Anuran Trypanosomes in Kyushu and Ryukyu Islands, with Descriptions of Six New Species

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Abstract: Ten species of frog captured in Kyushu and Ryukyu Islands were examined to find out haemoparasite, and from them, nine distinct species including six new species and five other types of anuran trypanosome were detected as listed below.
1) *Trypanosoma nagasakiense* n. sp. (*Hyla arborea japonica* in Nagasaki.) 2) *Trypanosoma rotatorium* (Mayer, 1843) (*Rana rugosa* and *Rana nigromaculata* in Nagasaki. *Rhacophorus japonicus* in Okinawa Island.) 3) *Trypanosoma loricatum* (Mayer, 1843) (*Rana narina* and *Rana holsti* in Okinawa Island. *Rana nigromaculata* in Nagasaki.) 4) *Trypanosoma ishigakiense* n. sp. (*Rana limnocharis limnocharis* in Ishigaki Island.) 5) *Trypanosoma miyagii* n. sp. (*Rana namiyei*, *Rana narina*, *Rana holsti*, and *Rana ishikawae* in Okinawa Island. *Rana subaspera* in Amami Island.) 6) *Trypanosoma tsukamotoi* n. sp. (*Rana namiyei* in Okinawa Island.) 7) *Trypanosoma rugosae* n. sp. (tadopole of *Rana rugosa* in Nagasaki.) 8) *Trypanosoma chattoni* Mathis and Leger, 1911 (*Rana holsti* and *Rana narina* in Okinawa Island. *Rana rugosa* in Nagasaki.) 9) *Trypanosoma tsunezomiyatai* n. sp. (*Rana rugosa* in Nagasaki. *Rana limnocharis limnocharis* in Ishigaki Island.). In addition to them, five more types of trypanosome were detected, which are apparently different morphologically from above species, however the types are not yet determined whether they are new species respectively or only varieties of known species.

Many authors have believed that only one species of anuran trypanosome, *Trypanosoma rotatorium* (Mayer, 1843), is present among various species of toads and frogs. This opinion was so strongly spread in literature that at first the present author believed that all trypanosomes detected from Japanese frogs belong to only one species (Miyata, 1976). However, recently he has had a good oportunity to study anuran trypanosomes in Ryukyu Islands with the co-operation of Prof. Ichiro Miyagi, College of Health Sciences, University of the Ryukyus. Prof. Miyagi collected many blood smears from various frogs in Ryukyu Islands, and he kindly gave the chance for the present author to examine all his collections. In addition to the collections, many preparations were taken from frogs captured in the vicinities of Nagasaki City with the co-operation of Dr. Masuhisa Tsukamoto and Mr. Akira

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Yamaguchi, and according to the examination results of those materials, the author gradually believed that anuran trypanosomes can not classify only one species. The trypanosomes can be separated into the following 14 types morphologically, and three of them were already named, but six species are described as distinct new species in the present paper. Other five types are different from those nine species morphologically, however the types are not yet determined whether they are new species respectively or only varieties of known species.

MATERIALS AND METHODS

The materials and methods used in the present study were described in the separate papers written by Miyata (1976) and Miyata, Miyagi, and Tsukamoto (1978). The scientific names of Japanese frogs used by Nakamura and Uéno (1974) were adopted in the present paper.

DESCRIPTIONS OF SPECIES

1) *Trypanosoma nagasakiense* n. sp. (Fig. 1, Fig. 11, a-b, and Table 1)

This trypanosome has been found from the blood smear taken from *Hyla arborea japonica*

Table 1. Size (in microns) of *Trypanosoma nagasakiense* n. sp. (typical form)

	TL	BL	P-K	K-Np	F	W	NL
	50.4	37.2	13.8	1.8	13.2	19.2	10.2
	52.2	39.0	12.0	0.6	13.2	20.4	10.8
	49.8	33.6	12.0	0.6	16.2	19.2	8.4
	30.0	24.0	10.2	1.2	6.0	16.8	9.0
	51.0	34.8	9.0	0.6	16.2	16.8	9.6
	50.4	30.0	13.8	1.8	20.4	15.6	10.2
	47.4	29.4	11.4	1.8	18.0	18.0	11.4
	48.0	36.0	9.6	0.9	12.0	18.6	10.5
Average	47.4	33.0	11.5	1.2	14.4	18.1	10.0
Minimum	30.0	24.0	9.0	0.6	6.0	15.6	8.4
Maximum	52.2	39.0	13.8	1.8	20.4	20.4	11.4

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
K-Np: Kinetoplast to posterior end of nucleus
P-K: Posterior end to kinetoplast
F: 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
see Miyata (1975)

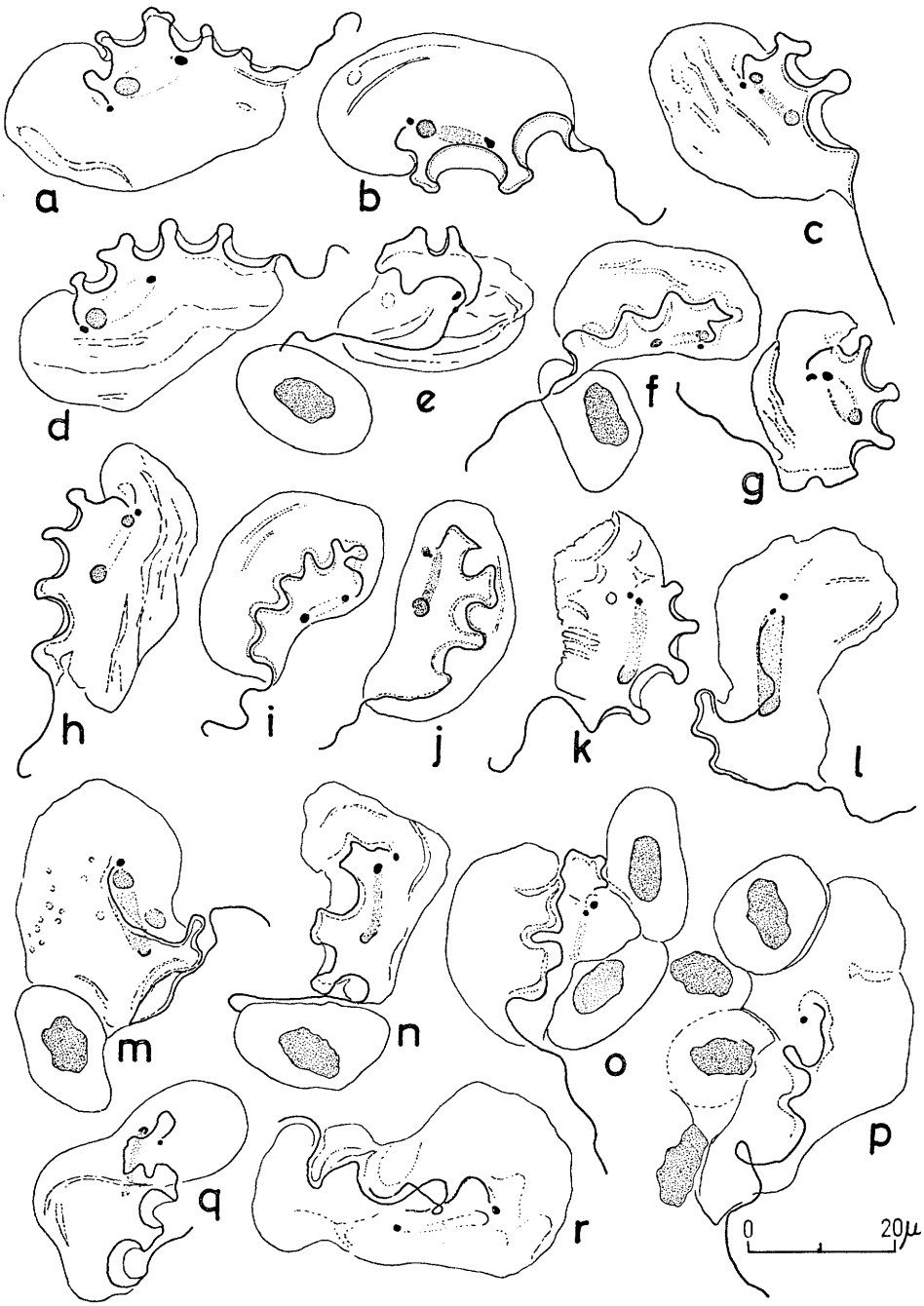

Fig. 1. *Trypanosoma nagasakiense* n. sp. (Host: *Hyla arborea japonica*)

which was captured in the vicinity of Isahaya, about 40 km east from Nagasaki City. The following description was based on typical forms shown in Fig. 1, a-d, and the size in parentheses is expressed as the average of 8 trypanosomes (Table 1).

