

Trypanosoma (Megatrypanum) ornata sp. n., a Parasite of the Eurasian Water Shrew *Neomys fodiens* (Pennant, 1771)

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Summary. Infection with the new trypanosome species *Trypanosoma ornata* sp. n. was detected in water shrews *Neomys fodiens* examined for presence of blood parasites in September 2003 and 2004 in Białowieża Forest (eastern Poland). The prevalence of infection was about 36.3% (4 out of 11 shrews examined). The parasites were described based on light microscopic characteristics, from 100 parasites isolated from blood of four infected animals. The trypanosomes occurred as trypomastigote forms only. Body length ranged from 20.50-38.26 μm (mean 26.66 μm), and width from 1.93-4.12 μm (mean 3.06 μm), with the free flagellum 3.12 ± 1.26 long, sometimes absent. Other morphological features of the species (small kinetoplast, sometimes the vermiform posterior end, the surface of the cell markedly striated with longitudinal "myonemes", short or absent free flagellum) support its affiliation to the *Megatrypanum* subgenus. The study provides the first detailed description of trypanosomes occurring in the Eurasian water shrew *Neomys fodiens*.

Key words: Białowieża Forest, Poland, *Neomys fodiens*, *Trypanosoma ornata* sp.n.

INTRODUCTION

The trypanosomes parasitizing wild mammals in the palearctic zone are not very well known. These are the species belonging to the Stercorarian group, from the subgenera *Megatrypanum*, *Herpetosoma* and *Schizotrypanum*. So far, 25 species of trypanosomes are noted as permanent components of parasitofauna of Euro-

pean mammals (Hoare 1972, Karbowski *et al.* 2001). However, trypanosomes parasitizing insectivorous mammals in the palearctic zone are poorly known. There is only a single report on the presence of a non-identified *Trypanosoma* in Eurasian water shrew *Neomys fodiens* from England from the beginning of 20th century (Henry 1913). Their pathogenic effects on hosts and life cycles are unknown. The vectors are probably fleas (Hoare 1972).

The goal of our study was description of the blood parasitofauna of shrews, and the morphological and morphometric characterisation of parasites found. The study is a base for the future research on their possible role as zoonotic foci of diseases.

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MATERIALS AND METHODS

Eurasian water shrew *Neomys fodiens* (Insectivora: Soricidae) is a small (max. 15 g body mass), semiaquatic mammal occurring across most of Palearctic. It lives in wet habitats along freshwater, and aquatic prey usually constitute over 50% of its diet. During its short life (mean life-span 13.5 months), the water shrew produces 2 or 3 litters of 6 or more (up to 15) young (Pucek 1981, Innes 1994, Rychlik 2000).

The material from water shrews was collected in September 2003 and 2004 in Białowieża Forest (eastern Poland, 52°42'N, 23°52'E). The shrews were caught in live-traps placed at permanent trap stations arranged in a rectangular grid or along a line. Traps were placed and set in the afternoon, checked four times per day, and blocked after a final check around midnight.

Blood samples taken from the tip of the tail were examined for the presence of trypanosomes using the microhaematocrit centrifugation technique (8 minutes, 6200 g) (Kingston *et al.* 1992). The flagellates accumulated above the WBCs fraction, and their movements were observed under a light microscope, using magnifications of 10 × 10 and 10 × 20 (eyepiece × objective).

Giemsa-stained blood films from the trypanosome fraction were used for morphological and mensural characteristics of trypanosomes. The smears were air-dried, fixed in 100% methyl alcohol (10 minutes) and stained for 1 hour with Giemsa (diluted 1:5 in phosphate buffer, pH 7.2). Slides were rinsed, dried and examined under a light microscope using magnifications of 10 × 100 and 12 × 100 (eyepiece × objective) with oil immersion.

For the measurements of parasites, the "Analysis" software, in combination with a video camera and Olympus BX50F4 microscope was used. This method provided possibilities for obtaining results accurate to 0.01 µm. In order to detail, characterize, and compare morphological features of parasites, the terminology commonly adopted by trypanosome researchers (Hoare 1972, Matthews *et al.* 1977, Kingston *et al.* 1992) was employed in the present study. Morphometric measurements were made on 100 parasite specimens, at a magnification of 1200×. Typical trypomastigote forms were selected for measurements. Weakly stained, damaged, or distorted individuals were ignored.

