# Trypanosoma (Megatrypanum) palawanense n. sp. (Protozoa: Trypanosomatidae) Detected from Rattus panglima Robinson in Palawan Island, the Philippines

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ABSTRACT: Trypanosoma (Megatrypanum) palawanense n. sp. from Rattus panglima Robinson, which was collected in the forest of Palawan Island, the Philippines, was described. The new species is very large with kinetoplast typically situated near the nucleus (KI=8.8~23.0) and far from the posterior end of the body, and approximately 70 microns in total length including the free flagellum and 5 microns in width. Thus, taxonomically the species belongs to the subgenus Megatrypanum Hoare, 1964. This trypanosome is well distinguishable from other described species from Rattus spp. in the large body size and the position of kinetoplast. The life cycle of T. palawanense is still unknown, but mites which have been observed on the body of the host rat, are the most suspicious as possible vectors.

A field survey on the human malaria was carried out in the Iwahig Penal Colony of Palawan Island, the Philippines, during a period from 1969 to 1973 (Nakabayashi et al., 1973 and 1974). At that time blood smears of small wild mammals were also taken for finding out haemoprotozoa in the same survey area (Miyata and Tsukamoto, 1975). A new species of trypanosome was discovered from the smears taken from *Rattus panglima* Robinson, which was trapped in forests. According to Hoare's monograph (1972), three species of trypanosomes, *Trypanosoma* (*Herpetosoma*) lewisi (Kent), *T*. (Schizotrypanum) cruzi Chagas, and *T*. (Megatrypanum) conorhini (Donovan), are known from *Rattus* spp., and all three belong to section Stercoraria. The species described herein as *Trypanosoma* (Megatrypanum) palawanense n. sp. is quite different from the above-mentioned three species and any other known trypanosomes. The description might stimulate other researchers having interests on the evolution and the systematic classification of the mammalian trypanosomes.

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## Trypanosoma (Megatrypanum) palawanense n. sp. (Table 1 & Figs. 1~4)

The morphological features of the trypanosome based on the Giemsa's stained blood-

smears are described below: The body usually slender; 70.4 microns in total length including a free flagellum and 5.0 microns in width at the widest point exclusive of the undulating membrane : the free flagellum rather short (6.0 microns); anterior and posterior ends of body narrow; pale purple cytoplasm usually free from granules or vacuoles; nucleus situated near kinetoplast and the average distance 1.9 microns; dark pink kinetoplast lying marginal, and rather small; shape of nucleus usually round or crescentic, and the colour light pink; the distance from the middle of nucleus to anterior end (27.4 microns) shorter than that to posterior end (36.7 microns).



Fig. 1. Trypanosoma palawanense n. sp. (Holotype smear).

According to Hoare (1972), the nuclear index and the kinetoplast index are useful to be described the morphological feature of trypanosomes. In the case of *T. palawanense*, the nuclear index (NI), the ratio of the distance from the posterior end of the body to the middle of the nucleus to the distance from the latter to the anterior end, is 1.4 in average, and the kinetoplast index (KI), arrived at by dividing the distance from the posterior end to the middle of the nucleus by the distance from the kinetoplast to the middle of mucleus, is  $8.8 \sim 23.0$  (12.4 in average).

Thus this new trypanosome belongs to the subgenus *Megatrypanum* (Hoare, 1964) in the morphological features such as body size and relative position of nucleus and kinetoplast.

The trypanosomes are very few in the host blood, and less than 15 typanosomes were detected in each thin smear, which was prepared from  $0.01 \sim 0.03$  ml of blood taken from heart. The measurement of 25 trypanosomes from a rat are shown in Table 1. In some trypanosomes the shape is moderately broad and the length is rather short as compared with those of the average size. Neither dividing forms nor immature trypanosomes has been observed.

Type smear: Holotype and paratype smears are in the collection of the author in the Department of Epidemiology, Institute for Tropical Medicine, Nagasaki University. Two paratype smears are deposited in the collection of Wellcome Museum of Medical Science, London.

Type host: *Rattus panglima* Robinson (Fig. 5). The host was identified by Dr. Godofredo L. Alcasid, Director of the National Museum, Manila. Several kinds of rats were examined for finding out any haemoprotozoa in the same place, but the trypanosome was never



Fig. 2. Try panosoma palawanense n. sp. (Holotype smear).

found from other rats than R. panglima.

