species of trypanosomatids may inhabit one organism of water strider and reported about the presence of other trypanosomatid species in bugs of the genus *Gerris*. These authors declared that *Blastocrithidia veliae*, *Crithidia flexonema*, *Leptomonas collosoma* and *L. costoris* might have been mistaken for life cycle stages of *Blastocrithidia gerridis* and redescribed the last species from this point of view, i.e. deleted promastigote-like cells, epimastigote with thick rounded body and small cyst-like nonflagellate forms from the redescription (Wallace et al., 1965).

The existing situation reflects two main problems of the trypanosomatid systematics: the lack of reliable morphological characters

to classify parasites of insects and the absence of the conception of host specificity range (Wallace et al., 1983; Podlipaev & Lobanov, 1996; Podlipaev & Bulat, 1998; Podlipaev et al., 1998).

The ultrastructure investigation of *Blastocrithidia* sp. from *Gerris lacustris* (Frolov et al., 1997) shows the presence of the whole set of morphotypes described by Patton (1908) including the cells which may belong to the species *Blastocrithidia gerridis*, as well as the cells coincident with *B. veliae*. Ultrastructure differences of any kind between two types of epimastigotes were not noted, but the presence of cyst-like amastigote cells, as in Patton's description, was confirmed (Frolov et al., 1997). The results of ultrastructure investigation more correspond to early reports (Patton, 1908; Porter, 1909) than to the later data (Wallace et al., 1965).

The presence of representatives of different species and genera in the same host does not contradict to the very high level of host specificity of trypanosomatids of insects which was demonstrated by using UP-PCR (Podlipaev & Bulat, 1998; Podlipaev et al., 1998).

Isolation of a culture is crucial to solve the problem whether there are several species of trypanosomatids inhabiting water striders or researchers deal with different polymorphic stages of one life cycle, but unfortunately there are some difficulties in isolation of *Blastocrithidia* cultures (Wallace et al., 1965; Peng & Wallace, 1981).

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