RESULTS

Infection with *Trypanosoma ornata* sp. n. was discovered in the blood of 4 out of 11 water shrews examined, prevalence of infection was 36.3%. Additionally, unidentified *Bartonella* sp. bacterias were detected in 5 shrews (prevalence about 45.4%), but there were no mixed infections. The trypanosomes occurred as pleomorphic trypomastigote forms only. The epimastigote, dividing forms or other developmental stages were not observed. The density of infection was low; only single parasites were observed in the haematocrit capillars and stained blood films.

The trypanosomes were broad, and in many cases had a tapering and pointed, sometimes somewhat vermiform, posterior end. A few specimens were characterized with a less sharp or blunt posterior end. The body was slightly curved, with the narrow undulating membrane located on the external side of the curvature. The surface of the cell was markedly longitudinally striated with dark-blue, "myonemes" (Figs 1, 2). The length of the body was 20.50-38.26 µm (mean 26.66 µm), the width was 1.93-4.12 µm (mean 3.06 µm). The free flagellum was relatively short, and varied from a stub to a short whip. The wide range of free flagellum length was readily noted, it was 1.0-9.18 µm (median length was 2.96 µm, mean 3.12 ± 1.26 µm), in some specimens free flagellum was too short to recognize. A few specimens had a longer flagellum, up to 9.18 µm. The undulating membrane was narrow, 0.55-1.09 µm. The oval nucleus was located in the middle or slightly displaced to the posterior part of the body (range of NI = 0.31-1.20, mean 0.69), parallel the longitudinal axis of body, and close to the margin of undulating membrane. The nucleus was 1.18-6.88 µm (mean 3.78) long, and 0.65-3.01 µm (mean 1.15) wide, in the Giemsa-stained smears it was violet coloured. The kinetoplast was particularly small, oval, 1.20 × 0.60 µm in size, usually lay close to the margin of the body. It was located near the posterior end of the body or in the middle of the PN distance (KI = 1.31-2.53, mean 1.68) (Figs 1, 2). The morphometric parameters are summarised in Table 1.

Taxonomic summary

Type host: Eurasian Water Shrew *Neomys fodiens* (Pennant, 1771)

Site of infection: Blood.

Trypomastigote form: Broad, slightly curved, with a tapering and pointed or vermiform posterior end; narrow undulating membrane located on the external side of the curvature; the surface of the cell marked with dark-blue, longitudinal "myonemes"; the length of the body 20.50-38.26 µm (mean 26.66 µm), the width 1.93-4.12 µm (mean 3.06 µm), free flagellum relatively short, varies in length from a stub to a short whip, range 1.0-9.18 µm, the median length 2.96 µm, mean 3.12 ± 1.26 µm, in some specimens too short to recognize; the undulating membrane narrow, 0.55-1.09 µm in width; nucleus oval, located in the middle or slightly displaced to the posterior part of the body, 1.18-6.88 µm (mean 3.78) long, and 0.65-3.01 µm (mean 1.15) wide, NI = 0.31-1.20, mean = 0.69; located along to the longitudinal axis