Type locality: The Montible Subcolony, Iwahig Penal Colony, Palawan Island, the Philip pines. The details on the type locality and survey method are described in a separate paper of the same issue by Miyata and Tsukamoto (1975). The host species was abundant in the forest area of the type locality and the parasite rate was 1/20 in January to February, 1971, and 1/19 in the same season of 1972, respectively.

Vector: The natural invertebrate host still remains to be found out, but a number of mites were found on the body of the host rats. The mites, which are one of suspicious vectors for this trypanosome, were not examined in the present survey.



Fig. 3. Body structure of Trypanosoma palawanense used for measurements shown in Table 1.



Fig. 4. Trypanosoma palawanense n. sp. (Paratype smears).

|         |      |      |              |     | -    |     |     |     |      |       |
|---------|------|------|--------------|-----|------|-----|-----|-----|------|-------|
|         | TL   | P-N  | A-N          | K-N | ΡK   | FF  | W   | NL  | KI   | NI    |
|         | 74.5 | 39.5 | 29           | 4.5 | 35   | 6   | 4   | 3   | 8.8  | 1.4   |
|         | 73.5 | 36.5 | 31           | 3.5 | 33   | 6   | 5   | 3   | 10.4 | 1.2   |
|         | 73   | 34   | 32           | 3   | 31   | 7   | 4   | 3   | 11.3 | 1.1   |
|         | 70   | 41   | 24           | 3   | 38   | 5   | 5   | 2   | 13.7 | 1.7   |
|         | 64   | 33   | 25           | 3   | 30   | 6   | 3.5 | 2.5 | 11.0 | 1.3   |
|         | 76.5 | 42.5 | 28           | 3   | 39.5 | 6   | 3.5 | 2.5 | 14.2 | 1.5   |
|         | 76   | 39   | 29           | 3   | 36   | 8   | 4   | 3   | 13.0 | 1.3   |
|         | 70   | 37   | $27^{\circ}$ | 2.5 | 34.5 | 6   | 4   | 2.5 | 14.8 | 1.4   |
|         | 64   | 30   | 28           | 3   | 27   | 6   | 4   | 3   | 10.0 | 1.1   |
|         | 63.5 | 34.5 | 24           | 2.5 | 32   | 5   | 4   | 2.5 | 13.8 | 1.4   |
|         | 66   | 35   | 26           | 3   | 32   | 5   | 4   | 2   | 11.7 | 1.3   |
|         | 71   | 38   | 28           | 3   | 35   | 5   | 4   | 2   | 12.7 | 1.3   |
|         | 67   | 36   | 26           | 2   | 34   | 5   | 4   | 2   | 18.0 | . 1.4 |
|         | 63   | 34   | 24           | 3   | 31   | 5   | 7   | 3   | 11.3 | 1.4   |
|         | 72   | 37   | 28           | 3   | 34   | 7   | 4   | 2   | 12.3 | 1.3   |
|         | 68   | 36   | 26           | 3   | 33   | 6   | 4   | 2   | 12.0 | 1.4   |
|         | 74   | 36   | 33           | 3   | 33   | 5   | 4   | 3   | 12.0 | 1.1   |
|         | 66   | 35   | 25           | 3.5 | 31.5 | 6   | 4   | 3   | 10.0 | 1.4   |
|         | 73   | 37   | 31           | 3.5 | 33.5 | 5   | 5   | 3   | 10.6 | 1.2   |
|         | 64   | 35   | 21           | 3   | 32   | 8   | 7   | 3   | 11.7 | 1.7   |
|         | 80   | 46   | 28           | 2   | 44   | 6   | 4   | 2   | 23.0 | 1.6   |
|         | 70   | 38   | 27           | 3   | 35   | 5   | 4   | 2   | 12.7 | 1.4   |
|         | 72   | 39   | <b>26</b>    | 4   | 35   | 7   | 8   | 4   | 9.8  | 1.5   |
|         | 69   | 35   | 28           | 3   | 32   | . 6 | 4   | 2   | 11.7 | 1.3   |
| e       | 71   | 34   | 30           | 3.5 | 30.5 | 7   | 4   | 3   | 9.7  | 1.1   |
| Average | 70.4 | 36.7 | 27.4         | 3.1 | 33.7 | 6.0 | 5.0 | 2.6 | 12.4 | 1.4   |
| Maximum | 80   | 46   | 33           | 4.5 | 44   | 8   | 8   | 4   | 23.0 | 1.7   |
| Minimum | 63   | 30   | 21           | 2   | 27   | 5   | 3.5 | 2   | 8.8  | 1.1   |
|         |      |      |              |     |      |     |     |     |      |       |

