*RAFFLES BULLETIN OF ZOOLOGY* 1994 **42**(3): 539-550

# FOUR NEW TRYPANOSOMES (PROTOZOA: TRYPANOSOMATIDAE) IN THE BLOOD OF A MALAYSIAN FROG, *RANA BLYTHI* (AMPHIBIA: RANIDAE)

**Akira Miyata** and **H. S. Yong**

*ABSTRACT.* - Four new amphibian trypanosomes, *Trypanosoma midaii, Trypanosoma ampanense, Trypanosoma maleisiense,* and *Trypanosoma bulat* were described in the blood of a Malaysian frog, *Rana blythi* from Peninsular Malaysia and Sarawak. We examined 190 individuals of the frog and in addition to four new species, we also detected *Trypanosoma rotatorium*-type and *Trypanosoma chattoni*-type. The prevalence of all six species of trypanosomes in *R. blythi* in Malaysia was summarized and discussed.

## INTRODUCTION

*Rana blythi* Boulenger (Ranidae) is a large forest frog easily found at night near small streams flowing through the primary and secondary forests in Peninsular Malaysia and Sarawak. Formerly, this frog was identified as a tuberculated narrow-headed form of *Rana macrodon* Dumeril & Bibron (see Berry, 1975). *Rana blythi* may grow to a large size; the largest individuals examined by us reached 14 centimeters in body length. The frog is sold in the local market and eaten by the non-Muslims.

We collected 190 individuals of *R. blythi* in various localities of Peninsular Malaysia and Sarawak in 1989 and 1990, and six species of trypanosomes were detected. Four of the trypanosomes are described herein as new species. The other two species resemble *Trypanosoma chattoni* (Mathis & Leger, 1911) and *Trypanosoma rotatorium* (Mayer, 1883) respectively, and as such we defer the determination of their species status until the completion of our comparative study on the morphology of these groups detected in various Malaysian frogs and toads.

**Akira Miyata** - Department of Biology, Medical College of Oita, Hazama, Oita 879-56, Japan. **H. S. Yong**- Department of Zoology, University of Malaya, 59100 Kuala Lumpur, Malaysia.

## MATERIALS AND METHODS

The blood survey was carried out at twelve localities (Table 1) in Malaysia during the period from August 1989 to October 1990. One hundred and ninety individuals of *Rana blythi* were collected from Selangor (Ulu Gombak, Templer Park, Ulu Langat and Ampang), Pahang (Bukit Rengit and Gua Musang of Taman Negara), Johor (Kota Tinggi), Penang (the Botanical Garden), and Sarawak (Sematan, Plaman Batang, Stinggang, and Matang). Two thin blood smears were prepared from the cardiac blood of each frog. After fixation in absolute methanol, the smears were stained in 3% Giemsa solution for one hour. They were examined at x200 and all the detected trypanosomes were photographed at x500 under oil immersion for the comparison of the morphology of each parasite. The measurements shown in micrometers in the descriptions of species and Tables were calculated from enlarged colour prints.

Type smears used in this study were deposited in the Department of Biology, Medical College of Oita, Japan, and the Department of Zoology, University of Malaya, Malaysia.