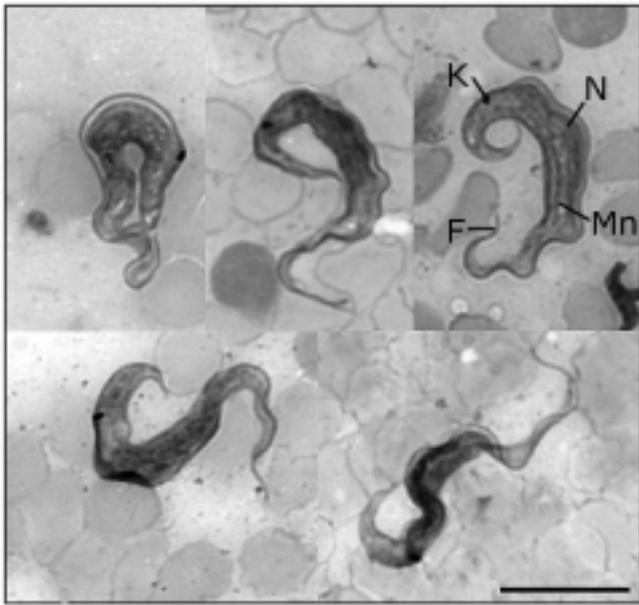


Fig. 1. Photomicrograph of Giemsa stained bloodstream forms of *Trypanosoma ornata* sp. n. from the Eurasian water shrew *Neomys fodiens*. F - free flagellum, K - kinetoplast, Mn - longitudinal "myonemes", N - nucleus. Scale bar 10 μ m.

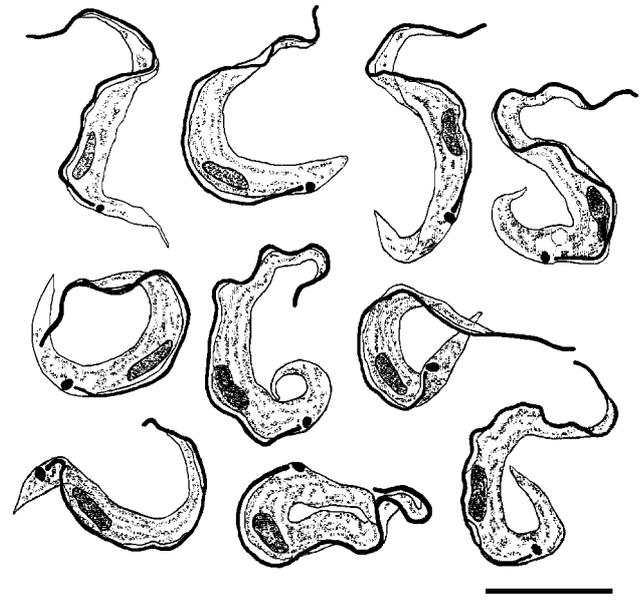


Fig. 2. The trypanosome *Trypanosoma ornata* sp. n. from the Eurasian water shrew *Neomys fodiens*. Scale bar 10 μ m.

Table 1. Dimensions (in μ m) of *Trypanosoma ornata* sp. n. from the Eurasian water shrew *Neomys fodiens* from Białowieża. PK - posterior end to kinetoplast, KN - kinetoplast to nucleus centre, PN - posterior end to nucleus centre, NA - nucleus centre to anterior end, BL - body length, FF - free flagellum length, L - total length, NI - nucleus length, Nw - nucleus width, W - width of body on the nucleus level excluding the undulating membrane. Indices: nuclear index NI = PN / NA, kinetoplastic index KI = PN / KN, flagellar index FF:BL. Measurements from 100 trypomastigote specimens.

Parameter	PK	KN	PN	NA	BL	FF*
mean	4.27 \pm 1.54	6.48 \pm 1.43	10.75 \pm 2.30	15.90 \pm 2.32	26.66 \pm 3.52	3.12** \pm 1.26
range	2.62-10.65	2.48-14.35	5.41-18.84	10.97-21.01	20.50-38.26	1.00-9.18

Parameter	L	NI	Nw	W	NI	KI	FF:BL*
mean	29.43 \pm 4.10	3.78 \pm 0.85	1.15 \pm 0.35	3.06 \pm 0.43	0.69 \pm 0.16	1.68 \pm 0.24	0.10 \pm 0.06
range	20.50-40.90	1.18-6.88	0.65-3.01	1.93-4.12	0.31-1.20	1.31-2.53	0.03-0.30

* - if the free flagellum is present; ** - the median is 2.96 μ m.

of body, on the side of undulating membrane. The kinetoplast particularly small, oval, 1.20 \times 0.60 μ m in size, lies close to the margin of the body, near the posterior end or in the middle of the body, KI = 1.31-2.53, mean 1.68.

Type locality: Białowieża National Park, eastern Poland, 52°42'N, 23°52'E.

