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# *Trypanosoma* (*Herpetosoma*) *grosi kosewiense* subsp. n., the Parasite of the Yellow-Necked Mouse *Apodemus flavicollis* (Melchior, 1834)

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**Summary.** Yellow-necked mice, *Apodemus flavicollis*, were examined for the presence of *Herpetosoma* trypanosomes at the Mazurian Lakeland, Poland. Bloodstream trypomastigotes were used for light microscopy investigations. In the host's blood, slender and stout trypomastigote stages of *Trypanosoma grosi kosewiense* subsp. n. were observed. The body length of the slender form ranged from 16.27 to 28.05 µm, mean 23.00 µm, and width from 0.79 to 2.56 µm, mean 1.43 µm. The free flagellum is 3.66-12.79 µm long, mean 7.59 µm. The kinetoplast is particularly large. Stout forms have a characteristic broad and short body and is 10.78-23.54 µm long, mean 14.74 µm, and 1.18-2.78 µm width, mean 1.76 µm. In contrast to the slender form of *T. grosi kosewiense* and many other trypanosomes, the broadest size of its body falls midway of the PN distance, not at the nuclear level. The free flagellum is 5.06-11.86 µm long. It is easy to notice the wide range of morphometric parameters. The results of light microscopy morphological description and morphometrical values of *Trypanosoma grosi kosewiense* subsp. n. were compared with those of *Trypanosoma grosi* Laveran *et* Pettit, 1909 parasitizing the wood mouse, *A. sylvaticus*, and revealed that trypanosomes parasitizing the yellow-necked mouse are different in many investigated parameters.

**Key words:** *Apodemus flavicollis*, morphology, *Trypanosoma* (*Herpetosoma*) *grosi kosewiense* subsp. n.

## INTRODUCTION

*Herpetosoma* trypanosomes occurring in West and Middle Europe are not very well known. The first records about trypanosomes parasitizing European small rodents were made in the beginning of the 20th century (Thiroux 1905, Laveran and Pettit 1909). Later these trypanosomes were investigated only in the U. S. A and in some Western-European countries (Davis 1952-53, Krampitz 1959, Šebek 1975, Molyneux 1976, Šebek *et al.* 1980, Noyes *et al.* 2002). The studies regarded morphology, primarily; few publications concerned the results of ecological and biochemical investigations. So far, in Europe about 15 species of *Herpetosoma* trypanosomes, parasitizing rodents and insectivores have been recorded. However, new species are constantly being described, as well as new localities of known trypanosomes (Karbowiak and Siński 1996, Karbowiak *et al.* 1998a,b; Macdonald *et al.* 1999). For the description of trypanosomes, Hoare (1972) recommended the following criteria: the host species, the vector species, the morphology and the biology of the trypanosome. Since then, ultrastructural and biochemical criteria were added. On account of the great variability within the *Herpetosoma* subspecies, and incomplete knowledge

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about these parasites, their recognition is difficult on the basis of morphological features only. Presently, for biochemical description, isoenzyme electrophoresis (Mohamed *et al.* 1987), lectin affinity (Maraghi *et al.* 1989) as well as 18S rRNA genotyping (Noyes *et al.* 2002) have been applied. Because a single criterion is insufficient for a majority of trypanosomes (Maraghi *et al.* 1989), the combination of various features (morphological, biological, biochemical) is recommended for the description and recognition of these parasites.

Until recently *Herpetosoma* trypanosomes were not investigated in Poland. Only in the last few years records have appeared about trypanosome infections of the bank-vole, *Clethrionomys glareolus*, and voles from genus *Microtus* (Karbowiak and Siński 1996, Bajer *et al.* 1998, Karbowiak *et al.* 1998b). Trypanosomes infecting yellow-necked mice *Apodemus flavicollis* are practically unknown; however, *Trypanosoma grosi*, infecting the wood mouse, *Apodemus sylvaticus*, is well described in England and Germany (Krampitz 1959, Hoare 1972, Noyes *et al.* 2002).