Body oval or elliptical; posterior end round; undulating membrane wide, with 3-4 folds; total length 30.0-52.0 microns (47.4 microns); body length 24.0-39.0 microns (33.0 microns); width at the widest part including undulating membrane 15.6-20.4 microns (18.1 microns); free flagellum 6.0-20.4 microns (14.4 microns); posterior end to kinetoplast 9.0-13.8 microns (11.5 microns); kinetoplast to posterior edge of nucleus 0.6-1.8 microns (1.2 microns); nucleus elongate fusiform, with pinkish chromatin granule in posterior end of nucleus and more darkly stained granule in anterior end of nucleus; nuclear length 8.4-11.4 microns (10.0 microns).

Type smear: Holotype and paratype smears are in the collection of the author in the

Table 2. Size (in microns) of *Trypanosoma rotatorium* (Mayer, 1843) (typical form)

	TL	BL	P-K	K-Np	F	W	NL
	54.0	36.0	4.2	1.8	24.0	12.0	15.6
	70.8	34.8	3.6	1.8	36.0	8.4	12.0
	61.8	42.0	4.2	0.6	19.8	12.0	13.2
	60.0	42.0	6.0	0.6	18.0	10.8	16.8
	60.0	37.2	3.6	0.6	22.8	8.4	12.0
	55.2	38.4	6.0	1.8	16.8	12.6	10.8
	62.4	37.2	3.6	1.8	25.2	12.0	12.0
	66.0	45.0	3.6	0.6	21.0	11.4	14.4
Average	61.3	39.1	4.4	1.2	23.0	11.0	13.4
Minimum	54.0	34.8	3.6	0.6	16.8	8.4	10.8
Maximum	70.8	45.0	6.0	1.8	36.0	12.6	16.8

Table 3. Size (in microns) of *Trypanosoma* sp. A (typical form)

	TL	BL	P-K	K-Np	F	W	NL
	44.4	31.8	3.6	3.6	12.6	10.2	15.0
	48.0	36.0	3.6	1.2	12.0	10.8	—
	37.8	30.0	6.6	—	7.8	11.4	—
	44.4	30.6	4.8	—	13.8	10.8	—
	42.0	36.0	4.8	—	6.0	10.8	—
	43.2	36.0	3.0	—	7.2	10.2	—
	54.0	36.0	4.8	—	24.0	18.0	—
	43.2	30.0	2.4	—	13.2	11.4	—
	49.2	33.6	1.2	3.0	15.6	18.0	15.0
	45.6	33.6	4.2	—	12.0	10.2	—
	37.2	31.2	4.8	—	6.0	15.6	—
Average	44.5	33.2	4.0	2.6	11.8	12.5	15.0
Minimum	37.2	30.0	1.2	1.2	6.0	10.2	15.0
Maximum	54.0	36.0	6.6	3.6	24.0	18.0	15.0

Department of Epidemiology, Institute for Tropical Medicine, Nagasaki University. Smear No. 1977-7-9-2 and 1977-7-9-1.

Type host: *Hyla arborea japonica* Günther, 1858

Type locality: Yunoo-machi, Isahaya City, Nagasaki Prefecture.

This trypanosome was detected from two out of 8 hosts collected in July, 1977, at Mr. Yamaguchi's house garden.

Remarks: *T. nagasakiense* n. sp. is apparently different from *Trypanosoma rotatorium*, *Trypanosoma arcei* Mazza, Gonzàlez, Franke and Alvarado, 1927, in body shape, width of body, distance between posterior end and kinetoplast, and nuclear length. From Italian subspecies of *Hyla arborea*, França (1908) described *Trypanosoma hylae*, however according to Diamond (1958 and 1965), França's species includes two different species, *Trypanosoma rotatorium* and *Trypanosoma chattoni*. *Trypanosoma borrelli* Marchoux and Salimbeni, 1907, was described from Brazilian *Hyla* sp. (similar to *Hyla lateristriga*), but this species has no free flagellum.

2) *Trypanosoma rotatorium* (Mayer, 1843) (Fig. 2, Fig. 10, a-d, Fig. 11, c-d, and Table 2)

This trypanosome is a common parasite of *Rana rugosa* in the vicinity of Nagasaki City, and the following descriptions are based on the Giemsa stained blood smears taken from the frog. The size in parentheses is expressed as the average of 8 typical trypanosomes such as Fig. 2, a-g.

Body elongate; posterior end round; undulationg membrane conspicuous, with 5-6 folds; total length 54.0-70.8 microns (61.3 microns); body length 34.8-45.0 microns (39.1 microns); width at the widest part of body including undulating membrane 8.4-12.6 microns (11.0 microns); free flagellum 16.8-36.0 microns (23.0 microns); posterior end to kinetoplast 3.6-6.0 microns (4.4 microns); kinetoplast to posterior edge of nucleus 0.6-1.8 microns (1.2 microns); nucleus elongate fusiform, with pinkish chromatin granule at each ends of nucleus; nuclear length 10.8-16.8 microns (13.4 microns).

Host: This trypanosome was detected from *Rana rugosa* Schlegel, 1838, captured at Mogi, the vicinity of Nagasaki City (see Miyata, 1976). From *Rana nigromaculata* Hallowell, 1860, a similar trypanosome was found (Fig. 10, d). Trypanosomes detected from *Rhacophorus japonicus* (Hallowell, 1860) captured in northern part of Okinawa Island, is identical with *Trypanosoma rotatorium* (Fig. 10, a-c).

Remarks: *Trypanosoma rotatorium* was shown by Brumpt (1928, Fig. 1, 9, in his paper), Schwetz (1930, Fig. 5, 7-8, and 11, in his paper), and other authors. The present reported species is identical with the trypanosome shown by those authors.

3) *Trypanosoma* sp. A (Fig. 3, Fig. 11, e-f, and Table 3)

This trypanosome was detected from the blood of *Rana limnocharis limnocharis* captured in Ishigaki Island by Prof. Ichiro Miyagi in July, 1976. The morphological features shown below are based on the Giemsa stained smears, and the size in parentheses is expressed as

