Anuran Haemoprotozoa Found in the Vicinity of Nagasaki City

1. *Trypanosoma rotatorium* (Mayer, 1843)

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ABSTRACT : During a period of July to August, 1974, blood examinations of frogs and tadpoles were carried out to find out any haemoparasite in Mogi, near Nagasaki City. The following results were obtained. 1) *Trypanosoma rotatorium* (Mayer, 1843) is detected from 50% of adults and 67% of tadpoles in *Rana rugosa*, and in the case of *Rana nigromaculata*, although only 5 adults could be collected, 2 of them had *T. rotatorium*. 2) Three morphological types were distiguished as follows; Type Ⅰ : This is the smallest among them, and the total length including free flagellum is 38∼49.5μ and the width at the widest point is 1.5∼2μ. Trypanosomes belonging to this type are usually observed in the blood of tadpoles, except only one case of this type was obtained from an apparently very young frog. Type Ⅱ : This type is a large form with the pointed posterior end and the narrow undulating membrane, measuring 60∼80μ in the total length including a short free flagellum and 3∼8μ in the width at the widest part. This type was found only from the tadpole. Type Ⅲ : This type includes various shapes of trypanosome detected from the adults, and most of the parasite have a very large round body and the round posterior end. Short free flagellum and narrow undulating membrane with many waves are usually observed, but absence of undulating membrane and free flagellum was observed in some individuals. This type is never found from the blood of tadpole. 3) The leeches, *Hirudo nipponia* were observed in the same place where the tadpoles were captured. Then the intestinal contents of the leeches were examined, in which few trypomastigotes and epimastigotes were detected. These forms might belong to *T. rotatorium*. 4) *Dactylosoma ranarum* (Kruse, 1890) were also detected from blood smears of some adult frogs of *R. rugosa*, as reported in the second paper of this series.

Trypanosoma rotatorium (Mayer, 1843) was originally described from European frogs (*Rana* spp.) as *Amoeba rotatoria*, and somewhat later of the description, Gruby (1843) erected the geuns *Trypanosoma* (type species: *Trypanosoma sanguinis* Gruby, 1843). *A. rotatoria* is believed identical with *T. sanguinis*, which became a synonym of *Trypanosoma rotatorium* (now type species of the genus). Lankester (1871) described *Undulina ranarum* from the frog blood, but this is also a synonym of *T. rotatorium*. At present more than 40 species of *Trypanosoma* from frogs and toads have been named by various authors (see Bardley

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and Harmsen, 1973). Diamond (1958) studied these trypanosomes and arranged to 26 species, but unfortunately the present author could not see the paper. Several species are distinguishable from others by morphological features, but most of anuran trypanosomes are polymorphic and very difficult to distinguish species.

T. rotatorium is a polymorphic and widely distributed species and from Japan, the following frogs were reported as hosts; *Rana esculenta* (maybe other species, because *R. esculenta* is not known from this country), *R. temporaria* (maybe *R. japonica japonica*), and *R. rugosa* by Koidzumi (1911). Tanabe (1931) described morphological features of *T. rotatorium*, which was detected from *R. nigromaculata* captured in Korea. According to his paper, four morphological types were distinguishable.

During a period of July to August, 1974, the present author had an opportunity to examine the blood smears of *Rana rugosa* and *R. nigromaculata*, which were collected in Mogi district, near Nagasaki City. From the blood smears, *Trypanosoma rotatorium* and *Dactylosoma ranarum* (Kruse, 1890) were detected. In the present paper, the author will describe morphological features of *T. rotatorium* detected from the smears, and in the second paper some observations concerning *D. ranarum* will be reported.

MATERIALS AND METHODS

A total of 22 adults and 12 tadpoles of *Rana rugosa* Schlegel and 5 adults of *Rana nigromaculata* Hallowell were collected in paddy fields of Mogi district, near Nagasaki City, between July to August, 1974. The blood was taken from heart, and before staining a small drop of the blood was examined to find out living trypanosomes or other parasite, then thin blood smears were prepared from each animal, and after fixation by absolute methyl alcohol, the smears were stained in 3% Giemsa's solution for 30 minutes. Examination of the stained smears were carried out by an immersion lense (×100) for 200 fields, then if being negative for parasites, the smear was checked by a low-power objective lenses (×20 or ×40). Stamp smears prepared from various organs were also stained in Giemsa's solution, and examined. The scientific names of the frog used by Nakamura and Ueno (1974) were adopted in the present paper.