The *Herpetosoma* trypanosomes are not very pathogenic and make no visual pathological effects to their hosts. The metacyclic forms of trypanosomes infecting mammals penetrate the skin through, or *per os*-when the host swallows the infected vector (Maraghi *et al.* 1995). This way of infection with trypanosomes was showed experimentally by Calvo *et al.* (1992) and Maraghi *et al.* (1995).

## MATERIALS AND METHODS

**Animals.** Yellow-necked mice *Apodemus flavicollis* were investigated in Kosewo Górne, Mazurian Lakeland, Poland, from May 1997 until June 1998. The animals were trapped monthly, using live traps containing oats as bait. After collection of material, the animals were numbered by toe clipping and released where they were trapped. Thus, many individuals were investigated repeatedly during the following months. In summary, 127 yellow-necked mice were examined.

**Detection of trypanosomes infection and morphometric measurements.** Blood samples were obtained from the tail tip of investigated animals. The trypanosomes were detected by centrifugation in microhaematocrit tubes (8 min, 6200 g). They accumulated above the WBCs fraction, and their movements were observed using a light microscopy, using magnifications 10 x 10 and 10 x 20 (eyepiece x objective). Smears were made from the fraction with trypanosomes, as well as from non-centrifuged blood. The smears were fixed in absolute methanol (10 min) and stained with Giemsa's stain, diluted 1:15 in 0.2 M phosphate buffer, pH 7.2 (60 min). Giemsa's reagent stains the cytoplasm of *Herpetosoma* trypanosomes light blue, the nucleus and kinetoplast a violet or purple-blue colour. Because the quality of staining with Giemsa's reagent was poor in the case of smears made from the centrifuged blood, the commercial kit Hemacolor (Kruszewicz 1994) was used.

For the measurements of parasites, the "Analysis" software in combination with a video camera and the Olympus BX50F4 microscopy was used. This method allows obtaining results accurate to 0.01 µm. Stained blood smears were analyzed at a magnification of 1200×. The nomenclature of morphometrics parameters of trypanosomes was that commonly used by other authors (Hoare 1972, Matthews *et al.* 1977, Kingston *et al.* 1992).

Typical trypomastigote forms were selected for measurements. Poorly stained, damaged or deformed individuals were ignored. The data obtained were compared with similar data given by Krampitz (1959), for *Trypanosoma grosi* Laveran *et* Pettit, 1909, parasitizing the *Apodemus sylvaticus*, related to the *Apodemus flavicollis*.

***In vitro* cultures.** The blood of infected mice was obtained by heart puncture and heparinized. Earlier the animals were anesthetized. The blood was inoculated into 4 ml of Veal Infusion Medium (VIM) and into 4 ml of Schneider's *Drosophila* Medium (SDM). The media were supplemented with 20% of foetal calf serum. Moreover, VIM medium also contained 0.2 mg/ml of vitamin $B_{12}$, 100 mg/ml of streptomycin and 100 units/ml of penicillin (Kingston 1973, Morton and Kingston 1976, McHolland-Raymond *et al.* 1978). SDM contained an addition of 100 mg/ml of gentamycin and 0.2 mg/ml of vitamin $B_{12}$ (Mohamed and Molyneux 1987). The cultures were incubated at 26°C and checked every second day, using light microscopy, magnification 1200x.

## RESULTS

Infection with *Trypanosoma grosi kosewiense* subsp. n. was discovered in 6 yellow-necked mice of the 127 caught. Morphometric measurements were made on 240 specimens of trypomastigotes, obtained from 4 heavily infected hosts.