Table 1. Prevalence of trypanosome species in *Rana blythi* in Malaysia

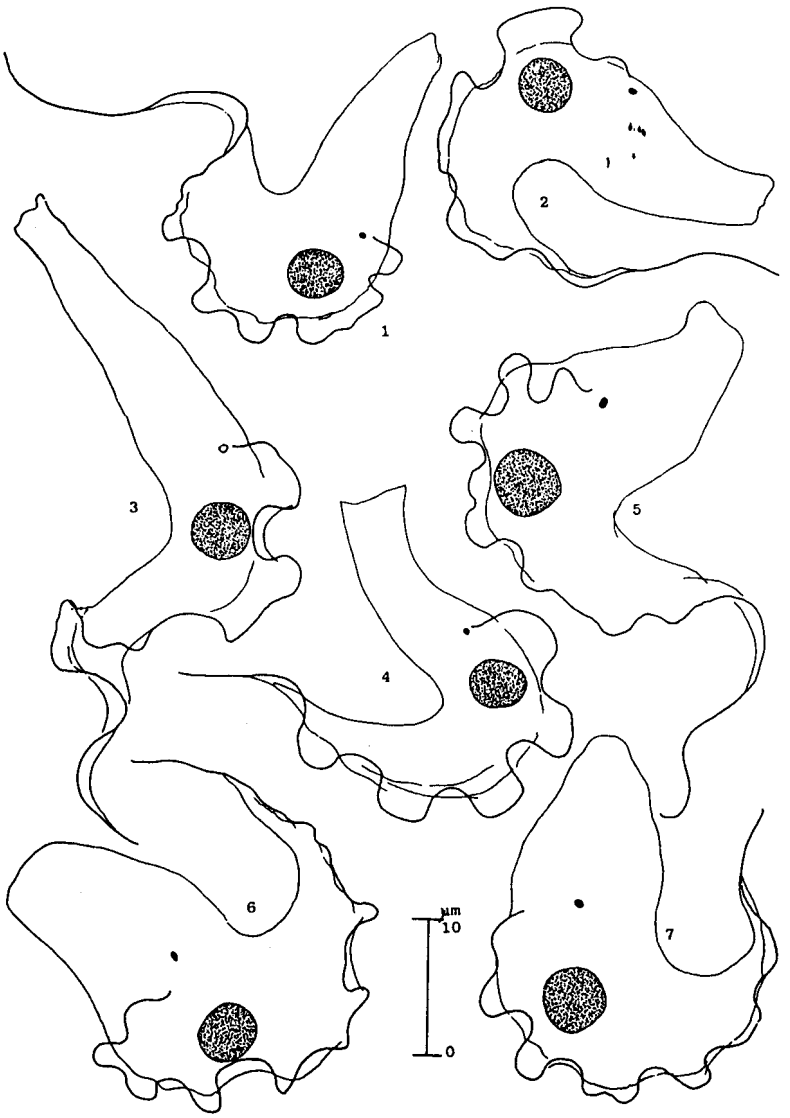
| Locality | No. Frogs | chat | rota | mida | ampa | male | bula |
|---|---|---|---|---|---|---|---|
| **Peninsular Malaysia** | | | | | | | |
| Gombak | 16 | 13 | 5 | 5 | | | 2 |
| Templer Park | 6 | 4 | | 1 | | | |
| Ampang | 70 | 32 | 11 | 3 | 2 | | 3 |
| Gua Musang | 8 | 3 | 1 | | | | |
| Bukit Rengit | 23 | 10 | 4 | 3 | | 9 | |
| Ulu Langat | 3 | 1 | 2 | 1 | | | |
| Kota Tinggi | 2 | 2 | 2 | 1 | | | |
| Penang | 17 | 2 | 1 | | | | |
| **Sarawak** | | | | | | | |
| Matang | 1 | | | | | | |
| Sematan | 41 | 25 | | | | | 7 |
| Plaman Batang | 1 | | | | | | |
| Stinggang | 2 | 1 | 1 | 1 | | 1 | 1 |
| Total | 190 | 93 | 34 | 15 | 2 | 10 | 13 |

chat: *Trypanosoma chattoni*-type; rota: *Trypanosoma rotatorium*-type; mida: *Trypanosoma midaii*, new species; male: *Trypanosoma maleisiense*, new species; ampa: *Trypanosoma ampanense*, new species; bula: *Trypanosoma bulat*, new species

## DESCRIPTIONS OF NEW SPECIES

### *Trypanosoma midaii*, new species
(Plate 1, Table 2)

***Description***. - Broad trypanosome (Plate 1, Figs. 5-7) with round or blunt posterior end, but slender form (typical form of this trypanosome; Plate 1, Figs. 1-4) with truncated posterior end; undulating membrane conspicuous; usually cytoplasm darkly stained with Giemsa except posterior end of slender form, and kinetoplast and nucleus difficult to observe; body length 39.4-58.6 micrometers; thickness at the thickest part of body 6.3-16.6




Pl. 1. *Trypanosoma midaii*, new species. Figs. 1-4, Slender form (typical form). Figs. 5-7, Broad form.