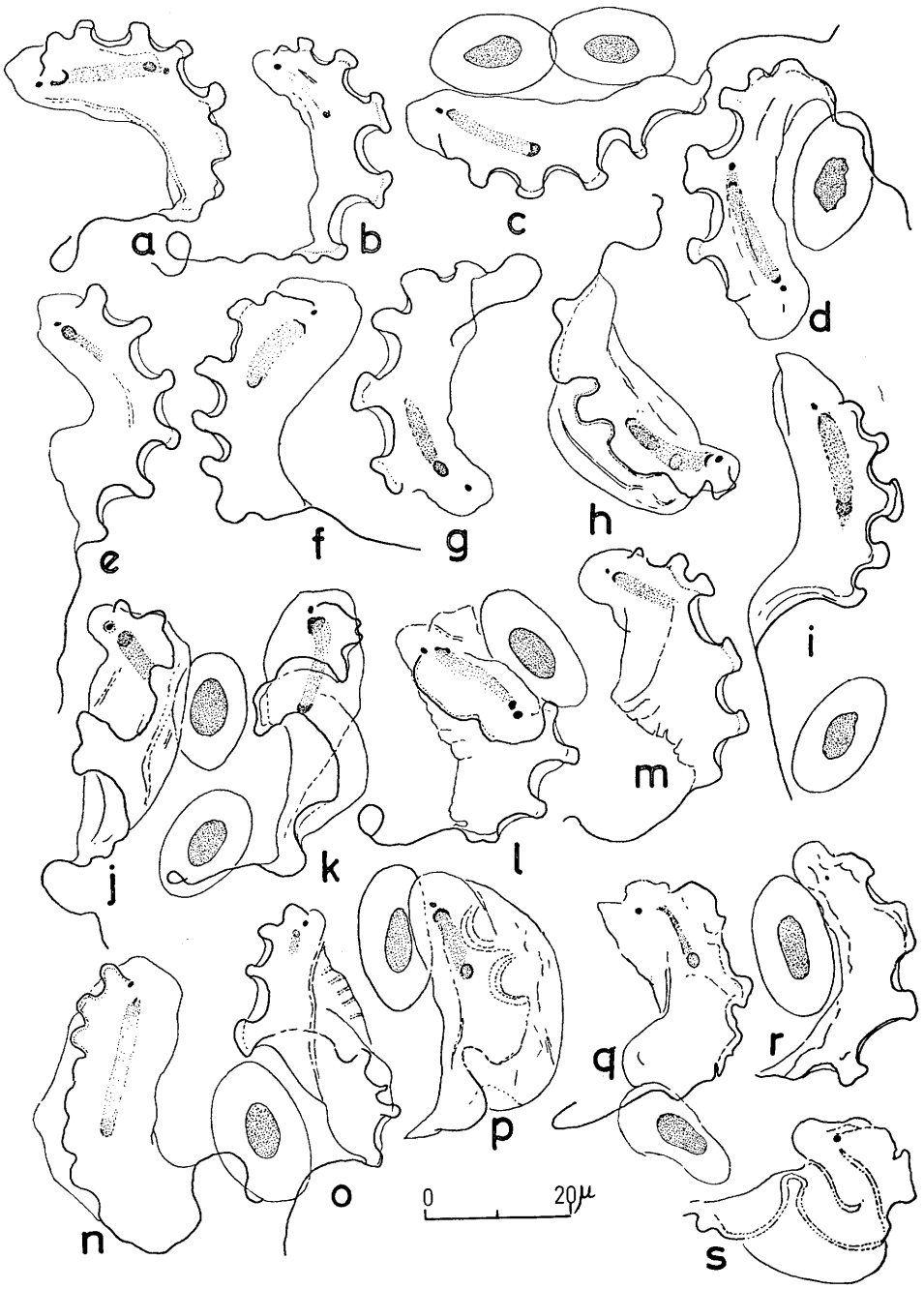

Fig. 2. *Trypanosoma rotatorium* (Mayer, 1843) (Host: *Rana rugosa*)

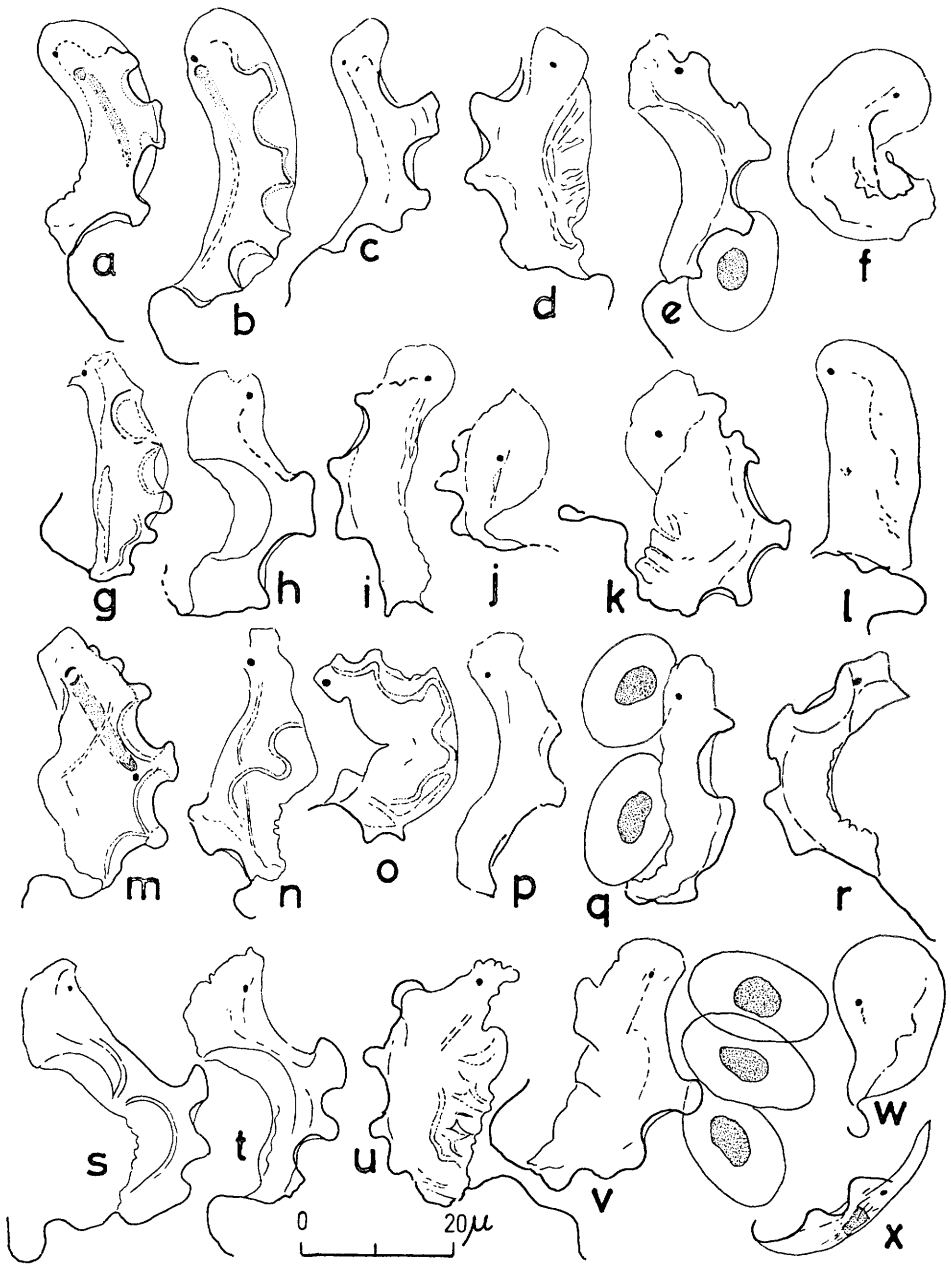

Fig. 3. *Trypanosoma* sp. A (Host: a–f, h–n, p–r, w, x. *Rana limnocharis limnocharis*; g, o. *Rana holsti*; s–v. *Rana rugosa*)

the average of 11 trypanosomes (Table 3).

Body similar to *Trypanosoma rotatorium*; posterior end round; total length 37.2–54.0 microns (44.5 microns); body length 30.0–36.0 microns (33.2 microns); width at the widest part of body including undulating membrane 10.2–18.0 microns (12.5 microns); free flagellum 6.0–24.0 microns (11.8 microns); posterior end to kinetoplast 1.2–6.6 microns (4.0 microns); kinetoplast to posterior edge of nucleus 1.2–3.6 microns (12.6 microns); nuleus elongate fusiform; nuclear length about 15.0 microns.

This species is usually stained darkly, then nucleus is sometimes indistinguishable.

Host: This trypanosome was found from the blood of *Rana limnocharis limnocharis* Wiegmann, 1835, captured in Ishigaki Island. Similar trypanosome was detected from the blood smears taken from *Rana holsti* Boulenger, 1892, captured in northern part of Okinawa Island (Fig. 3, g). From *Rana rugosa* Schlegel, 1838, captured at Mogi, Nagasaki City, also similar trypanosome was detected as shown in Fig. 3, s-u.

Remarks: This species resembles *Trypanosoma rotatorium*, however cytoplasm of *Trypanosoma* sp. A in Giemsa stained specimens is darker than that of *Trypanosoma rotatorium*, then in many individuals of *Trypanosoma* sp. A, nuclear length could not be measured. Body length and length of free flagellum are apparently different in each species.