RESULTS

Trypanosoma rotatorium (Mayer, 1843) is detected from 11 (50%) of adults and from 8 (67%) of tadpoles in *Rana rugosa*. In *R. nigromaculata*, only five adults could be examined and two of them had *T. rotatorium* in their bloods. In any case, parasitaemia is very low, particullarly in adults. In Table 1 and 2, the results of the microscopic examination were summarized.

a. Morphology

Trypanosomes detected from *R. rugosa* and *R. nigromaculata* are a polymorphic species. Apparently adult frogs had large well-grown trypanosomes as shown in Fig. 1, a～h, but

Table 1. Detection rate of haemoparasites from frogs and tadpoles

Host	No. examined	*Trypanosoma rotatorium*	*Dactylosoma ranarum*
Rana nigromaculata			
Adult	5	2 (40%)	0
Rana rugosa			
Adult	22	11 (50%)	8 (36%)
Tadpole	12	8 (67%)	0

Table 2. Detailed records on positive case of haemoparasites from frogs

Host No.	Size* (cm)	*Trypanosoma rotatorium*		*Dactylosoma ranarum*	
		Types of trypanosomes	Figure	Infection rate in 1000 erythrocytes	Figure
Rana nigromaculata					
Adult					
1974−14	4.8	Ⅲ	1 (a, b)**	—	
1974−23	8.9	Ⅲ	1 (f)	—	
Rana rugosa					
Adult					
1974−1	>4	Ⅲ		2	3**(c,d,i,n,r,t) 4 (e,i)
1974−4	>4	Ⅲ		<1	
1974−5	>4	Ⅲ		5	
1974−13	4.6	Ⅲ	1 (b)	—	
1974−17	5.5	Ⅲ	1 (c)	7	3 (a,b,e,h,j,m,o,q,s,v,w) 4 (b,f,h,j)
1974−18	4.4	—	1 (e)	<1	
1974−19	3.9	—		2	
1974−26	4.6	Ⅲ		1	
1974−27	4.3	Ⅲ	1 (g)	14	3 (u,w) 4 (a,c,d,k,l)
1974−29	3.9	—		—	
1974−33	2.3	Ⅰ	1 (i, k)	—	
1974−34	4.0	Ⅲ	1 (h)	—	
Tadpole					
1974−35	2 legs	Ⅰ		—	
1974−36	2 legs	Ⅰ		—	
1974−37	2 legs	Ⅱ	2 (a, b)	—	
1974−40	2 legs	Ⅰ	2 (k)	—	
1974−47	2 legs	Ⅱ	2 (c, e, g)	—	
1974−51	no leg	Ⅰ	2 (i)	—	
1974−52	no leg	Ⅰ		—	
1974−55	no leg	Ⅰ, Ⅱ	2 (b, h, j)	—	

* Size: measured from nose to anus

** 1 (a, b) : Trypanosomes detected from the blood of frog No. 1974−14 are shown in Fig. 1, a and b. Figs. 3 and 4 means Figs. 1 and 2 in the second paper. (Trop. Med., 18(3), 138−139, 1976)