Table 2. Measurements of *Trypanosoma midaii*, new species in *Rana blythi*

| No. | BL | TN | PK | KN | AN | F | NL |
|---|---|---|---|---|---|---|---|
| 1 | 42.9 | 12.0 | 10.0 | 5.1 | 22.9 | 10.0 | 4.3 |
| 2 | 40.0 | 11.4 | 11.4 | 3.4 | 20.0 | 8.6 | 4.6 |
| 3 | 51.4 | 6.3 | 21.4 | 3.4 | 23.7 | 8.6 | 4.0 |
| 4 | 40.0 | 6.6 | 11.4 | 2.9 | 34.3 | 2.9 | 3.4 |
| 5 | 42.9 | 10.0 | 8.6 | 4.6 | 25.7 | - | 4.0 |
| 6 | 42.9 | 11.1 | 11.4 | 8.6 | 11.4 | 12.9 | 3.7 |
| 7 | 58.6 | 13.1 | 17.1 | 5.7 | 32.9 | - | 4.6 |
| 8 | 45.7 | 16.6 | 10.0 | 7.1 | 20.0 | 14.3 | 2.9 |
| 9 | 45.7 | 16.6 | 10.0 | 7.1 | 20.0 | 14.3 | 3.4 |
| 10 | 41.4 | 8.6 | 12.0 | 4.3 | 21.4 | 9.1 | 3.7 |
| 11 | 39.4 | 9.4 | 14.3 | 6.9 | 20.0 | 10.0 | 4.0 |
| AVG | 44.6 | 10.3 | 12.7 | 5.2 | 23.2 | 9.1 | 3.9 |
| MAX | 58.6 | 16.1 | 21.4 | 8.6 | 34.3 | 14.3 | 4.6 |
| MIN | 39.4 | 6.3 | 8.6 | 2.9 | 11.4 | 2.9 | 2.9 |
| SD | 5.50 | 2.86 | 3.50 | 1.69 | 6.01 | 3.22 | 0.50 |
| VAR | 30.27 | 8.16 | 12.28 | 2.86 | 36.11 | 10.40 | 0.25 |

BL: Body length excluding free flagellum; B: Breadth at broadest part of body; TN: Thickness of body at thickest part; FF: Length of free flagellum; PN: Distance from posterior end of body to posterior edge of nucleus; PK: Posterior end of body to kinetoplast; KN: Kinetoplast to posterior edge of nucleus; AN: Anterior end of body to anterior edge of nucleus; N: Diameter of nucleus; AVG: Average; Max: Maximum; Min: Minimum; SD: Standard deviation; VAR: Variance

micrometers; length of free flagellum 2.9-14.3 micrometers; distance from posterior end of body to kinetoplast 8.6-21.4 micrometers; distance from kinetoplast to posterior edge of nucleus 2.9-8.6 micrometers; diameter of round nucleus 2.9-4.6 micrometers.

***Type smear***. - Holotype smear: Slide No. 89-10-16-22 from Gombak. Paratype smears: Slide No. 89-10-18-21 from Ulu Langat, No. 89-09-15-08 from Kota Tinggi, No. 89-10-18-32 from Gombak, and No. 90-01-20-01 from Gombak.

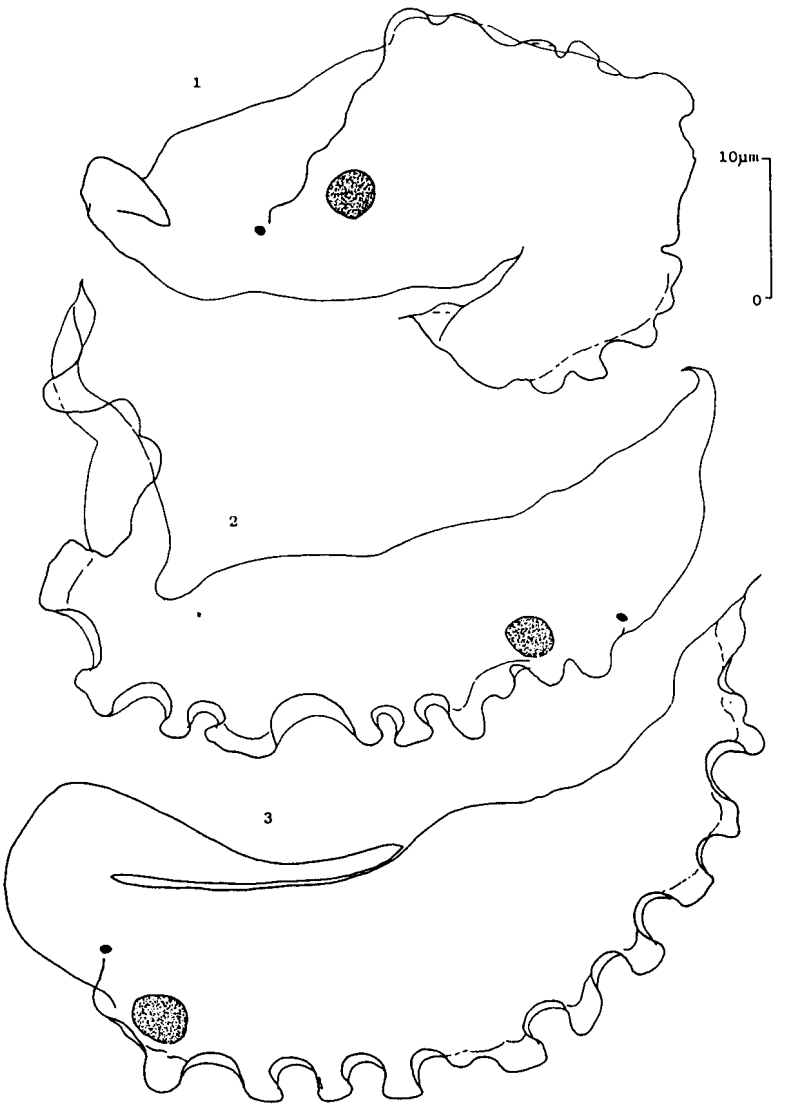
***Remarks***. - *Trypanosoma midaii* is a very common species in Peninsular Malaysia, but it is very rare in Sarawak as shown in Table 1. We found several individuals of *T. midaii* in a blood smear of *R. blythi* caught at Stinggang, Sarawak. This trypanosome is easily distinguished from other species found in amphibians by the truncated posterior end of the slender form. Its movement in fresh materials is very sluggish.