Particular form (temporally call Type I of *Trypanosoma* sp. A) figured in Fig. 3, a and b, is usually not seen in *Trypanosoma rotatorium*. In other prominent form of *Trypanosoma* sp. A, posterior edge broader like a fan, and the edge opposite of undulating membrane, in appearence, folds on the body surface. Individuals belonging to this type (Type II of *Trypanosoma* sp. A) are shown in Fig. 3, e and h. This type is also usually not observed

Table 4. Size (in microns) of *Trypanosoma loricatum* (Mayer, 1843)

	BL	P-K	K-Np	W	NL
	43.8	18.6	5.4	24.0	3.6
	54.0	20.4	4.2	22.8	3.6
	42.0	19.8	4.8	27.0	3.9
	39.0	15.0	4.2	30.0	3.0
	45.0	15.6	4.8	28.8	3.6
	48.0	24.6	3.6	30.0	4.2
	36.0	18.0	1.2	19.8	3.0
	48.0	13.2	6.0	28.8	3.9
	45.0	15.0	4.8	26.4	3.6
	30.0	13.2	3.6	19.8	3.0
	30.6	13.2	4.8	24.0	4.2
	42.0	10.8	5.4	26.4	3.0
	48.0	21.0	1.8	22.8	4.2
	42.0	15.0	3.6	24.0	4.8
	42.0	18.0	3.9	30.0	3.0
Average	42.4	16.8	4.1	25.6	3.6
Minimum	30.0	10.8	1.2	19.8	3.0
Maximum	54.0	24.6	6.0	30.0	4.8

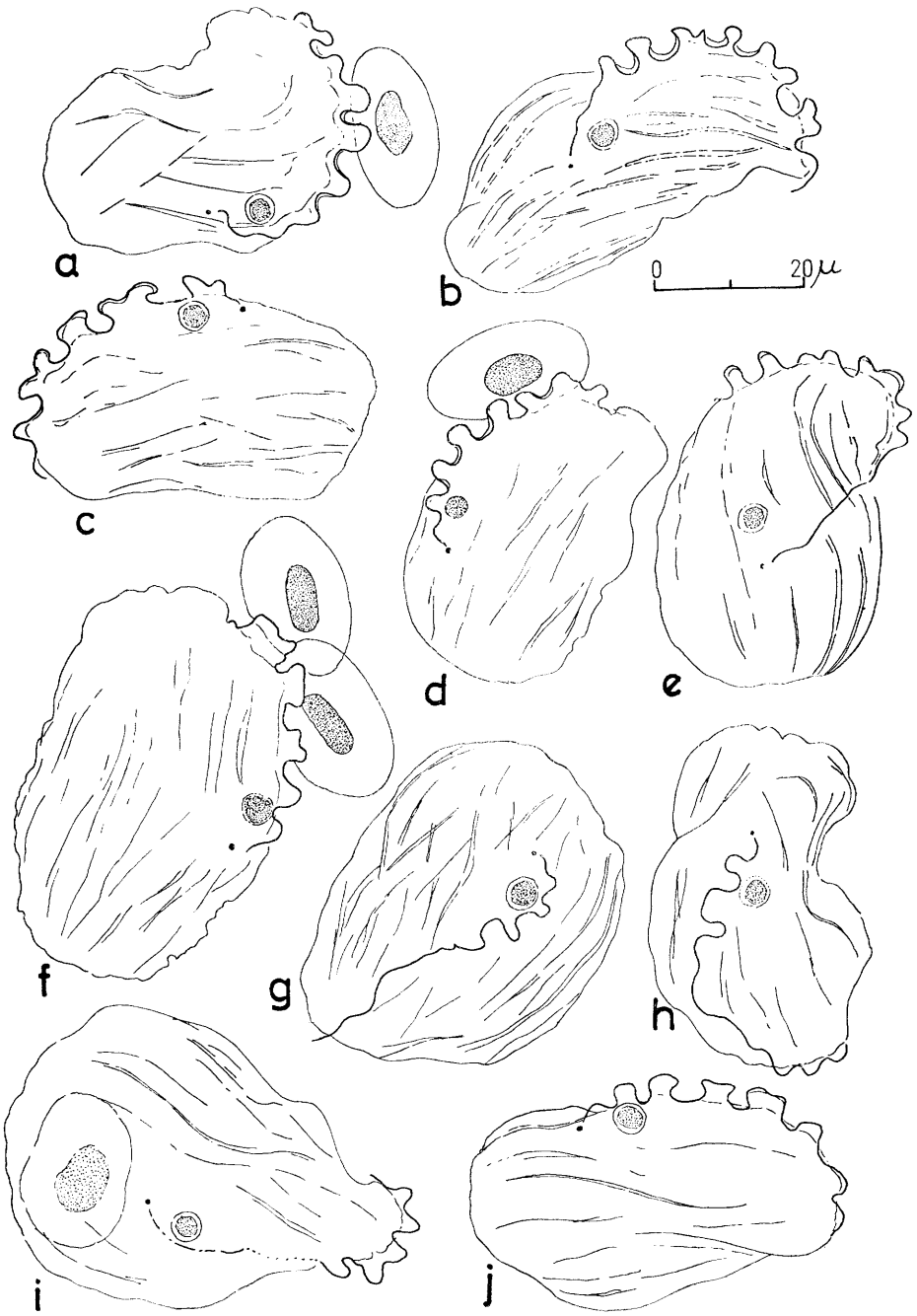

Fig. 4. *Trypanosoma loricatum* (Mayer, 1843) (Host: a–f, i, j. *Rana holsti*; g, h. *Rana narina*)

in *Trypanosoma rotatorium*. From one of *Rana rugosa* captured in Mogi, Type II was detected as shown in Fig. 3, s and t.

In future, *Trypanosoma* sp. A might be separated as an independent species from *Trypanosoma rotatorium*, but at present the present author has enough evidence other than above introduced materials.

4) *Trypanosoma* sp. B (Fig. 10, e-f, and Fig. 11, g-h)

This trypanosome was detected from the blood of *Hyla arborea japonica* which was captured in the vicinity of Isahaya, about 40 km east from Nagasaki City. Unfortunately, only two trypanosomes were found on the blood smear, then it is not clear whether this is a new species or a form of already known species.

Body elongate and strongly arched; posterior end narrow; kinetoplast situated near posterior end; cytoplasm darkly stained; body length 33 microns (Fig. 10, e) and 36 microns (Fig. 10, f); free flagellum about 18 microns; nucleus elongate fusiform; length of nucleus 9.6 microns.

Host: *Hyla arborea japonica* Günther, 1858, captured at Yunoo-machi, Isahaya City, Nagasaki Prefecture, in June, 1977. Smear No. 1977-6-23-2.

Remarks: This species is apparently different from *Trypanosoma nagasakiense* n. sp. in the shape of body and measurements. *Trypanosoma rotatorium* and *Trypanosoma* sp. A resemble *Trypanosoma* sp. B in shape of nucleus, however the body shape of *Trypanosoma* sp. B is apparently distinguishable from other species of *rotatorium*-type. The trypanosome shown in Fig. 11, h, might belong to *Trypanosoma* sp. B. In future study, this species will be described as a distinct species.

5) *Trypanosoma* sp. C. (Fig. 10, g)

From *Rana holsti*, small pyriform trypanosome was detected as shown in Fig. 10, g. Unfortunately, only one individual was found, then at present the author can not determine

Table 5. Size (in microns) of *Trypanosoma ishigakiense* n. sp.

	BL	P-N	A-N	K-N	P-K	W	NL	KI	NI
	33.0	15.0	18.0	5.4	12.0	12.6	3.0	2.7	0.8
	41.4	17.4	24.0	6.6	9.6	12.0	1.8	2.6	0.7
	46.8	18.0	28.8	5.4	15.0	6.0	2.4	3.3	0.6
	30.6	15.0	15.6	7.2	12.0	8.4	1.8	2.0	1.0
	34.8	16.8	18.0	7.2	12.0	15.6	1.8	2.6	0.9
	33.0	12.0	21.0	7.2	9.0	13.2	1.2	1.6	0.6
	41.4	18.6	22.8	6.6	13.2	11.4	2.1	2.8	0.8
	45.6	24.6	21.0	6.6	18.0	7.8	0.9	3.7	1.2
	42.0	21.0	21.0	7.2	15.6	7.2	1.2	2.9	1.0
Average	38.7	17.6	21.1	6.6	12.9	10.5	1.8	2.7	0.8
Minimum	30.6	12.0	15.6	5.4	9.0	6.0	0.9	1.6	0.6
Maximum	46.8	24.6	28.8	7.2	18.0	15.6	3.0	3.7	1.2