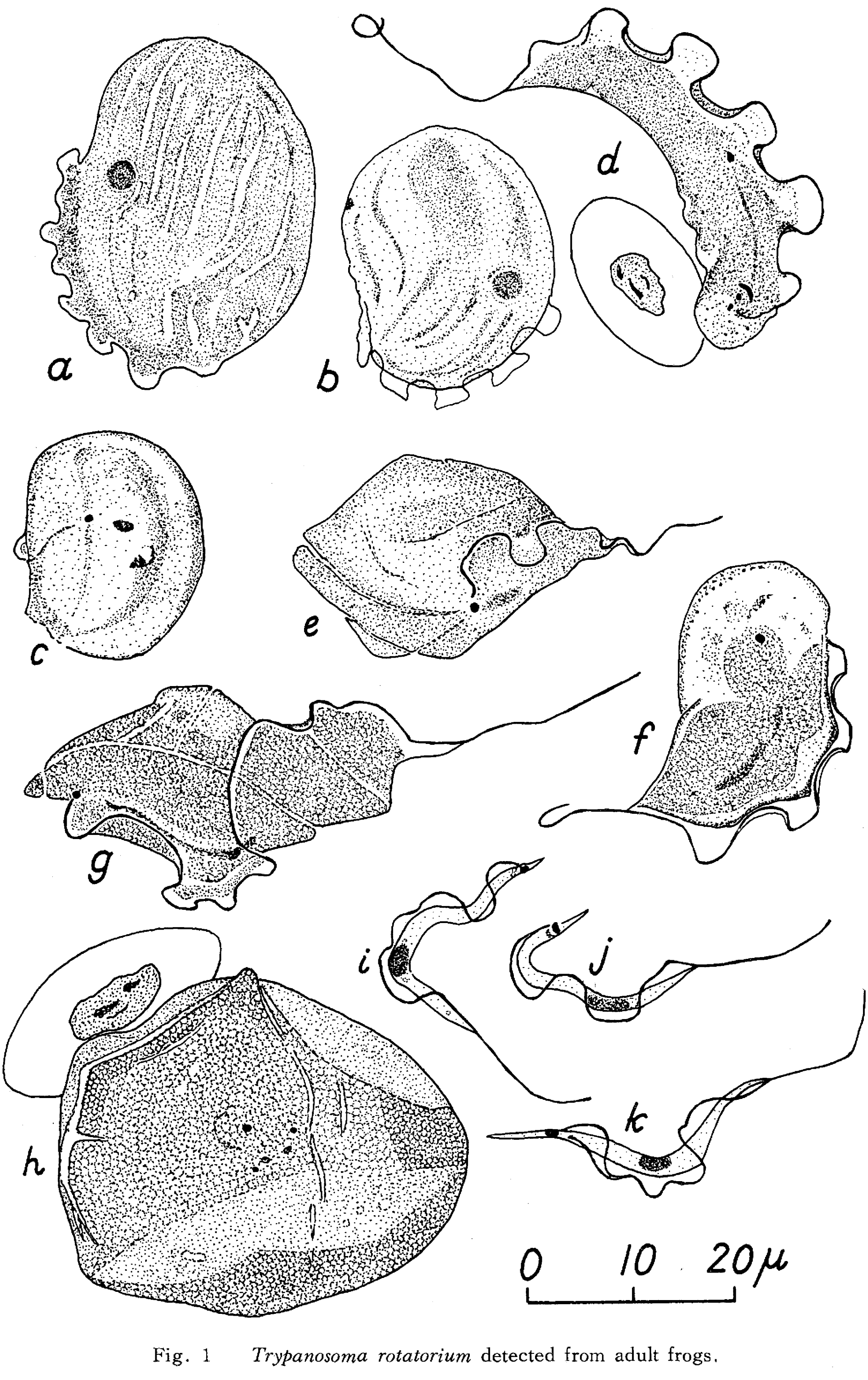

Fig. 1 *Trypanosoma rotatorium* detected from adult frogs.

from one of small frogs, small trypanosomes were detected (Fig. 1, i～k). Tadpoles of *R. rugosa* had various shapes of trypanosomes, but most of parasites were apparently small size as shown in Fig. 2, h～k. The detected trypanosomes can be distinguished morphologically into the following three types:

Type I: (Fig. I, i～k and Fig. 2, h～j) This is the smallest form and the following dimensions were obtained: posterior end to kinetoplast=2.5～5.5μ; kinetoplast to posterior margin of nucleus=7.5～16.5μ; length of nucleus=2～2.5μ; anterior margin of nucleus to anterior end of body=11～17μ; length of free flagellum=13～19μ; total body length including free flagellum=38～49.5μ; width at the widest part of the body=1.5～2μ. Nucleus is ellipsoidal and undulating membrane is rather wide with 3～4 waves. The body is lightly

