

First record of *Trypanosoma* infection in Mediterranean mouse (*Mus macedonicus* Petrov & Ružić, 1983) in Bulgaria

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Abstract. The study presents a *Trypanosoma* infection in Mediterranean mouse (*Mus macedonicus* Petrov & Ružić, 1983) from Plovdiv region (Bulgaria). The average established prevalence of the parasite in *Mus macedonicus* was 23.5 %, whereas the infection rate was lower in female (11.1%) compared to male (37.5%) individuals. All observed parasite forms are trypomastigotes, possessing the morphological characteristics of the sub-genus *Herpetosoma* (*Stercoraria* section), to which *T. lewisi*-like parasites belong. These features are: free flagellum, “C shape” of the parasite with size about $25 \pm 5 \mu\text{m}$, clear visible undulating membrane, subterminal kinetoplast with oval shape, anterior located nucleus. These morphological characteristics and the absence of infection in the other investigated rodents, hosting other *Trypanosoma* species, allow us to provide evidence-based assumption that the registered parasite is *Trypanosoma musculi*. Further studies with molecular methods are required to confirm the registered species.

Key words: *Mus macedonicus*, *Trypanosoma musculi*, parasitology, acridine orange.

Introduction

Trypanosomes are digenetic protozoans of the Trypanosomatida order of Phylum Euglenozoa (Adl *et al.* 2012) that infect domestic and wild animals, as well as humans. They cause serious vector-borne diseases, which turns them into a major public health concern. There are many species of trypanosomes that infect virtually all vertebrate taxa. They typically cycle between blood-sucking insects or leech vectors and vertebrate hosts and undergo biochemical and morphological changes in the process. These parasites have evolved a variety of strategies to evade or modulate immunity of endothermic and ectothermic vertebrates hosts (Oladiran & Belosevic 2012).

Some trypanosomes normally infect rodents and utilize fleas as vectors. *Herpetosoma* is a homogenous subgenus of several dozen no name species that are often described as morphologically indistinguishable *T. lewisi*-like parasites (Jittapalapong *et al.* 2008). The rodent trypanosomes are well adapted to their respective hosts and have a high degree of host peculiarity – *Trypanosoma lewisi* in rats, *Trypanosoma musculi* in mice, *Trypanosoma grosi* in some species of *Apodemus*, *Trypanosoma microti* in voles from genus *Microtus*, *Trypanosoma evotomys* in *Clethrionomys glareolus* (Noyes *et al.* 2002, Sato *et al.* 2003). Since these trypanosomes are extracellular parasites they are exposed to the humoral immune responds of the host at all times. Although the subgenus *Herpetosoma* is considered as non-pathogenic to normal hosts, there are rare occasions reported in association with human diseases (Jittapalapong *et al.* 2008; Truc *et al.* 2013).

Besides the *Trypanosoma equiperdum* (Golemansky 1990), there are no more reports of trypanosome infections in rodents or other representatives of the wild fauna in Bulgaria. This protozoan parasite causes the disease “dourine” in horses, known also as equine syphilis.

The main goal of our investigation is to present preliminary data on the distribution and morphological characteristic of *Trypanosoma* species in Mediterranean mouse *Mus macedonicus* in Bulgaria.

Material and Methods

The area of study (Fig. 1) covers a region near Plovdiv, located in the northern part of the Western Rhodopes, at 230 m asl. The natural forest vegetation was completely destroyed, only fractions of scattered mosaic mixed deciduous forests, bushy and grassy components are still preserved. The main part of the territory is occupied by agricultural ecosystems. A lead-zinc smelting factory (KCM-Plovdiv) as well as other industrial enterprises are evenly distributed on both sides of the road to Asenovgrad. Different trapping sites according to the species biotope adherence inside the area were selected.



Fig. 1. Location of the study area with investigated sites of *Trypanosoma* infection – site 1 (N42°3'58.68"; E24°49'18.57") and site 2 (N42°3'13.49"; E24°49'39.89").