Deposition of the type slides: The protozoological collection of the Witold Stefański Institute of Parasitology, Polish Academy of Sciences, Twarda str. 51/55, 00-

818 Warsaw, Poland. Contact with corresponding author.

Etymology: After Latin *ornata* = decorated, alluding to the longitudinal striped pattern on the body surface of trypanosomes.

DISCUSSION

According to Hoare (1972) two *Trypanosoma* subgenera, *Megatrypanum* and *Herpetosoma*, are represented in small mammals of Europe. The characteristic features of *Megatrypanum* trypanosomes are: relatively large size; relatively wide body; frequent presence of the filiform "tail" at the posterior end; short or absent free flagellum; small kinetoplast, situated close to the nucleus and far from the posterior end of body (index $KI \geq 2.0$); reproduction in epimastigote stage in mammalian host. The diagnostic features of trypomastigotes of *Herpetosoma* are: medium size; slender and curved body; long and pronounced free flagellum; anterior nuclear position or in the middle of the body; large and often rod-shaped kinetoplast, lying nearer to the posterior extremity than to the nucleus ($KI = 1.2-2.0$, average 1.5) (Hoare 1972).

There are two species of *Trypanosoma* described in insectivorous mammals in Europe: *Trypanosoma talpae* Nabarro, 1907 and *Trypanosoma crocidurae* Brumpt, 1923. *Trypanosoma talpae* is described from the blood of the mole *Talpa europaea* in western Europe, and is included presently in the *Megatrypanum* subgenus (Hoare 1972, Baker 1974, Podlipaev and Krylov 1990). The trypanosomes found in *N. fodiens* differed from *T. talpae* in some features: different localization of nucleus and kinetoplast in the cell (in *T. ornata* sp.n. both lie on the same side of the body, in *T. talpae* both lie on the opposite side of the body), proportionally shorter PK and longer KN distances, and a narrower body in *T. ornata* sp.n. *Trypanosoma crocidurae* is described from the blood of greater white-toothed shrew *Crocidura russula* and common shrew *Sorex araneus* in Belgium, Germany and France (Krampitz 1961, Hoare 1972) as well as from the lesser white-toothed shrew *Crocidura suaveolens* in the former Czechoslovakia (Šebek 1975). This species is included in the *Herpetosoma* subgenus; however, the classification based on the morphological analysis of data given in the descriptions by several authors and collected by Hoare (1972) can be discussed. The size of *T. crocidurae* (about 20 μm by Šebek 1960, 25-32 μm after Hoare 1972) is similar to the

medium size of species of the *Herpetosoma* subgenus, but the long PK distance (11 μm) and presence of the filiform "tail" at the posterior end relate this species to *Megatrypanum* subgenus. The only feature of *T. crocidurae* similar to the trypanosomes found in *N. fodiens* in the present study is the filiform "tail" on the posterior end of the body; the other parameters of these two species are different. *T. ornata* sp.n. differ from *T. crocidurae* in different localization of nucleus, bigger size and wider body. Moreover, Šebek (1960) reported high density of infection with *T. crocidurae*, not observed in the case of *T. ornata* sp. n.

It is impossible to compare our results with the record of Henry (1913) on the presence of trypanosomes in *N. fodiens* in England due to the absence of any description.

The trypanosomes found in *N. fodiens* fulfil some of the conditions of subgenus *Megatrypanum*. The morphological features, namely broad body, small kinetoplast, short free flagellum, presence of the vermiform "tail" and "myonemes", the lack of free flagellum in some specimens, the value of KI index in some cases bigger than 2 (till 2.53) (Table 1) are characteristic of *Megatrypanum*. Only few parameters (the body size, KI index smaller than 2.0 in many specimens) (Table 1) support its affiliation to *Herpetosoma* subgenus, the most common in small mammals of Europe (Hoare 1972, Baker 1974, Karbowiak *et al.* 2001).

In conclusion, the morphological and biological characteristics of *Trypanosoma ornata* sp.n. found in *N. fodiens* from Białowieża Forest place this parasite within *Megatrypanum* subgenus. However, only a few specimens of water shrews have been investigated, and the developmental cycle of the parasite, its invertebrate vector and ecology remain unknown.

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