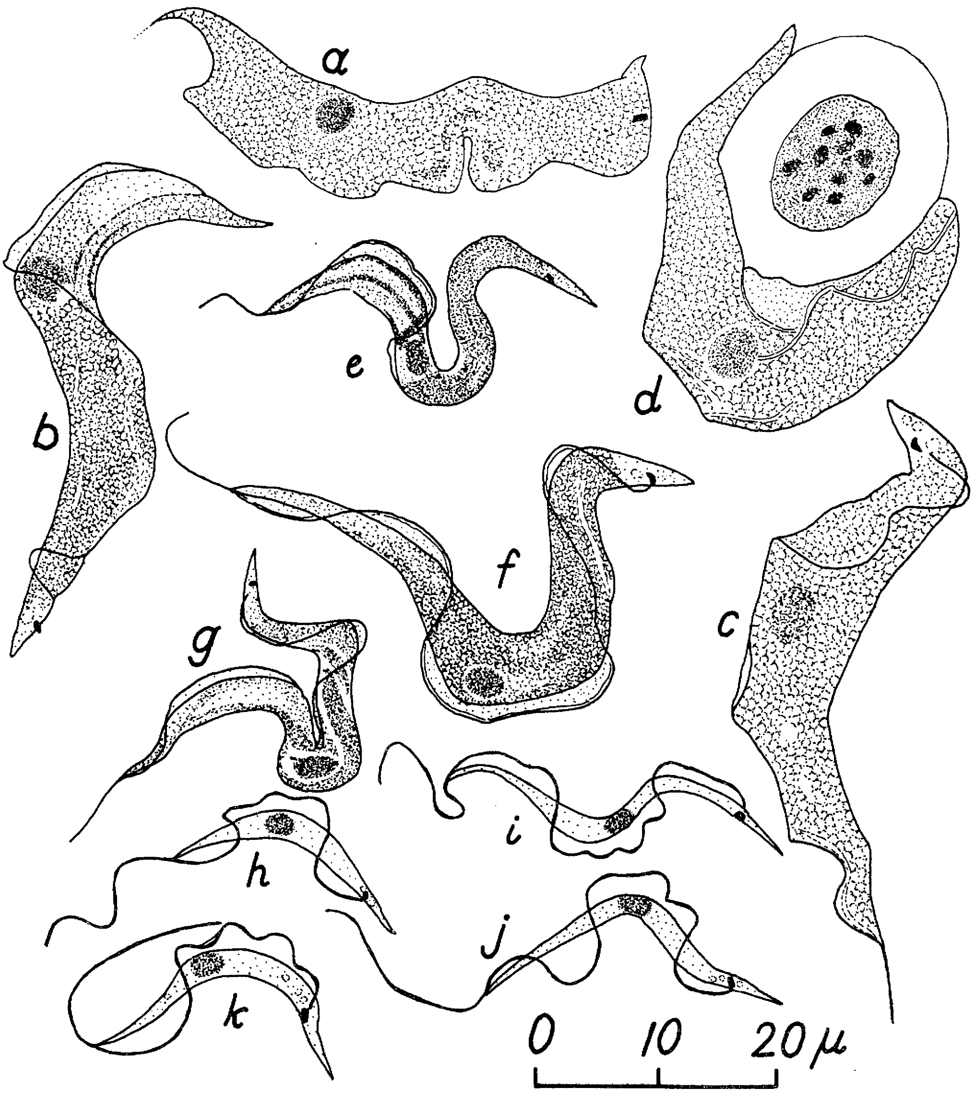

Fig. 2 *Trypanosoma rotator ium* detected from tadpoles.

stained with Giemsa.

Type Ⅱ : (Fig. 2, a～g) This is the large trypanosome with the pointed posterior end, narrow undulating membrane and small round nucleus, measuring 60～80μ in total length including short free flagellum and 3～8μ in width at the widest point. The body is rather darkly stained except posterior end and anterior part.

Type Ⅲ : (Fig. 1, a～h) This type include various shapes of trypanosomes, and these polymorphic parasites can be distinguished into two or three sub-types furthermore. Most of parasites have the round posterior end and the round body with short free flagellum and narrow undulating membrane with many waves.

Type Ⅲ is detected from frogs but is never found in the blood of tadpole. In contrast, type I is predominant in the tadpole, namely, 6 out of 8 trypanosome-positive cases were observed. In frogs, type I is detected from only one case, and the frog is apparently young and recently finished the metamorphosis. Type Ⅱ is also predominant in tadpoles but is not yet detected from adults. A trypanosome shown in Fig. 1, d, is similar to type Ⅱ, but the round posterior end and many waves in undulating membrane are different from type Ⅱ. In some cases, however, the round body without undulating membrane nor free flagelum was seen as shown in Fig. 1, h. It appears that trypanosome grows type Ⅰ to type Ⅲ in relation to the growth of the host animal (Fig. 4). Types of trypanosomes detected from each animal examined were shown in Table 2.