**Morphological characterization.** The trypanosomes in the blood of the yellow-necked mouse occur as trypomastigote forms. Two different morphological forms were observed. A slender form is typical for *Trypanosoma lewisi* group. It has a narrow, long body, with a sharpened posterior end. The length of the body is 16.27-28.05 µm, mean 23.00 µm, the width is 0.79-2.56 µm, mean 1.43 µm. The free flagellum is 3.66-12.79 µm long, mean 7.59 µm. The undulating membrane is weakly developed, however always visible. The nucleus is oval in shape and is located in the middle or in the anterior part of the body, parallel to the long axis. It is 1.42-3.71 µm long, mean 2.68 µm. The kinetoplast is particularly large. It is visible on the blood smears as oval in shape, 0.35-1.46 µm in diameter, mean 0.87 µm, or rod-shaped, 0.42 x 0.75 up to 1.18 x 2.07 µm in size, mean 0.69 x 1.02 µm. It is located near the posterior end of the body (Figs 1, 2A). A wide range of morphometrics parameters (Table 1) was readily noted.

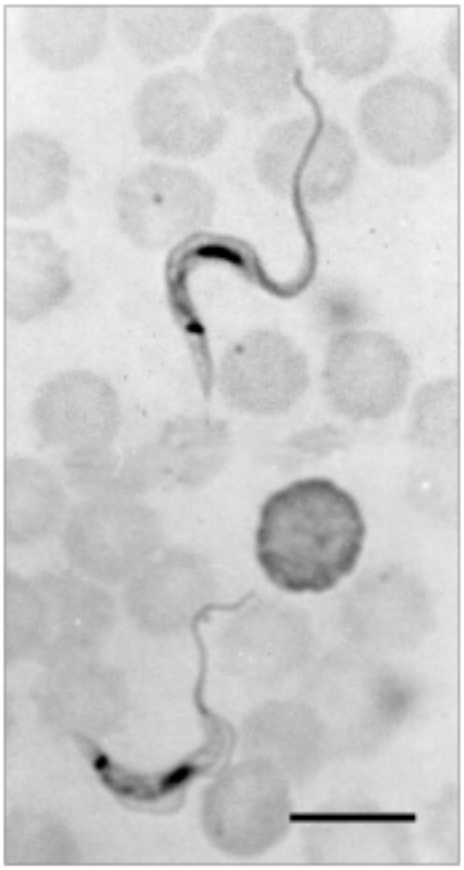

**Fig. 1.** Photomicrographs of bloodstream trypomastigotes of *Trypanosoma grosi kosewiense* subsp. n., from the yellow-necked mouse, *Apodemus flavicollis*, in the Mazurian Lakeland, Poland. Scale bar 10 µm.

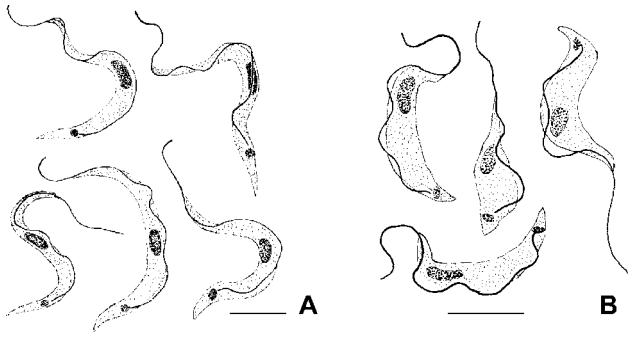


**Fig. 2.** Drawings of Giemsa-stained typical bloodstream trypomastigotes of *Trypanosoma grosi kosewiense* subsp. n., from the yellow-necked mouse, *Apodemus flavicollis*. **A** - slender forms; **B** - stout forms. Scale bars 10 µm.

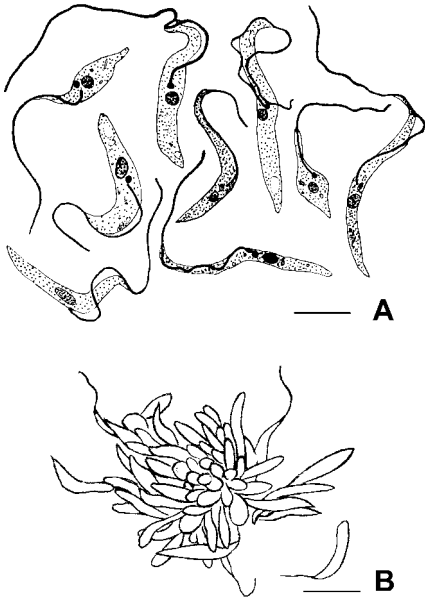


**Fig. 3.** The *Trypanosoma grosi kosewiense* subsp. n., culture forms seen in Schneider's medium. **A** - epimastigotes and promastigotes; **B** - the rosettes of epimastigotes. Scale bars 10 µm.