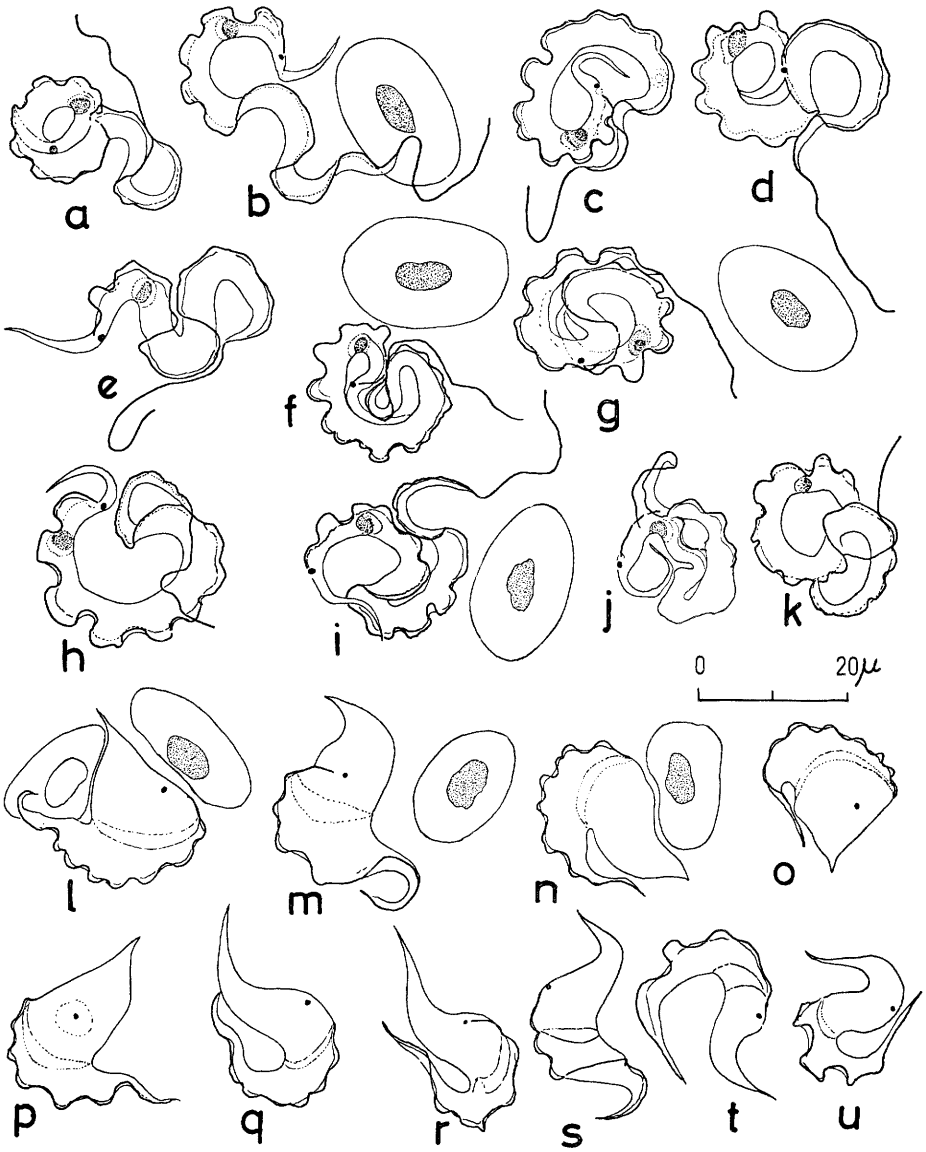

Fig. 5. *Trypanosoma tsukamotoi* n. sp. (Host: a–k. *Rana namiyei*) and *Trypanosoma ishigakiense* n. sp. (Host: l–u. *Rana limnocharis limnocharis*)

the systematic position. This trypanosome has a fusiform nucleus.

Host: *Rana holsti* Boulenger, 1892, which was captured at Yona, Kunigamison, northern part of Okinawa Island. Smear No. 1976-6-19-38.

Remarks: *Trypanosoma* sp. C somewhat resembles *Trypanosoma* sp. A in its nuclear shape, but body shape is apparently different.

6) *Trypanosoma loricatum* (Mayer, 1843) (Fig. 4, Fig. 11, i-k, and Table 4)

The morphological features ot this trypanosome described below are based on the Giemsa stained blood smears prepared from *Rana narina* and *Rana holsti* captured in Okinawa Island, and the size in parentheses is expressed as the average of 15 trypanosomes.

Monomorphic trypanosome with elliptical or oval body; surface costate; both ends round; undulating membrane with many folds; body length 30.0-54.0 microns (42.4 microns); width at the widest part of body including undulating membrane 19.8-30.0 microns (25.6 microns); free flagellum usually invisible or, if present, very short; kinetoplast to posterior edge of nucleus 1.2-6.0 microns (4.1 microns); posterior end to kinetoplast 10.8-24.6 microns (16.8 microns); nucleus round and stained pinkish; diameter of nucleus 3.0-4.8 microns (3.6 microns); nucleus and kinetoplast usually present peripheral part of body.

Host: This trypanosome was detected from *Rana narina* Stejneger, 1901, and *Rana holsti* Boulenger, 1892, captured in northern part of Okinawa Island, and *Rana nigromaculata* Hallowell, 1860, captured in the vicinities of Nagasaki City. The trypanosome detected from the third host is somewhat smaller than that obtained from other two hosts.

Remarks: This trypanosome was shown in Miyata's paper (1976, Fig. 1, a-b in his paper) under the name of *Trypanosoma rotatorium*. Type II of *Trypanosoma ryukyuense* Miyata, 1977c, which was detected from the lizard, *Eublepharis kuroiwae kuroiwae* (Namie, 1912), somewhat resembles *Trypanosoma loricatum*, but differs in situation of nucleus, distance between nucleus and kinetoplast and length of free flagellum.

Table 6. Size (in microns) of *Trypanosoma miyagii* n. sp.

	TL	BL	P-N	A-N	K-N	P-K	F	W	NL	KI	NI
	80.4	72.0	31.2	40.8	16.2	18.0	8.4	15.0	2.4	1.9	0.8
	—	75.0	36.0	39.0	21.0	18.0	—	12.6	3.0	1.7	0.9
	84.6	78.0	30.0	48.0	13.8	12.0	6.6	13.8	2.4	2.2	0.6
	—	73.2	36.0	37.2	19.2	18.0	—	16.8	4.8	1.9	1.0
	87.0	81.6	28.8	52.8	15.0	18.0	5.4	16.2	4.2	1.9	0.5
	—	84.0	36.0	48.0	16.8	18.0	—	14.4	3.6	2.1	0.8
	—	78.0	40.8	37.2	13.8	22.8	—	16.2	3.0	3.0	1.1
	90.0	84.0	40.8	43.2	15.6	19.2	6.0	15.0	3.0	2.6	0.9
	—	79.2	36.0	49.2	16.2	30.0	—	15.6	4.2	2.2	0.7
	79.8	75.0	30.0	45.0	16.2	18.0	4.8	15.0	3.0	1.9	0.7
Average	84.4	78.0	34.6	44.0	16.4	19.2	6.2	15.1	3.4	2.1	0.8
Minimum	79.8	72.0	28.8	37.2	13.8	12.0	4.8	12.6	2.4	1.7	0.5
Maximum	90.0	84.0	40.8	49.2	21.0	30.0	8.4	16.8	4.8	3.0	1.1