Table 1. Size, in microns, of Trypanosoma palawanense n. sp. from Rattus panglima

TL : Total length including free flagellum

P -N : Posterior end to middle of nucleus

A-N : Anterior end to middle of nucleus

K-N : Kinetoplast to middle of nucleus

P-K : Posterior end to kinetoplast

FF : Free flagellum

W : Width at the widest point

NL : Nuclear length at the longest point

KI : Kinetoplast Index=P-N/K-N

NI : Nuclear Index=P·N/A·N

RELATION BETWEEN THE NEW SPE-CIES AND OTHER KNOWN TRYPANO-SOMES

Totally 29 species of the subgenus Megatrypanum had been described from various mammals (Hoare, 1972). Morerecently, Trypanosoma cyclops Weinman, 1972, which might belong to the subgenus, was recorded from Malaysian



Fig. 5. *Rattus panglima* Robinson (dorsal: brownish grey; ventral: white)

monkeys, Macaca nemestrina and M. ira. Then, T. palawanense is the 31st species of the members of the subgenus, and is easily distinguishable from others in the following two features. 1) T. palawanense is the largest species in the average length (average 70 microns) among the known mammalian trypanosomes except T. ingens Bruce, Hamerton, Bateman, and Mackie, 1909, (the total length of the latter is  $72\sim130$  microns). 2) The kinetoplast situates adjuscent to the nucleus and KI is  $8.8\sim23.0$  (average 12.4) which is the highest value ever recorded from mammalian trypanosomes.

Trypanosomes of *Rattus* spp.: According to Hoare (1972) and his personal cominucation (1974), three species of trypanosomes were known from *Rattus* spp., and among them, *Trypanosoma* (*Herpetosoma*) *lewisi* (Kent) and *T*. (*Schizotrypanum*) *cruzi* Chagas are quite different from *T*. (*Megatrypanum*) *palawanense* in size and shape. The former two species are smaller than the present trypanosome.

T. (Megatrypanum) conorhini (Donovan) likewise resembles morphologically T. palawanense. T. conorhini was originally described from triatomine bugs, Triatoma rubrofasciata (syn. Conorhinus rubrofasciatus), from Madras, India, and was named Crithidia conorrhini by Donovan in 1909. The natural vertebrate host of this trypanosome was first discovered by Bonne (1937). He found the host, Rattus rattus diardi, by xenodiagnosis with laboratory-bred larvae of the bugs, because no trypanosomes could be detected by direct examination of the blood of the rats. Concerning this trypanosomes, Morishita (1935) studied the complete life cycle in Formosa, and showed beautiful colour figures of murine blood-stage of the parasite. According to his work, the kinetoplast of the trypanosome is rather large. In addition, a vacuole-like structure, which is stained almost uniformly reddish or pinkish with Giemsa staining and nearly as large as the nucleus, is usually seen in front of the kinetoplast. This feature is not seen in T. palawanense; and moreover T. conorhini is rather small in total length  $(36.0 \sim 62.0 \text{ micron})$  and free flagellum is rather long  $(7.3 \sim 15.0 \text{ microns})$ , though the trypanosome is similar to T. palawanense in the situation of kinetoplast and nucleus and in slender shape of the body. Thus, T. palawanense is an unrecorded species from Rattus spp.

In addition, the natural vertebrate host of *T. conorhini* was discovered by xenodiagnosis, and also in the case of *T. (Megatrypanum) theodori* Hoare, 1931, trypanosome of Palestinian goat, *Capra hircus mambrica*, the blood stage is still unknown, but the trypanosome is known only by xenodiagnosis with goat keds, *Lipoptena capreoli*. Those facts mean that parasitaemia in the host blood is very low. Morishita (1935) mentioned as follows: "Since this trypanosome (T. conorhini) does not undergo active multiplication in the mammalian blood, it is quite difficult to discover them in the peripheral blood stream in case of slight infection."

(see p. 497 in his paper). In *T. palawanense*, very few parasites were detected from each blood smear, and also most of species belonging to the subgenus *Megatrypanum* are difficult to detect by the direct examination of the blood smear. Originally *T. palawanense* was found during examination of a thick blood smear for microfilariae under low magnification. If an immersion lens would be used for detection of haemoprotozoa, this large trypanosome could not be detected. It can be assumed that the same trypanosome of *Rattus* spp. might be widely distributed in other parts of East Asia, particularly Malay Archipelago, though where no information is available concerning large-type trypanosomes other than *T. conorhini*.