***Etymology***. - The species name is derived from the late Mr. Midai Ak. Ninbon, in memory of his co-operation with us. He was an excellent collector of amphibians and worked at the Sarawak Museum as a technician for almost 30 years.

***Trypanosoma ampanense*, new species**
(Plate 2, Table 3)

***Description***. - Large species; both body ends pointed; undulating membrane conspicuous with 10-13 peaks; body length 62.8-80.0 micrometers; thickness at thickest part of body 12.0-20.3 micrometers; kinetoplast close to nucleus; distance from posterior end of body to kinetoplast 13.7-18.6 micrometers; distance from kinetoplast to posterior edge of nucleus 2.6-4.9 micrometers; length of free flagellum 3.4-13.7 micrometers; small circular nucleus present near base of undulating membrane; length of nucleus 3.9-6.3 micrometers.

***Type smears***. - Holotype Smear: No. 90-01-08-04 from Ampang Forest Reserve. Paratype Smear: No. 90-01-08-49 from Ampang Forest Reserve.




Pl. 2. *Trypanosoma ampanense*, new species.

Table 3. Measurements of *Trypanosoma ampanense*, new species, in *Rana blythi*

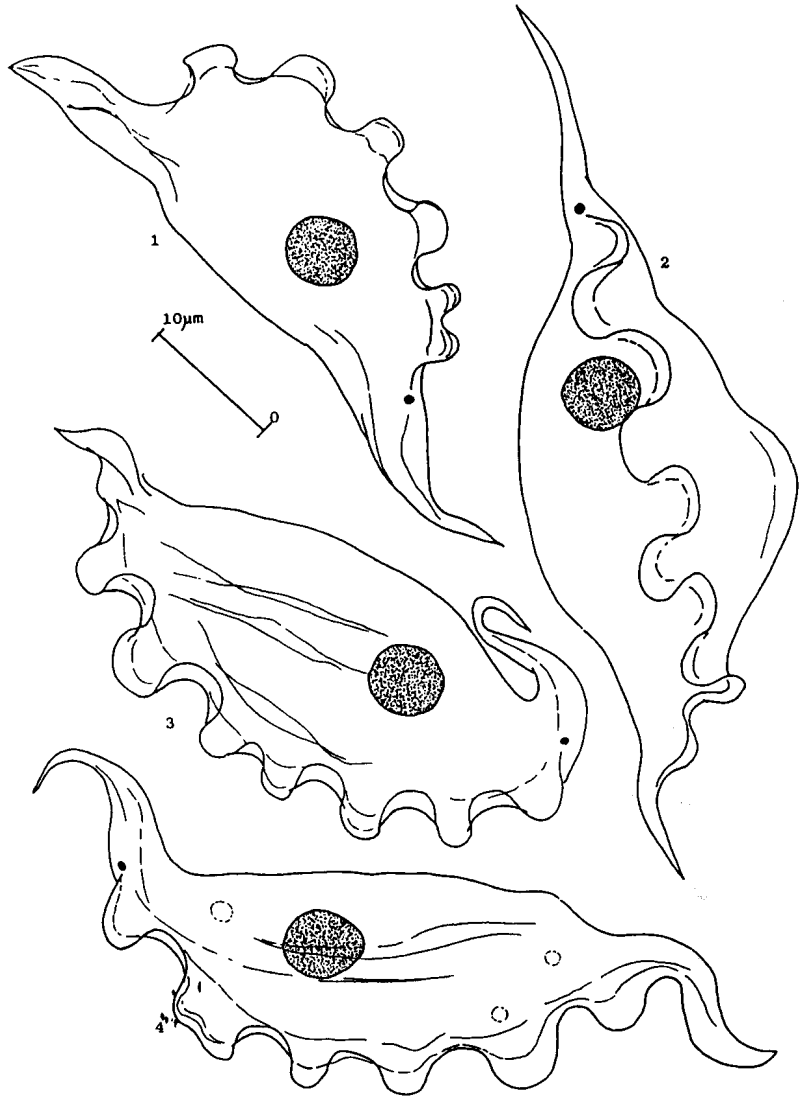
| No. | BL | TN | KN | PK | NL | F |
|---|---|---|---|---|---|---|
| 1 | 72.0 | 14.3 | 2.8 | 15.7 | 4.0 | 10.9 |
| 2 | 66.0 | 17.1 | 3.1 | 14.3 | 4.6 | 8.9 |
| 3 | 72.0 | 13.4 | 3.3 | 14.9 | 4.3 | 9.4 |
| 4 | 80.0 | 13.7 | 4.0 | 14.3 | 4.9 | 3.4 |
| 5 | 71.4 | 13.7 | 2.6 | 16.9 | 4.6 | 8.6 |
| 6 | 73.7 | 12.0 | 3.1 | 15.7 | 4.0 | - |
| 7 | 77.7 | 14.9 | 3.9 | 15.7 | 3.9 | - |
| 8 | 71.4 | 14.3 | 2.9 | 14.3 | 4.9 | 13.7 |
| 9 | 77.1 | 12.3 | 4.6 | 15.7 | 3.9 | 8.6 |
| 10 | 73.7 | 13.4 | 4.6 | 14.3 | 4.3 | 6.3 |
| 11 | 74.3 | 14.0 | 3.4 | 15.7 | 4.3 | - |
| 12 | 76.6 | 12.0 | 4.9 | 17.1 | 4.3 | 9.1 |
| 13 | 68.6 | 13.7 | 3.1 | 13.7 | 4.9 | - |
| 14 | 71.4 | 13.4 | 4.6 | 14.3 | 4.9 | 7.1 |
| 15 | 65.7 | 12.3 | 3.1 | 17.1 | 4.6 | 6.3 |
| 16 | 62.8 | 20.3 | 4.0 | 15.7 | 4.9 | - |
| 17 | 65.7 | 15.7 | 3.6 | 18.6 | 6.3 | - |
| AVG | 71.8 | 14.1 | 3.6 | 15.5 | 4.6 | 8.4 |
| MAX | 80.0 | 20.3 | 4.9 | 18.6 | 6.3 | 13.7 |
| MIN | 62.8 | 12.0 | 2.6 | 13.7 | 3.9 | 3.4 |
| SD | 4.64 | 2.00 | 0.70 | 1.28 | 0.56 | 2.55 |
| VAR | 21.56 | 4.00 | 0.49 | 1.63 | 0.32 | 6.50 |