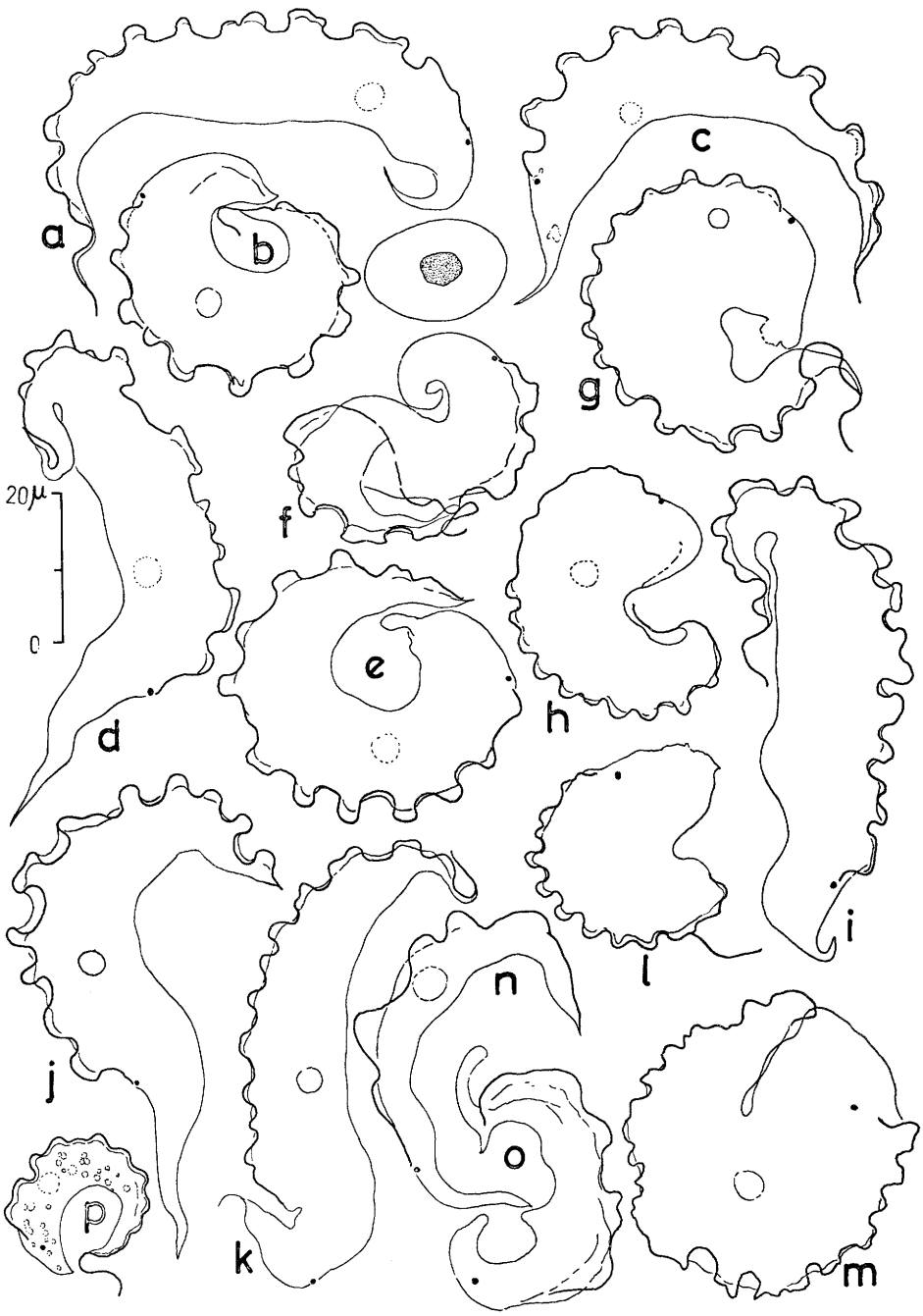

Fig. 6. *Trypanosoma miyagii* n. sp. (Host: a-k, m, p. *Rana namiyei*; l, n. *Rana holsti*; o. *Rana narina*)

7) *Trypanosoma ishigakiense* n. sp. (Fig. 5, l-u, Fig. 12, e-g, and Table 5)

This is a *mega*-type trypanosome, which has been detected from blood smear of *Rana limnocharis limnocharis* captured in Ishigaki Island by Prof. Ichiro Miyagi in March, 1976. The morphological features shown below are based on the Giemsa stained smears taken from the frog, and the size in parentheses is expressed as the average of 9 trypanosomes.

Body slender in young individual, but older trypanosome somewhat broader; both ends pointed; body length 30.6-46.8 microns (38.7 microns); width at the widest part of body 6.0-15.6 microns (10.5 microns); undulating membrane unconspicuous; free flagellum not seen in this specimen; posterior end to kinetoplast 9.0-18.0 microns (12.9 microns); nucleus elliptical or lens shaped, and its long axis transverse body; length of nucleus 0.9-3.0 microns (1.8 microns), width of nucleus about same as body width; posterior end to middle of nucleus 12.0-24.6 microns (17.6 microns); anterior end to middle of nucleus 15.6-28.8 microns (21.1 microns); the kinetoplast index=1.6-3.7(2.7); the nuclear index=0.6-1.2 (0.8).

Young slender trypanosomes are usually stained darkly and the colour of pre- and post-nuclear portions are not different. But, in mature trypanosome, the post-nuclear portion is more lightly stained than pre-nuclear portion.

Type smear: Holotype and paratype smears are in the collection of the author in the Department of Epidemiology, Institute for Tropical Medicine, Nagasaki University. Smear No. 1976-6-24-5.

Type host: *Rana limnocharis limnocharis* Wiegmann, 1835.

Type locality: Ishigaki Island, southern part of Ryukyu Archipelago.

Remarks: According to Dutton, Todd, and Tobey (1907), and Schwetz (1930), *Trypanosoma mega* Dutton and Todd, 1903, was known from *Bufo regularis* and *Rana oxyrhynchus* in Africa. *Trypanosoma ishigakiense* n. sp. is somewhat similar to *Trypanosoma mega* in the shape of nucleus, but the new species is smaller than *Trypanosoma mega*, and is different in distance

Table 7. Size (in microns) of *Trypanosoma tsukamotoi* n. sp.

	TL	BL	P-N	A-N	K-N	P-K	F	W	NL	KI	NI
	97.8	70.8	18.0	52.8	6.0	12.0	27.0	4.2	1.8	3.0	0.3
	102.0	78.0	21.0	57.0	12.0	9.0	24.0	5.4	2.4	1.8	0.4
	91.8	63.0	24.0	39.0	12.0	12.0	28.8	6.0	2.4	2.0	0.6
	99.6	66.0	18.0	48.0	9.0	9.0	33.6	4.2	1.8	2.0	0.4
	87.0	69.0	15.0	54.0	9.0	6.0	18.0	4.8	3.6	1.7	0.3
	100.8	76.8	16.8	60.0	4.8	12.0	24.0	6.0	2.4	3.5	0.3
	96.0	66.0	18.0	48.0	11.4	6.6	30.0	4.8	3.0	1.6	0.4
	88.8	70.8	16.8	54.0	4.8	12.0	18.0	4.8	2.4	3.5	0.3
	87.6	69.6	16.8	52.8	9.6	7.2	18.0	3.6	1.8	1.8	0.3
	84.0	66.0	18.0	48.0	6.0	12.0	18.0	4.2	1.8	3.0	0.4
Average	93.5	69.6	18.2	51.4	8.5	9.8	23.9	4.8	2.3	2.2	0.4
Minimum	84.0	63.0	15.0	39.0	4.8	6.0	18.0	3.6	1.8	1.6	0.3
Maximum	102.0	78.0	24.0	60.0	12.0	12.0	33.6	6.0	3.6	3.5	0.6

between kinetoplast and nucleus, and between posterior end and middle of nucleus. *Trypanosoma ishigakiense* n. sp. might be the first record of *mega*-type trypanosome in the outside of Africa.

8) *Trypanosoma miyagii* n. sp. (Fig. 6, Fig. 10, h-l, Fig. 12, h-j, and Table 6)

This trypanosome is very common species in the blood of *Rana namiyei*, *Rana narina* and *Rana holsti*, which distribute in the forest area in Okinawa Island. The following description is based on the Giemsa stained smears taken from the frogs, and the size in parentheses is expressed as the average of 10 trypanosomes as shown in Table 6.

Monomorphic trypanosome with short free flagellum ; undulating membrane with many folds ; both ends pointed ; body length 72.0-84.0 microns (78.0 microns) ; width at the widest part of body including undulating membrane 12.6-16.8 microns (15.1 microns) ; posterior end to kinetoplast 12.0-30.0 microns (19.2 microns) ; posterior end to middle of nucleus 28.8-40.8 microns (34.6 microns) ; anterior end to middle of nucleus 37.2-49.2 microns (44.0 microns) ; in many individuals free flagellum not seen, but if seen, the length 4.8-8.4 microns (6.2 microns) ; small circular nucleus present near center of body, and its diameter 2.4-4.8 microns (3.4 microns) ; the kinetoplast index=1.7-3.0 (2.1) ; the nuclear index=0.5-1.1 (0.8).

Cytoplasm of the trypanosome is finely granulated and the colour is dark blue or bluish purple, then in the darkly stained parasite, its nucleus can not be distinguished. In some individuals, round posterior end as shown in Fig. 6, m, was observed, but these individuals also belong to *Trypanosoma miyagii* n. sp., because due to the observation of some smears, posterior end sometimes turned to opposite side of the side having an undulating membrane as shown in Fig. 6, h. Younger form is slender as shown in Fig. 6, n-o. From a smear taken from *Rana namiyei* (No. 1976-7-3-9), a small trypanosome was detected as shown in Fig. 6, p. This individual might belong to a younger form of *Trypanosoma miyagii* n. sp.