Other trypanosomes of subgenus Megatrypanum detected from various mammals: T. palawanense shows a general resemblance to T. (M.) pifanoi Marinkelle and Duarte, T.(M.) hoarei Davis, and T. (M.) leonidasdeanei Zeledon and Rosabal. Especially T. pifanoi found from Colombian bats, Artibeus lituratus and Phyllostomus hastatus, is quite similar to T. palawanense in the slender body, the short free flagellum, and the kinetoplast index. From the description by Marinkelle and Duarte (1968) and Hoare (1972), however, the total length of T. pifanoi is a range of  $30.8 \sim 53.6$  microns including the short free flagellum ( $1.5 \sim 4.8$  microns)and KI is  $5.7 \sim 15.4$ , which is the highest record except that T. palawanense. According to my estimation based on the original description of this species, KI is a range of  $5.7 \sim$ 15.4(8.0 in average). In Hoare's book (1972), however, KI of T. pitanoi is written to be 8  $\sim 33$ . Dr. Hoare informed me that his calculations of KN and PN were based on the distances to the edge of the nucleus (not the middle). If the method is applied to T. palawanense, the range of KI would be  $17 \sim 46$  instead of  $8.8 \sim 23.0$ .

In *T. hoarei*, discovered by Davis (1969) in the blood of American shrews (*Sorex* spp.) from California, the total length and width of the trypanosomes are about 34 microns and 6 microns respectively, and the distance between the kinetoplast and the middle of the nucleus is 6 microns (KI=2). The total length and width of *T. leonidasdenei*, which was described from an insectivorus white-lined bat (*Saccopteryx bilineata*) in Costa Rica (Zeledon and Rosabal, 1969), are about 44 microns and 5 microns (KI=4.8~8.0). In both species the free flagellum is very short, and the kinetoplast lies near the nucleus, but these trypanosomes are much smaller than *T. palawanense*.

From the above pointed reasons, it is concluded that T. *palawanense* is a well established species distinguishable from other described trypanosomes. The new species is named after the type locality, Palawan Island, the Philippines.

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フィリピン・パラワン島産野生ネズミ Rattus panglima Robinson から検出された大型トリパノゾーマの新種 Trypanosoma (Megatrypanum) palawanense n. sp. (原生動物・トリパノゾーマ科)
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フィリピン・パラワン島の森林内で、 捕獲した野生ネズミ Rattus panglima Robinson の血液中から 発見した大型のトリパノゾーマは、 今まで全く知られていない種であるので、 ここに Trypanosoma (Megatrypanum) palawanense n. sp. と命名記載した. 従来, Rattus 属のネズミからは, 全世界的 に分布する T (Herbetosoma) lewisi の他に, 中南米に分布し, 人体にも感染する T (Schizotrypanum) cruzi と, アジアおよびアメリカから記録されている T. (Megatrypanum) conorhini の3 種類のトリパノゾーマが知られている. T. (M.)palawanense は、 鞭毛を含む体長が70ミクロン, 体 幅は5ミクロン,核は体中央よりやや前方に位置し,核指数1.4,キネトブラストは小さく,核の2ミ クロン後方にあり、遊離鞭毛は短かく、6.0ミクロンであるなど、多くの点で、上記3種とは明瞭に区 別できる.本種のキネトプラスト指数は、8.8~23.0(平均12.4)で、既知の哺乳類のトリパノゾーマ のうちでは、最も高い値をしめす.また平均体長では、T. (Megatrypanum) ingens (体長 72~130 ミ クロン)についで大きい. 一般的外観では, 中南米のコウモリやキネズミのトリパノゾーマと似てい るが、新しく記載した種類は、大きさ、 キネトプラストと核の距離、 核の位置などから、既知種と全 く異なり、爬虫類のトリパノゾーマのあるものと形態的に似ている.本種は、単に Rattus 属に寄生す る第4番目のトリパノゾーマであるというだけではなく、実験室でも Rattus 属に感染するこのような 大型の種類は知られていないので、 このトリパノゾーマの生活史を明らかにし、 実験系統として確立 できるならば、原虫研究者にとって大変有益であろう.

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