***Remarks***. - This species closely resembles *Trypanosoma miyagii* Miyata, 1978 from Okinawa Island and *Trypanosoma pseudomiyagii* Molan *et al.*, 1989 from Iraq, in body size and shape, but apparently different in the situation of nucleus, which, in the case of *T. ampanense*, is located near the posterior part of the body and is very close to the kinetoplast. This trypanosome was found in *R. blythi* collected at Ampang Forest Reserve. In addition to it, it was detected in a blood smear of a frog taken by Mr. Rosni Sarjan at Gua Musang, Taman Negara, in 1990; the host frog specimen (No. GM15) was lost before identification but it might be *R. blythi*.

***Etymology***. - The species is named after the type locality, Ampang Forest Reserve.

***Trypanosoma maleisiense*, new species**
(Plate 3, Table 4)

***Description***. - Fusiform trypanosome; both ends of body gradually pointed at about one third from anterior end of body; several ridges present along body axis; length of body 51.4-74.2 micrometers; thickness of body at thickest part 10.9-22.9 micrometers; undulating membrane conspicuous with 7-8 peaks; distance from posterior end of body to kinetoplast 8.6-24.3 micrometers; distance from kinetoplast to posterior edge of nucleus 5.7-22.9 micrometers; length of nucleus 4.0-6.0 micrometers; kinetoplast very small; free flagellum usually absent.




Pl. 3. *Trypanosoma maleisiense*, new species. Figs. 1, 3, 4, Lateral View; Fig. 2, Dorsal View.