Table 8. Size (in microns) of *Trypanosoma rugosae* n. sp.

	TL	BL	P-N	A-N	K-N	P-K	F	W	NL	KI	NI
	51.0	43.8	24.0	19.8	20.4	3.6	7.2	5.4	2.4	1.2	1.2
	49.8	42.0	24.0	18.0	20.4	3.6	7.8	5.7	2.4	1.2	1.3
	45.6	39.6	21.6	18.0	16.2	5.4	6.0	5.4	2.4	1.3	1.2
	46.8	40.8	22.8	18.0	18.6	4.2	6.0	5.7	3.0	1.2	1.3
	56.4	49.2	24.6	24.6	21.0	3.6	7.2	5.4	2.4	1.2	1.0
	52.2	43.2	20.4	22.8	16.8	3.6	9.0	6.0	2.4	1.2	0.9
	45.0	37.8	18.0	19.8	14.4	3.6	7.2	4.8	2.4	1.3	1.1
	48.6	41.4	24.0	17.4	20.1	3.9	7.2	4.8	2.4	1.2	1.4
	49.2	43.2	25.2	18.0	20.7	4.5	6.0	6.0	2.4	1.2	1.4
	49.2	43.2	19.2	24.0	15.0	4.2	6.0	4.8	2.4	1.3	0.8
Average	49.4	42.4	22.4	20.0	18.4	4.0	7.0	5.4	2.5	1.2	1.1
Minimum	45.0	37.8	18.0	17.4	14.4	3.6	6.0	4.8	2.4	1.2	0.8
Maximum	56.4	49.2	25.2	24.6	21.0	5.4	9.0	6.0	3.0	1.3	1.4

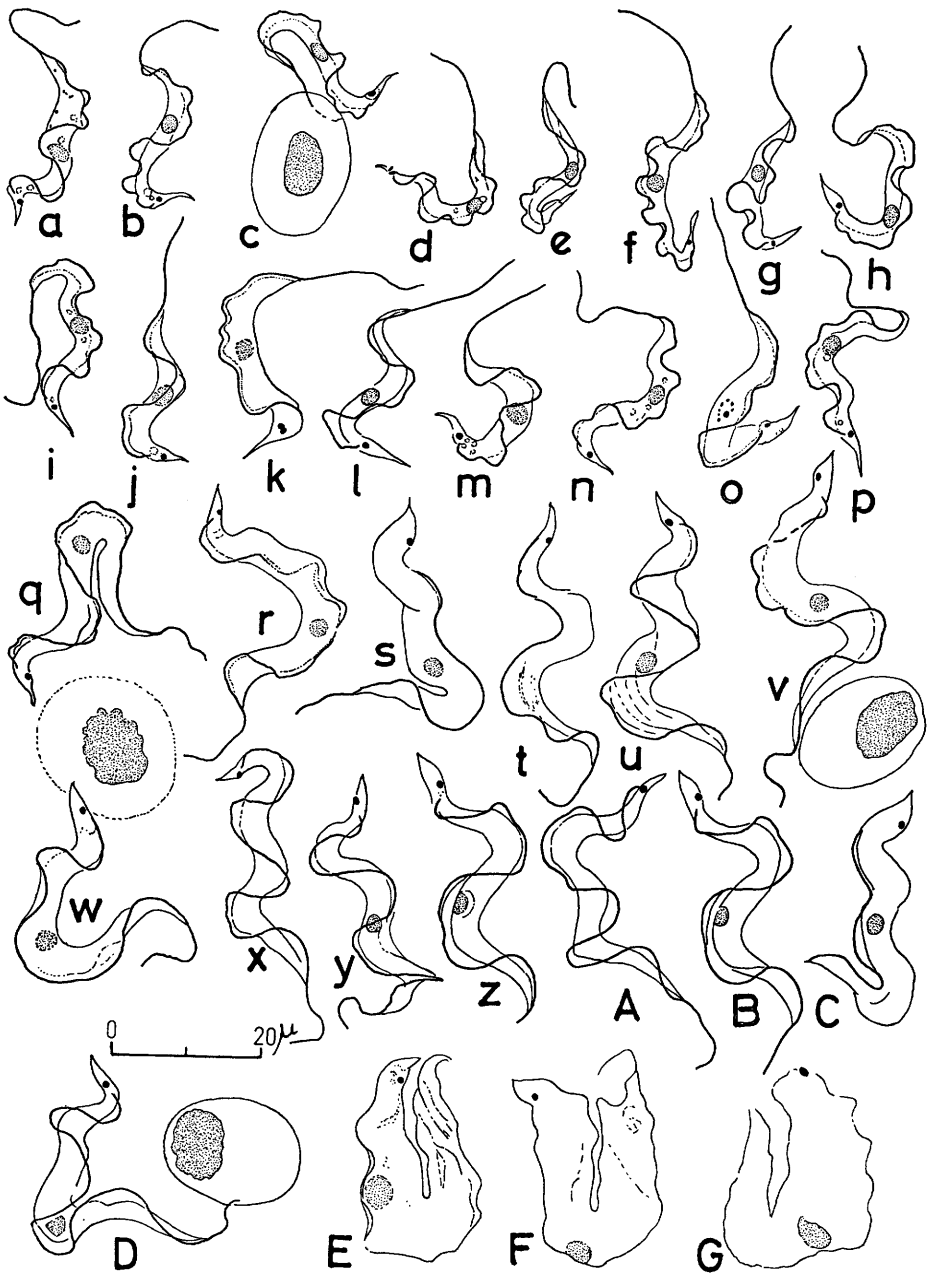

Fig. 7. *Trypanosoma* sp. E (Host: *Rana rugosa*. a–g. adult; h–p. larva) and *Trypanosoma rugosae* n. sp. (Host: q–G. *Rana rugosa*. larva)

Type smear: Holotype and paratype smears are in the collection of the author in the Department of Epidemiology, Institute for Tropical Medicine, Nagasaki University. Smear No. 1976-6-19-25, No. 1976-7-1-4, and others.

Type host: *Rana namiyei* Stejneger, 1901.

Type locality: Yona, Kunigamison, northern part of Okinawa Island.

Other hosts: This new species was detected from *Rana narina* Stejneger, 1901, and *Rana holsti* Boulenger, 1892, captured in northern part of Okinawa Island. From *Rana ishikawae* (Stejneger, 1901) captured at Yona, similar trypanosome was detected (Fig. 10, h-j). *Rana subaspera* Barbour, 1908, which was captured in Amami Island by Mr. Hiroshi Suzuki, are also infected with a trypanosome (Fig. 10, k-l).

Remarks: *Trypanosoma diamondi* Pérez-Reyes, 1969, and *Trypanosoma grandis* Pérez-Reyes, 1969, both described from Mexican frog, *Rana pipiens*, and *Trypanosoma bufophlebotomi* Ayala, 1970, from North American toad, *Bufo boreas halophilus*, somewhat resemble *Trypanosoma miyagii* n. sp., however in those three species, kinetoplast situates very near to nucleus.

The new species is named after Prof. Ichiro Miyagi. This work was carried out with his co-operation in Ryukyu Islands.

9) *Trypanosoma tsukamotoi* n. sp. (Fig. 5, a-k, Fig. 12, a-c, and Table 7)

A very long trypanosome like a snake has been detected from *Rana namiyei* captured in northern part of Okinawa Island. The following morphological description is due to the Giemsa stained smears, and the size in parentheses is expressed as the average of 10 trypanosomes shown in Table 7.