In total 65 specimens of 3 rodent species were collected (Table 1). The trapping was performed between September 2010 and November 2013. Sherman live traps were placed at dusk, left active overnight, and collected the next morning. The rodents were brought to the laboratory, where they were sexed and weighed. Blood samples were obtained from the

caudal vena cava. A drop of blood was placed onto the pre-cleaned microscope slide and using a pusher slide a thin smear was prepared. Two peripheral blood smears from each animal were made. Slides were air dried at room temperature for 24 h and fixed in absolute methanol for 10 min. The coded slides were stained with fluorochrome dye acridine orange (0.1% solution supplemented in Sørensen's phosphate buffer) for 1 min, rinsed and cover-glassed immediately before evaluation with fluorescence microscopy Leica DM 1000 equipped with appropriate for acridine orange I3 filter. Acridine orange staining method has a high diagnostic capacity to detect different parasites in blood smears because of its higher speed of reading and sensitivity when compared with common brightfield microscopy using Giemsa staining and the opportunity to detect DNA/RNA through dyeing in different color. The technique is recommended for a fast diagnosis especially in countries with endemic areas where the disease like malaria, sleeping sickness, Lyme disease, babesiosis, spirochetemia occurs. The acridine orange is appropriate dye for detecting cases of low-level parasitaemias (Fig. 2).

Table 1. Number of investigated species in the studied area.

Species	Gender		Total
	male	female	
Mediterranean mouse (<i>Mus macedonicus</i> Petrov & Ružić, 1983)	16	18	34
Yellow-necked mouse (<i>Apodemus flavicollis</i> Melchior, 1834)	14	10	24
Common vole (<i>Microtus arvalis</i> Pallas, 1778)	4	3	7
			65

Results

From the 3 investigated rodent species trypanosome infection was registered only in the blood smears of Mediterranean mouse *Mus macedonicus* from two of the trapping sites (Fig. 1). The other two studied species – *Apodemus flavicollis* and *Microtus arvalis*, that inhabit the same habitats, demonstrated a complete lack of this parasite in their blood. The established prevalence in *Mus macedonicus* was 23.5 % (8 infected specimens from 34 investigated). A lower infection rate of female (2 infected from the total 18 female – 11.1%) as compared with male (6 infected from the total 16 male – 37.5%) was found.

The registered species of *Trypanosoma* is a trypomastigote form, which lives extracellular and circulate freely among red blood cells (Fig. 3). The average size of the parasite was $25 \pm 5 \mu\text{m}$ (n=273). The smallest parasite observed was $14 \mu\text{m}$ and the largest – $32 \mu\text{m}$. The kinetoplast was oval shaped, subterminal located in the very sharp and thin posterior end. Nucleus was more in the anterior part of the body. In all observed forms flagellum was free and the undulating membrane was not large but clear visible. These characteristic features (Fig. 4) were compared with described morphological characteristics of different rodent's *Trypanosoma* species.