Table 4. Measurements of *Trypanosoma maleisiense*, new species, in *Rana blythi*

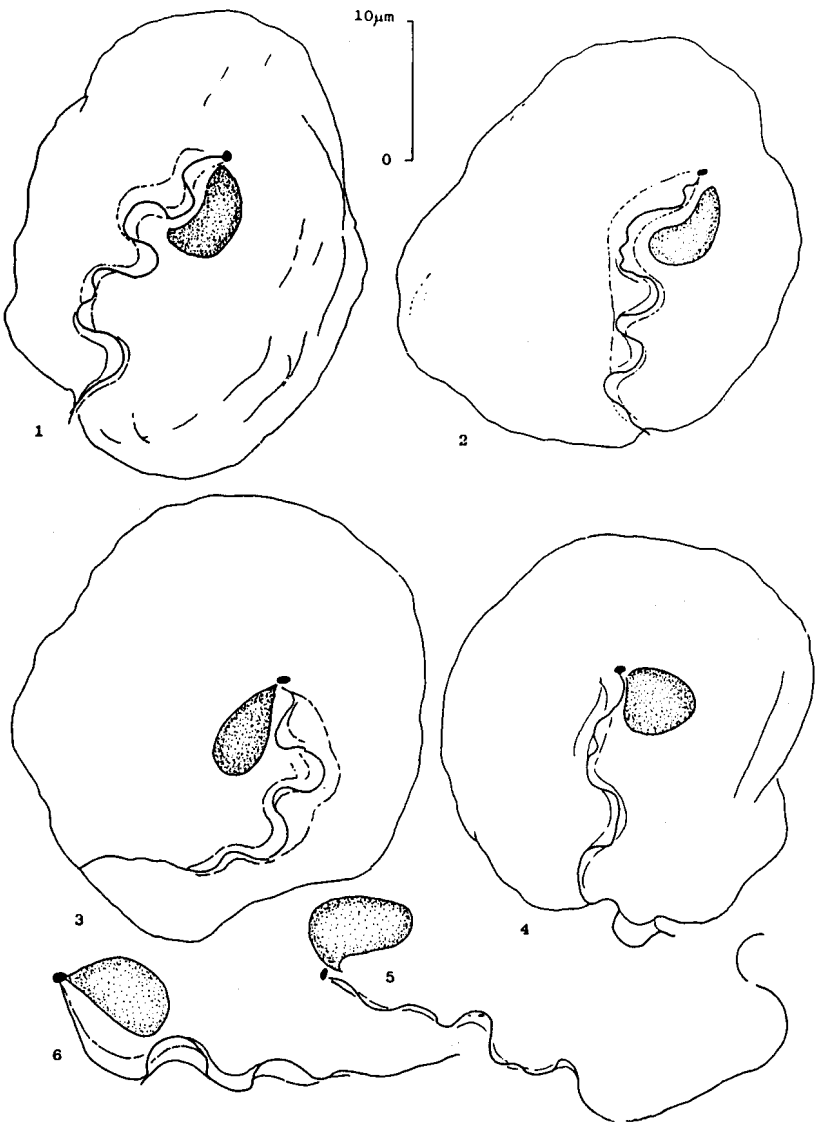
| No. | BL | TN | PK | KN | AN | NL |
|---|---|---|---|---|---|---|
| 1 | 72.4 | 12.8 | 14.3 | 5.7 | 20.0 | 4.6 |
| 2 | 51.4 | 13.4 | 14.3 | 8.6 | 22.9 | 5.1 |
| 3 | 57.1 | 14.9 | 14.3 | 8.6 | 25.7 | 4.9 |
| 4 | 60.0 | 15.7 | 17.1 | 11.4 | 25.7 | 5.6 |
| 5 | 65.7 | 15.1 | 24.3 | 5.7 | 28.6 | 5.1 |
| 6 | 65.7 | 18.9 | 14.3 | 8.6 | 32.9 | 5.1 |
| 7 | 67.1 | 17.7 | 12.9 | 5.7 | 40.0 | 5.1 |
| 8 | 60.0 | 16.6 | 14.3 | 5.7 | 32.9 | 5.1 |
| 9 | 54.3 | 17.7 | 12.9 | 11.4 | 22.9 | 5.7 |
| 10 | 62.9 | 14.3 | 12.9 | 6.3 | 34.3 | 5.3 |
| 11 | 62.9 | 16.0 | 13.7 | 5.7 | 35.7 | 5.1 |
| 12 | 57.1 | 14.9 | 17.1 | 8.6 | 25.7 | 5.1 |
| 13 | 65.7 | 12.9 | 17.1 | 22.9 | 20.0 | 4.3 |
| 14 | 54.3 | 12.3 | 17.1 | 11.4 | 22.9 | 4.3 |
| 15 | 62.9 | 16.9 | 17.1 | 11.4 | 28.6 | 4.9 |
| 16 | 61.4 | 13.7 | 18.6 | 15.7 | 25.7 | 4.6 |
| 17 | 61.4 | 14.3 | 15.7 | 5.7 | 34.3 | 4.9 |
| 18 | 60.0 | 16.6 | 14.3 | 5.7 | 32.9 | 5.1 |
| 19 | 51.4 | 16.0 | 14.3 | 8.6 | 20.0 | 4.9 |
| 20 | 54.3 | 11.4 | 8.6 | 10.0 | 31.4 | 4.0 |
| 21 | 58.6 | 14.3 | 14.3 | 11.4 | 27.1 | 4.6 |
| 22 | 52.9 | 14.9 | 14.3 | 6.3 | 31.4 | 4.3 |
| 23 | 62.9 | 13.7 | 17.1 | 8.6 | 28.6 | 4.9 |
| 24 | 57.1 | 16.6 | 14.9 | 17.1 | 21.4 | 5.1 |
| 25 | 64.3 | 18.3 | 14.3 | 5.7 | 42.3 | 4.6 |
| 26 | 60.0 | 15.4 | 14.3 | 13.7 | 21.4 | 5.1 |
| 27 | 62.9 | 16.6 | 17.1 | 11.4 | 28.6 | 5.1 |
| 28 | 64.3 | 13.7 | 14.3 | 10.0 | 34.3 | 4.9 |
| 29 | 62.9 | 10.9 | 10.9 | 10.0 | 32.9 | 5.0 |
| 30 | 54.3 | 16.9 | 12.9 | 8.6 | 27.1 | 5.1 |
| 31 | 51.4 | 14.3 | 14.3 | 8.6 | 22.9 | 4.9 |
| 32 | 62.9 | 14.3 | 17.1 | 8.6 | 34.3 | 4.3 |
| 33 | 58.6 | 15.1 | 17.1 | 9.7 | 25.7 | 5.1 |
| 34 | 54.3 | 14.9 | 14.3 | 7.1 | 30.0 | 4.6 |
| 35 | 65.7 | 14.3 | 14.3 | 14.3 | 32.9 | 5.7 |
| 36 | 51.4 | 15.7 | 17.1 | 14.3 | 22.9 | 4.6 |
| 37 | 61.4 | 16.0 | 14.3 | 5.7 | 27.1 | 5.4 |
| 38 | 60.0 | 22.9 | 18.6 | 8.6 | 25.7 | 6.0 |
| AVG | 59.9 | 15.3 | 15.2 | 9.6 | 28.4 | 5.0 |
| MAX | 74.2 | 22.9 | 24.3 | 22.9 | 42.3 | 6.0 |
| MIN | 51.4 | 10.9 | 8.6 | 5.7 | 20.0 | 4.0 |
| SD | 5.18 | 2.17 | 2.52 | 3.72 | 5.51 | 0.42 |
| VAR | 26.87 | 4.70 | 6.34 | 13.85 | 30.32 | 0.17 |