Stout forms (Fig. 2B) have a characteristic broad and short body. The posterior end is more or less sharp or blunt. The cell is 10.78-23.54 µm long, mean 14.74 µm, and 1.18-2.78 µm in width, mean 1.76 µm. In contrast to the slender form of *T. grosi kosewiense* and many other trypanosomes, the broadest size of its body occurs in the middle of the PN distance, not at the nuclear level. The free flagellum is 5.06-11.86 µm long, mean 8.56 µm. The undulating membrane is well developed. The nucleus, slightly oval or round in shape, is shifted to the anterior end of body. If the nucleus is oval, it is placed parallel, oblique, or in extremely broad individuals, perpendicular to the long axis of the body. The nucleus is 1.64-3.35 µm long, mean 2.38 µm. The kinetoplast is oval or rod-shaped, 0.25 x 0.30 µm to 1.27 x 1.34 µm in size. It is located nearer to the posterior end of the body in stout forms, than in the slender forms. Similarly to the slender forms, there is a wide range of morphometric parameters visible. The data obtained for trypanosomes isolated from the yellow-necked mouse *Apodemus flavicollis* were compared with the same parameters of *Trypanosoma grosi*, presented by Krampitz (1959) and Hoare (1972) (Table1).

**The morphology of *T. grosi kosewiense* subsp. n. in *in vitro* cultures.** *T. grosi kosewiense* subsp. n., isolated from the blood of yellow-necked mouse, grew well in Schneider's medium, and weaker in VIM medium. The trypanosomes occur in *in vitro* cultures in the

**Table 1.** Dimensions (in µm) of slender and stout forms of *Trypanosoma grosi kosewiense* subsp. n. from Mazurian Lakeland compared with *T. grosi* Laveran *et* Pettit, 1909, description by Krampitz (1959).

| Parameter | | PK | KN | PN | NA | BL | FF | No |
|---|---|---|---|---|---|---|---|---|
| *T. grosi kosewiense* f. slender | mean | 3.87 ± 0.65* | 8.34 ± 0.79 | 12.21 ± 1.97 | 10.79 ± 1.98 | 23.00 ± 2.31 | 7.59 ± 1.35 | 160 |
| | range | 2.32-5.78 | 5.73-11.10 | 8.73-15.57 | 5.84-15.42 | 16.27-28.05 | 3.66-12.79 | |
| *T. grosi kosewiense* f. stout | mean | 2.08 ± 0.66 | 7.30 ± 1.42 | 9.37 ± 1.67 | 5.37 ± 1.14 | 14.74 ± 2.29 | 8.56 ± 1.51 | 80 |
| | range | 0.93-4.01 | 3.98-11.12 | 5.71-15.06 | 3.49-8.88 | 10.78-23.54 | 5.06-11.86 | |
| *T. grosi* (Krampitz 1959) | mean | 1.90 | 7.30 | *9.20* | 12.30 | *21.5* | 4.55 | |
| | range | 1.00-2.50 | 6.00-9.00 | - | 10.00-17.50 | - | 1.5-7.6 | |

| Parameter | | L | N | W | NI | KI | FF:BL | No |
|---|---|---|---|---|---|---|---|---|
| *T. grosi kosewiense* f. slender | mean | 30.59 ± 2.23 | 2.68 ± 0.36 | 1.43 ± 0.30 | 1.17 ± 0.25 | 1.47 ± 0.09 | 3.15 ± 0.74 | 160 |
| | range | 23.69-36.17 | 1.42-3.71 | 0.79-2.56 | 0.70-1.95 | 1.23-1.75 | 1.54-6.04 | |
| *T. grosi kosewiense* f. stout | mean | 23.33 ± 2.66 | 2.38 ± 0.34 | 1.76 ± 0.30 | 1.80 ± 0.39 | 1.30 ± 0.11 | 1.77 ± 0.44 | 80 |
| | range | 17.88-31.85 | 1.64-3.35 | 1.18-2.78 | 0.94-2.68 | 1.14-1.74 | 1.04-3.11 | |
| *T. grosi* (Krampitz 1959) | mean | 23.80 | - | - | *0.74* | *1.26* | *4.72* | - |
| | range | 21.00-28.00 | 1.50-3.00 | - | - | - | - | |