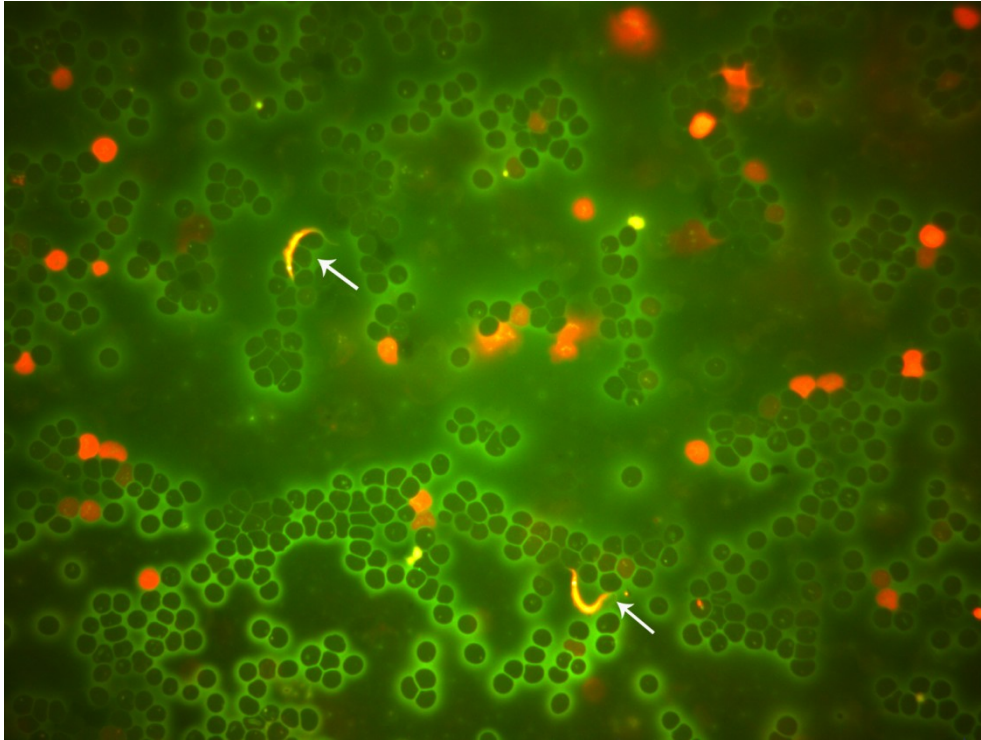


Fig. 2. A blood smear showing trypanosomes (white arrows) stained with acridine orange. Parasites (orange) are easily recognizable alongside red blood cells (mature – dark green; young – red) (magnification 400x).

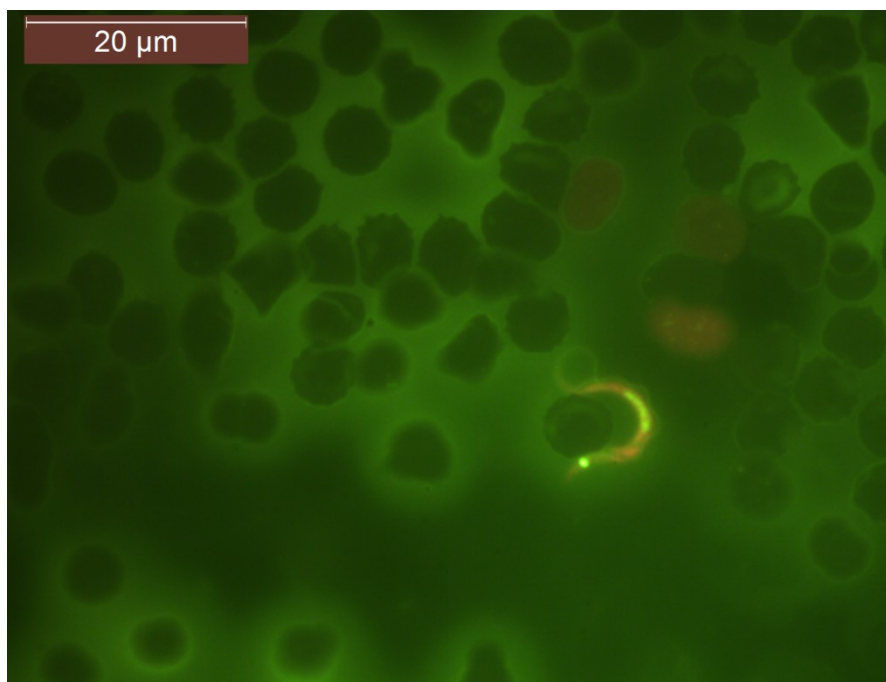


Fig. 3. Microphotograph of *Trypanosoma musculi* from *Mus macedonicus* near Plovdiv in a thin blood smear (magnification 1000x).

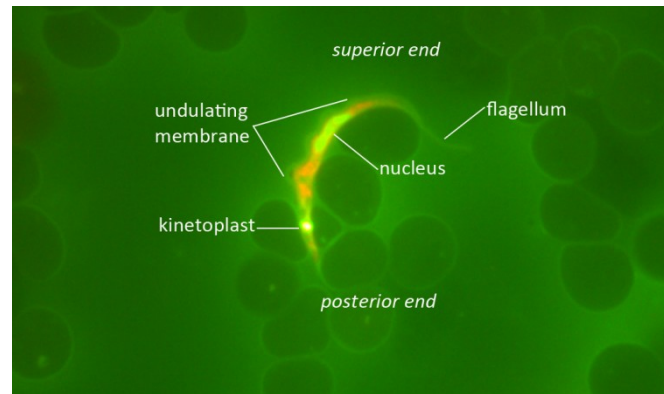


Fig. 4. Morphological characteristics of the adult trypomastigote form of *Trypanosoma musculi*.