***Type Smears.*** - Holotype Smear: Slide No. 90-05-26-43 from Bukit Rengit. Paratype Smears: Slide No. 99-05-26-41, No. 90-02-06-04, No. 90-02-06-09, and No. 90-02-06-03 all from Bukit Rengit; No. 90-05-08-18 from Stinggang, Sarawak.

***Remarks.*** - This trypanosome is quite distinct in the body shape (Plate 3) and we could not find any similar species among several hundreds of known trypanosomes in the world. *T. maleisiense* was detected only in Bukit Rengit, Peninsular Malaysia, and Stinggang, Sarawak.

***Etymology.*** - This species is named after Malesia.

***Trypanosoma bulat*, new species**
(Plate 4, Table 5)




Pl. 4. *Trypanosoma bulat*, new species. Figs. 1-4, Lateral View; Figs. 5-6, Connection of kinetoplast and nucleus observed outside destroyed trypanosome.

Table 5. Measurements of *Trypanosoma bulat*, new species, in *Rana blythi*

| No. | BL | PK | B | NL | F |
|---|---|---|---|---|---|
| 1 | 27.4 | 8.6 | 25.7 | 5.7 | 8.6 |
| 2 | 26.6 | 8.0 | 22.9 | 5.7 | - |
| 3 | 21.7 | 6.6 | 22.9 | 4.9 | - |
| 4 | 27.1 | 7.1 | 20.6 | 4.9 | - |
| 5 | 30.0 | 13.7 | 24.6 | 5.7 | - |
| 6 | 29.7 | 12.3 | 25.1 | 4.9 | - |
| 7 | 28.9 | 10.3 | 22.9 | 5.4 | - |
| 8 | 27.1 | 7.7 | 24.3 | 6.3 | - |
| 9 | 29.7 | 14.0 | 22.3 | 5.1 | 4.9 |
| 10 | 28.6 | 6.9 | 28.6 | 6.9 | - |
| 11 | 30.9 | 11.1 | 28.6 | 6.9 | - |
| 12 | 30.9 | 9.7 | 24.6 | 6.3 | - |
| 13 | 29.7 | 9.7 | 25.7 | 5.1 | - |
| 14 | 31.1 | 10.9 | 24.3 | 7.4 | - |
| AVG | 28.5 | 9.8 | 24.5 | 5.8 | - |
| MAX | 31.1 | 14.0 | 28.6 | 7.4 | - |
| MIN | 21.7 | 6.6 | 20.6 | 4.9 | - |
| SD | 2.39 | 2.35 | 2.15 | 0.81 | - |
| VAR | 5.71 | 5.52 | 4.62 | 0.65 | - |