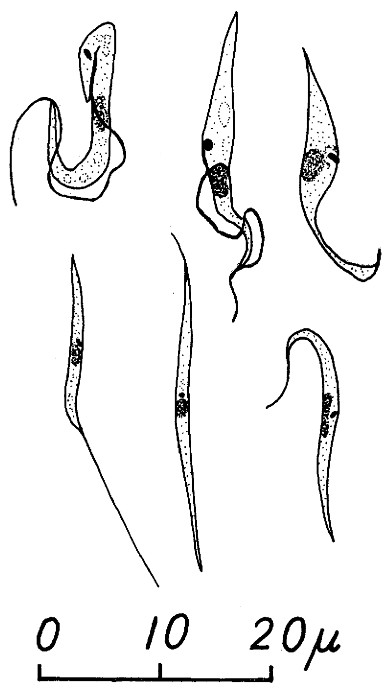

Fig. 3 *Trypanosoma rotatorium* detected from leeches.

b. Vector

In the field, several leeches belonging to *Hirudo nipponia* could be collected together with tadpoles. The leeches intensely took blood from tadpoles. The leeches were killed and several smears of the intestinal contents were stained with 3% Giemsa's solution after fixation by methyl alchohol. From the smears very few trypanosomes were found as shown in Fig. 3. These flagellates might belong to *T. rotatorium*, but further study is needed to the identification.