Discussion

The established characteristic features of registered trypanosomes coincides with the morphological characteristics described for so called *T. lewisi*-like group of subgenus *Herpetosoma* (*Stercoraria* section). *T. lewisi* has a stringent species specificity and cannot grow in other rodents such as mice (Desquesnes *et al.* 2002). *Trypanosoma musculi* (synonymous *T. duttoni*) is a parasite specific to mice, which resides in the blood and lacks intracellular stages (Monroy & Dusanic 2000). “C shape” of the parasite, and free flagellum are the most striking features of the adult forms belonging to *Herpetosoma* (Truc *et al.* 2013). The both characteristics are undoubtedly visible in the microphotographs (Figs 2; 3 and 4). These facts together with its host specificity and the characteristic morphological features allowed us to provide evidence-based assume that the registered parasite is *T. musculi*. The complete lack of this parasite in the other investigated rodents species – *Apodemus flavicollis* could confirm the host specific of rodent’s trypanosomes.

The results of our investigation demonstrate that the male mice with infection rate of 37.5% are more than threefold more susceptible than the female (11.11%). This coincides with the results of some previous studies. Sex dependence of resistance to *T. rhodesiense* infection in mice has been studied by Greenblatt & Rosenstreich (1984), which suggested that the decreased resistance of males was due to their relative inability to control parasite growth, whence female mice were markedly more resistant than male mice. According to Šima *et al.* (2011) the different susceptibility to *T. brucei* in males and females is due to dissimilar genetic regulation in certain genetic combinations. It is possible that the different susceptibility of *Mus macedonicus* to *T. musculi* in males and females may also be the fact of different genetic regulation in both sexes.

The two classical forms of human trypanosomoses are sleeping sickness and Chagas disease. Also humans possess an innate protection against most *Trypanosoma* species (Vanhamme *et al.* 2003) that naturally occur and infect livestock and many mammalian species in wild fauna. In recent years a number of atypical human infections caused by other *T. species* (or sub-species) have been reported, namely due to *T. brucei brucei*, *T. vivax*, *T. congolense*, *T. evansi*, *T. lewisi*, and *T. lewisi*-like. These cases are reviewed from Truc *et al.* (2013). It is believed that *T. lewisi* in rats is a worldwide non-pathogenic parasite transmitted by fleas, however in recent years *T. lewisi* and other species like *T. evansi* have emerged as potentially pathogenic for humans. Recently, a *T. lewisi*-like infection was detected in a sick Thai infant, thus the objective of this study was to investigate the prevalence of *T. lewisi* infections among different rodents indigenous to Thailand in order to identify possible sources of human cases (Jittapalapong *et al.* 2008). These data give importance to the established trypanosomes infection in *Mus macedonicus*. Habitats where rodents were collected are significantly close to Plovdiv and its industrial zone and

agricultural areas and we suggest that the degree of anthropization may influence the transmission of *Trypanosoma sp.* and may increase the risk of human infection.

This is the first report of *Trypanosoma musculi* in the blood of Mediterranean mouse *Mus macedonicus* in Bulgaria. Among several species of mammals captured in the region near Plovdiv (*Mus macedonicus*, *Apodemus flavicollis*, *Microtus arvalis*) *Trypanosoma* was detected in 23.5 % of the *Mus macedonicus* individuals collected near Plovdiv. This finding confirms the important role of Mediterranean mice as a reservoir for trypanosome infection.

In order to characterize the established *Trypanosoma* species further studies with molecular methods as well as additional investigations on potential vectors, reservoirs (wild and domestic animals), and infection pathway are required.

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