***Description***. - Oval or elliptical trypanosome; body very thin, sheet-like; both ends rounded; undulating membrane seen on dorsal surface; length of body 21.7-31.1 micrometers; width of body 20.6-28.6 micrometers; distance from posterior end of body to kinetoplast 6.6-14.0 micrometers; kinetoplast very close or connected to posterior edge of nucleus; nucleus elliptical and posterior end of nucleus somewhat pointed like Japanese persimmon (kaki) stone; length of nucleus 4.9-7.4 micrometers; undulating membrane with 4-5 peaks; free flagellum usually absent but if present its length 4.9-8.6 micrometers.

***Type smear***. - Holotype smear: Slide No. 90-01-08-43 from Ampang Forest Reserve. Paratype smears: Slide No. 89-10-18-28 from Gombak, No. 90-01-08-01 from Ampang Forest Reserve, and No. 90-01-08-73 from Ampang Forest Reserve.

***Remarks***. - The body of *T. bulat* is very thin and can only be observed on its dorsal view as shown in Plate 4. Usually the dorsal view of trypanosomes is very difficult to observe and therefore in the present paper the lateral view was used for the descriptions of the other three new species as shown in Plates 1-3. *T. bulat* somewhat resembles *Trypanosoma chattoni* in the outline of the body shape, but the former species is easily distinguished from the latter by its conspicuous undulating membrane.

***Etymology***. - The species name "*bulat*" means "round" in Malay. The name is used as a noun in apposition.

***Trypanosoma* cf. *rotatorium***
**and *Trypanosoma* cf. *chattoni***

These two species resemble *T. rotatorium* and *T. chattoni*, respectively, in the shape of the body. Trypanosomes belonging to *T. rotatorium*-type and *T. chattoni*-type are very common in various frogs and toads, and some of them may be new species. However, at present we reserve the analysis of these species until the completion of our survey of the amphibian trypanosome fauna in East Asia.

## DISCUSSION

*Rana blythi* is rather common in the forest areas of Peninsular Malaysia and many individuals are captured and sold as food in the local market. Because of its wide distribution the trypanosome fauna of *R. blythi* is expected to be uniform in all localities. However, some trypanosomes occur widely but others appear to be limited to one or two localities. The prevalence of all the six species of trypanosomes recorded in *R. blythi* in Malaysia is summarized in Table 1. Both the prevalence and trypanosome species differed from locality to locality. *Trypanosoma chattoni*-type was detected in ten localities and was very common in *R. blythi*. *Trypanosoma rotatorium*-type was present in nine localities, but its detection rate was lower than that of the former type. *Trypanosoma midaii* was more common in Peninsular Malaysia than Sarawak. *Trypanosoma bulat* was also detected in Peninsular Malaysia and Sarawak, but the detection rate was quite low except Sematan, Sarawak. *Trypanosoma ampanense* and *Trypanosoma maleisiense* were each limited to one locality, with the former being the rarest species among the six trypanosomes. *Trypanosoma maleisiense* was rather common at Bukit Rengit.

Among the four new species, *T. ampanense* is noteworthy because similar trypanosomes are reported over a wide range of Asia, from Iraq to Okinawa. One of them, *Trypanosoma miyagii,* is very common in several frog species belonging to the genus *Rana* in Okinawa. However, *T. ampanense* is very rare in Peninsular Malaysia and Sarawak and has not been detected in other frogs captured at the Ampang Forest Reserve and other localities. Also the distribution of *T. maleisiense* is apparently limited to Bukit Rengit in Peninsular Malaysia, and in spite of our extensive study, we could not detect the species in other frogs.

## ACKNOWLEDGEMENTS

We are deeply indebted to the Vice-Chancelor, University of Malaya, the Socio-Economic Research Unit of the Prime Minister's Department of Malaysia, and the Director of Sarawak Museum, for supporting this research, and to Dr. Hideo Hasegawa, Ryukyu University, the late Mr. Midai Ak. Ninbon, Sarawak Museum, Mr. Ng Cheong Kee, Mr. Rosni Sarjan and Che Rohani Haji Che Wil, Department of Zoology, University of Malaya, for their assistance.

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