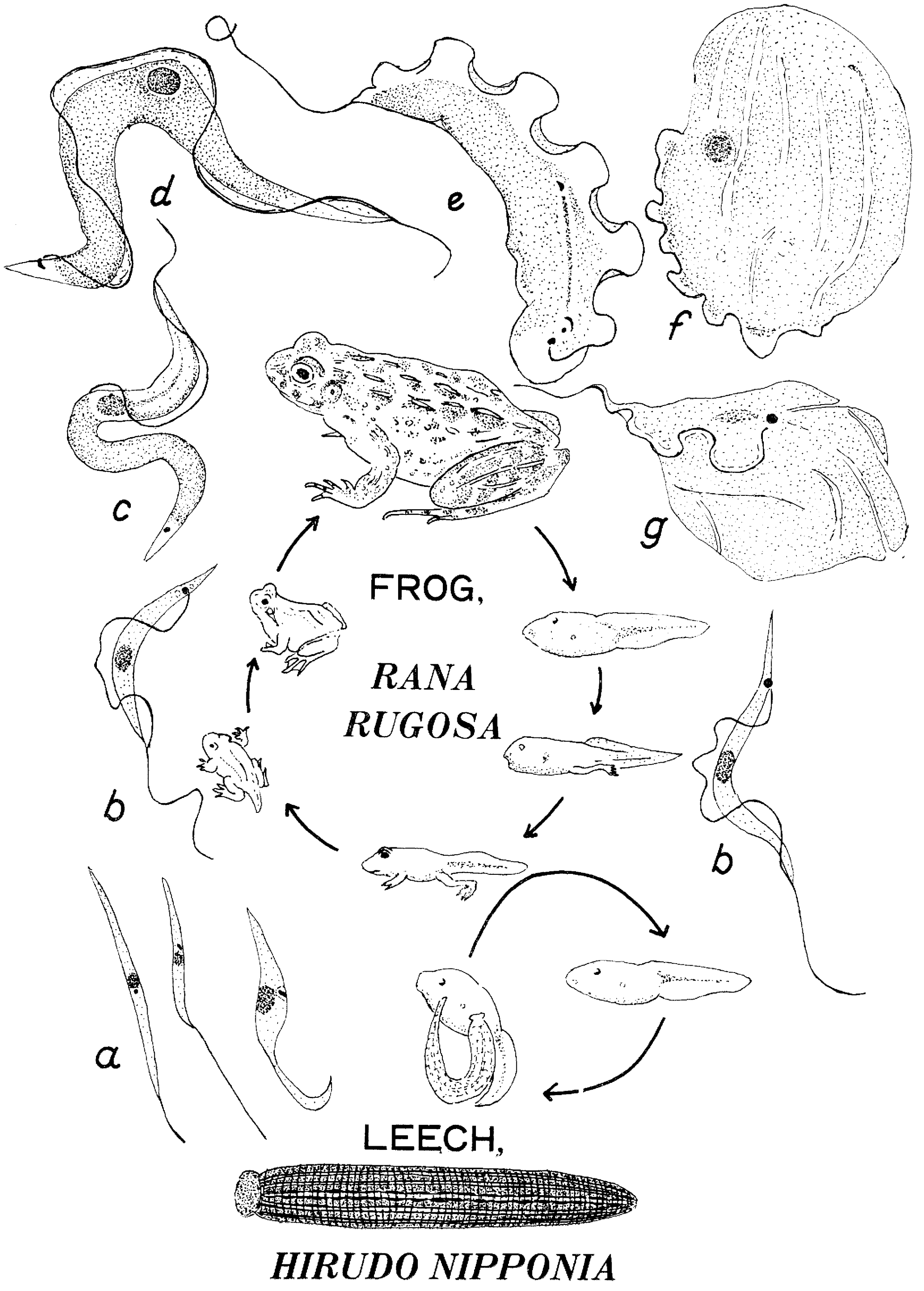

Fig. 4 Possible life cycle of *Trypanosoma rotatorium*.

DISCUSSION

Trypanosoma rotatorium (Mayer, 1843) is a very complicated species, and under this name different type of trypanosomes were reported for example Desser *et al.* (1973) and Fantham *et al.* (1942). Therefore, the present author could not determine whether all individuals shown in Figs. 1 and 2 in this paper belong to one species or not. Nöller (1913) and Wenyon (1926) had described similar trypanosomes as *T. rotatorium*, and Koidzumi (1911) and Tanabe (1931) also identified their materials as this species. At present, the author also follows their conclusions until new evidence enough to separate species will be obtained. *T. rotatorium* was detected from *Rana rugosa* and *R. nigromaculata* in the present study, and a similar trypanosome was also found in the blood of *Rana limnocharis limnocharis* in Okinawa and *R. subapsera* in Amami Island by the present author, however, materials of the later two frogs are not eonugh to study morphological features.

According to Tanabe (1931), *T. rotatorium* was found from the blood of *R. nigromaculata* in Korea, and he showed four morphological types as shown in Fig. 5. Among them, type A was detected only from the bone marrow of infected frogs, and the other types were never found from the bone marrow. Type B was seen in the blood, and the morphological features are as follows; the body shape is similar to the type A (type I in the present paper), it ranges 40～59μ in length, and 3～5μ in width, and flagellum is about a half of the body length; its most characteristic feature is that kinetoplast is situated very near the nucleus as shown in Fig. 5. In the figures shown by Nöller (1913) or Wenyon (1926), this type of trypanosomes were not seen under *T. rotatorium*, and the present author also could not detect such type in Nagasaki, whereas other three types shown by Tanabe were commonly seen.