Data in italic-unpublished (calculated here on the base of previous data); *-standard deviation; PK-posterior end to kinetoplast; KN-kinetoplast to nucleus center; PN-posterior end to nucleus center; NA-nucleus centre to anterior end; BL-body length; L-total length; FF-free flagellum length; N-nucleus length; No-number of individuals investigated; W-width of body on the nucleus level, excluding the undulating membrane; and indices: nuclear index NI = PN / NA; kinetoplastic index KI = PN / KN; flagellar index FF:BL (= BL/FF). Index NI = 1 demonstrates the central position of the nucleus in the body; NI < 1 demonstrates the position of the nucleus nearer the posterior end of the body; NI > 1 demonstrates the position of the nucleus nearer anterior end of the body. Analogous-KI = 2 demonstrates the location of the kinetoplast midway between the nucleus and the posterior end of the body; KI < 2 the location of the kinetoplast nearer to the posterior end; KI > 2 nearer to the nucleus (Keymer 1967, Hoare 1972). Index FF:BL defines the proportion of the free flagellum to the length of body.

epimastigote and promastigote forms. They are motile for about 10 weeks, later symptoms of degeneration are observed, and the cultures are lost. In the lag phase of culture, rosettes occur. In Schneider's medium they are bigger and more numerous than in VIM medium. After 2-3 weeks, free epi-and promastigote forms appear. At this point the density of trypanosomes reached 5 x $10^6$ of individuals in 1 ml of medium. After 4-5 weeks the size of trypanosomes diminished, the range of dimensions is smaller, and finally the trypanosomes disappeared.

The body length of epimastigotes (Fig. 3A) is 13.00-25.00 µm. The nucleus is oval in shape, the distance of the nucleus center to the anterior end of body is 3-10 µm, and most often was no greater than 5 µm. The distance of the nucleus center from posterior end is 8-15 µm. The kinetoplast is oval and is easily observed stained on slides. The free flagellum is 5-15 µm long. The epimastigotes were often connected in rosette form with the anterior ends (Fig. 3 B).

The shape, body length of promastigotes, and length of flagellum and organelles' size are similar to those found in epimastigotes. Some promastigotes are characterized by a great swelling in the middle of body (Fig. 3A).

**The prevalence, seasonal dynamics and intensity of infection.** The infection of yellow-necked mice with trypanosomes was detected in August, September and October 1997. The prevalence of infection reached

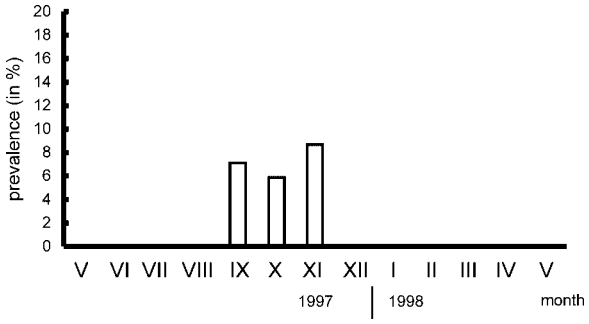


**Fig. 4.** The dynamics of infection of yellow-necked mouse *A. flavicollis* with *Trypanosoma grosi kosewiense* subsp. n., during the period May 1997-May 1998.