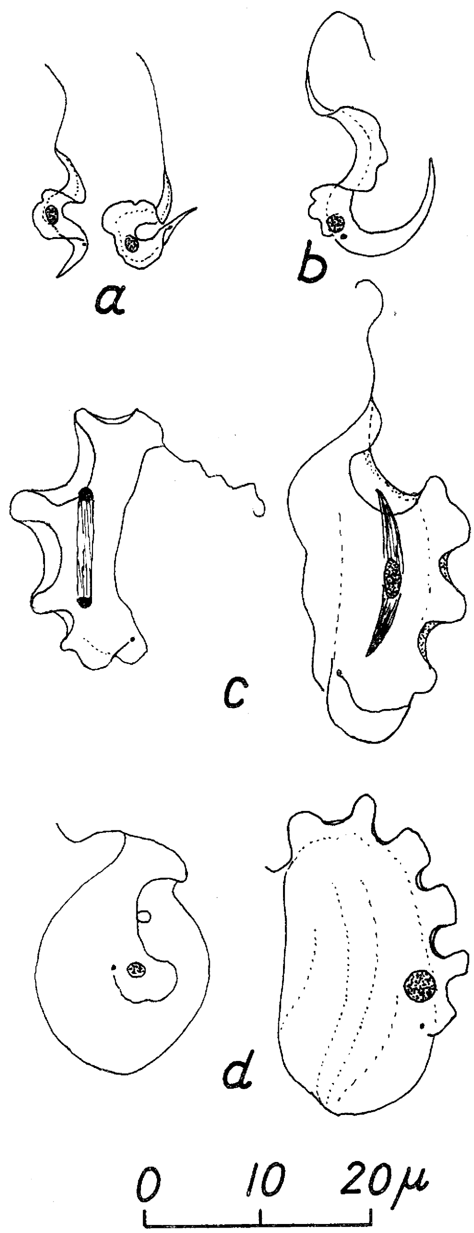

Fig. 5 Four types of *Trypanosoma rototorium* (modified after Tanabe, 1931).

The leech is a well-known vector for *T. rotatorium*, and Nöller (1913) recorded a kind of leeches, *Hemiclepsis marginata* as a vector. In the present study, from intestine of *Hirudo nipponia*, trypanosomes were detected. The leeches is commonly seen in paddy fields in Nagasaki and apparently this is the most suspicious vector for *T. rotatorium*. *H. nipponia* usually takes blood from tadpoles, and both trypanosomes detected from tadpoles and the leeches are very similar to each other.

The life cycle of *T. rotatorium* might be shown diagramatically as Fig. 4. Several types of trypanosomes were detected from adult frogs and tadpoles, but these trypanosomes might belong to a single species, because occasionally the young frogs show typical tadpole forms (type I in this paper), while adult frogs raised from the tadpoles showed only the large trypanosomes (type III in this paper). The most possible way of infection is from tadpoles to tadpoles by leeches, but from frogs to tadpoles by leeches is also possible, but such case must be apparently very few, because young trypanosomes (type I) is very rare in adult frogs.

Recently several workers reported that the mosquito is suspicious as possible vectors for *T. rotatorium*. Baily (1962) described intestinal forms of *T. rotatorium* in *Aedes aegypti*. Desser *et al.* (1973) described intestinal forms of *T. rotatorium* in *Culex territans*. Those trypanosomes illustrated by them are somewhat different from *T. rotatorium* of the present author in their morphology. But these observations might be very important in concerning the life cycle of anuran trypanosomes.

Further studies are needed to solve many problems concerning *T. rotatorium*, which is very interesting species to understand the evolution of the genus *Trypanosoma* Gruby, 1843.

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長崎市郊外で発見された無尾類の住血原虫　1. *Trypanosoma rotatorium* (Mayer, 1843)

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無尾類の住血原虫類は，多くの点で原始的特徴を備えており，広く寄生原虫類の進化を理解する上で大変重要である．また医動物学の手近な実験材料としても注目されている．筆者は，1974年7～8月，たまたま長崎市郊外茂木で無尾類を入手し，その住血原虫を調べることができたので，その成績を報告しておく． 1) *Trypanosoma rotatorium* (Mayer 1843) が，ツチガエル (*Rana rugosa*) の成体の50%から，またオタマジャクシの67%から検出された． トノサマガエル (*Rana nigromaculata*) は5匹検査しただけであるが，そのうち2匹から *T. rotatorium* がみつかった． 2) 検出されたトリパノゾーマは，次の3型にわけることができる．第1型はもっとも小さく，虫体体長は38～49.5ミクロン，体幅は 1.5～2ミクロンである．この型はオタマジャクシの血液中にみられ，1例のみ非常に若いツチガエルの成体から検出された．第Ⅱ型は，体長60～80ミクロン，体幅3～8ミクロンのトリパノゾーマで，体後端がとがっている．この型はオタマジャクシにのみみられた．第Ⅲ型は，さまざまの形態のトリパノゾーマを含み，さらにいくつかの亜型にわけることができる．概して虫体は大きくまるく，特に虫体後端もまるい．この型は成体からのみ検出され，オタマジャクシからは発見されなかった． 3) チスイビル (*Hirudo nipponia*) が，オタマジャクシとともに発見され，盛んにオタマジャクシを吸血するのが観察された．このヒルの消化管内にもトリパノゾーマが発見された．このヒルが，*T. rotatorium* の媒介者ではないかと考えられる． 4) *Dactylosoma ranarum* (Kruse, 1890) も，ツチガエル成体の赤血球内に寄生しているのが観察された．この原虫については，続報で詳しく報告する．

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