8.7% (Fig. 4). In other months the infection with trypanosomes was not detected The intensity of infection was very variable, from 150 trypanosomes up to 300 000 in 1 ml of blood. Both morphological forms of trypanosomes were recovered in the blood of 5 of 6 investigated mice. Their proportions were about 20:1 (slender/stout form), and were similar in each host. The last (sixth) mouse had a low intensity of infection, with slender forms only. The infected mice were caught again in the few following months, however in no individuals was the infection with trypanosomes detected for the second time.

## DISCUSSION

**Morphology.** So far, the trypanosomes parasitizing in yellow-necked mouse, *A. flavicollis*, were seldom found and were recognized originally as *T. grosi* Laveran *et* Pettit, 1909 (Krampitz 1959; Šebek 1960, 1975; Hoare 1972, Šebek *et al.* 1980). However, these recognitions were supported by the similarity of hosts, because the peculiar host of *T. grosi*, the wood mouse *A. sylvaticus* is a close relative to *A. flavicollis*. Other criteria were not considered at the time. The results of our investigations show that trypanosomes parasitizing the yellow-necked mouse are bigger than *T. grosi*. This phenomenon is evident in every investigated parameter. Indices NI and KI show that the nucleus is located closer to the body centre, and the distance between the kinetoplast and the posterior end of the body is longer. The morphological differences between trypanosomes parasitizing *A. sylvaticus* and *A. flavicollis* also were noted by Šebek (1960). Moreover, by the polyphyletic character of *Trypanosoma grosi* parasitizing wood mice (*Apodemus sylvaticus*) in Great Britain was demonstrated (Noyes *et al.* 2002).

The characteristic feature of this parasite is the pleomorphism of trypomastigota forms living in the host's blood, which manifests itself with two morphological forms: slender and stout. This pleomorphism wasn't observed in other rodent's trypanosomes, but is known in *T. (Herpetosoma) rangeli* Tejera, 1920 and in many trypanosomes from the Salivaria section, especially from the *Trypanozoon* subgenus (Hoare 1972, Urdaneta-Morales 1983). The reason for pleomorphism of *T. grosi kosewiense* is not clear at the present. In many cases the morphological differentiation is in correlation with the age of trypanosomes. However, the dividing forms, described in *T. grosi* (Krampitz 1959) and in other trypanosomes parasitizing rodents from the family Muridae, were absent in the blood of trypanosome infected yellow-necked mice.

**Conclusions.** In spite of the described differences between *T. grosi kosewiense* subsp. n., parasitizing *Apodemus flavicollis*, and *Trypanosoma grosi* Laveran *et* Pettit, 1909, there are some counterarguments against a description of a new species. The differences in the morphology may be induced by the peculiarity of their hosts. The variability of morphometric parameters, induced by different hosts was described in other species of trypanosomes (Mathew *et al.* 1992, Dávila *et al.* 1998), however, the change of host has no influence on the location of dividing forms in his body. It was mentioned above, that dividing forms of *T. grosi kosewiense* subsp. n. were not revealed in the peripheral blood of *A. flavicollis*.

The second counterargument is the close relationship between *A. sylvaticus* and *A. flavicollis*, which implicates the possibility of cross infections between these hosts. It is necessary to note that the wood mouse and the yellow-necked mouse live in different habitats. The yellow-necked mice prefer deciduous and coniferous forests, and choose well sheltered and moist habitats. Wood mice favour warm and dry habitats (Pucek 1984). In the case of overlapping areas, the wood mouse is gradually pushed out from the areas attended by yellow-necked mouse individuals (Hoffmeyer 1973). Consequently, the interactions between individuals of the different species are too rare and short, for the efficient exchange of ectoparasites and cross infections with trypanosomes. It is also important that the wood mouse is more numerous than the yellow-necked mouse in the

western Europe, and the yellow-necked mouse is more numerous in the east (Pucek 1984). These factors can have an influence on the morphology and biology of trypanosomes parasitizing these closely related rodents.

In view of the above arguments and counterarguments, we can recognize trypanosomes found in the yellow-necked mouse *A. flavicollis* as a new subspecies, *Trypanosoma (Herpetosoma) grosi kosewiense* subsp. n.

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