Body very long; both ends tapered gradually to points; total length 84.0-102.0 microns

Table 9. Size (in microns) of *Trypanosoma chattoni* Mathis and Leger, 1911

	Longest Length	Shortest Length	NL
	38.4	36.0	8.4
	42.0	34.2	6.0
	39.0	36.0	5.4
	46.2	30.0	4.8
	42.6	27.0	7.2
	57.0	30.0	5.4
	51.0	28.8	6.0
	37.2	32.4	6.0
	46.2	31.8	4.8
	33.0	32.4	7.2
	42.0	34.2	—
Average	40.0	32.1	6.1
Minimum	33.0	27.0	4.8
Maximum	57.0	36.0	8.4

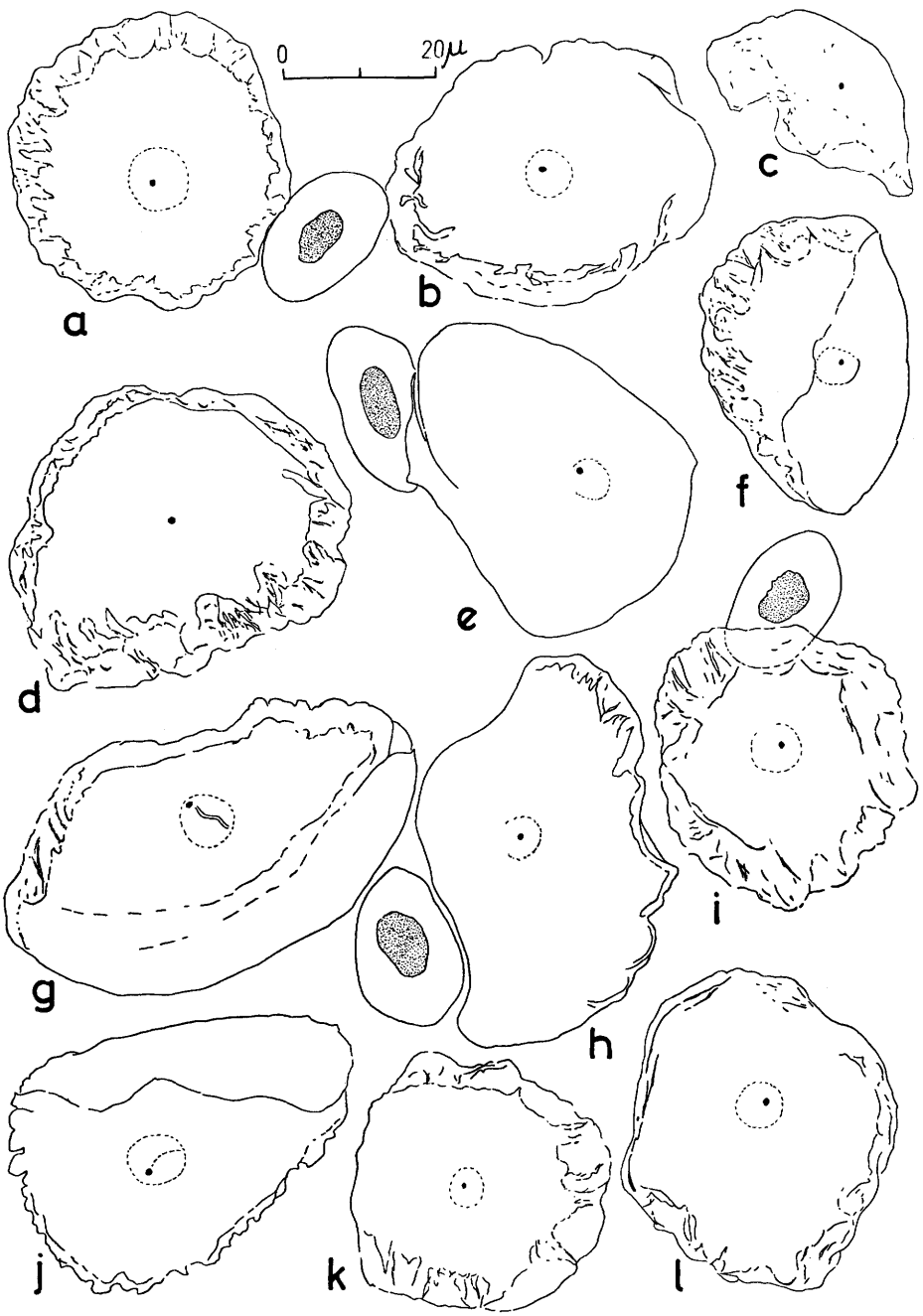

Fig. 8. *Trypanosoma chattoni* Mathis and Leger, 1911 (Host: a–g, j. *Rana holsti*. h, i, k, l. *Rana narina*)

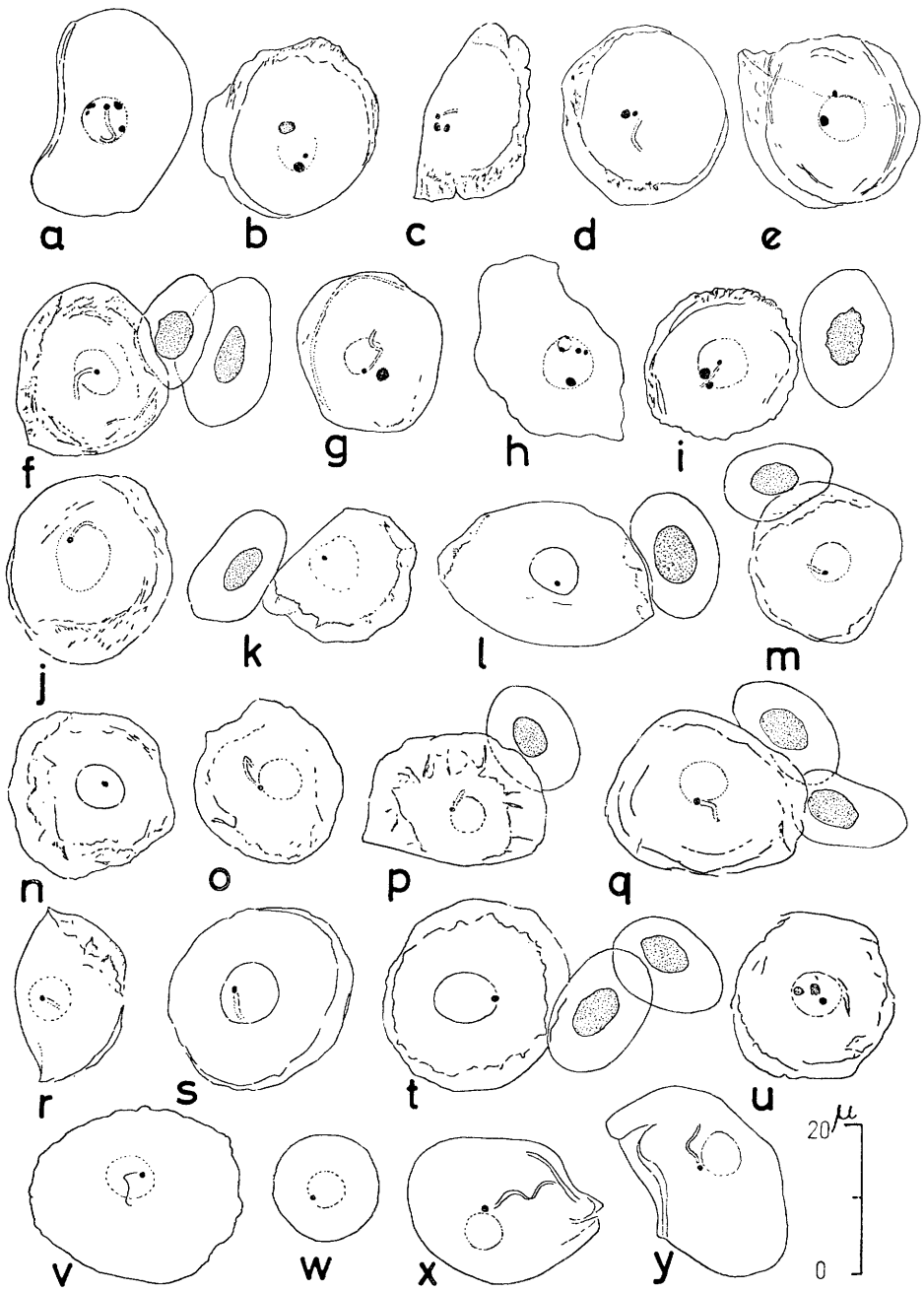

Fig. 9. *Trypanosoma tsunezomiyatai* n. sp. (Host: a–i. *Rana rugosa*; j–y. *Rana limnocharis limnocharis*)

(93.5 microns); body length 63.0–78.0 microns (69.6 microns); width at the widest part of body including undulating membrane 3.6–6.0 microns (4.8 microns); length of free flagellum 18.0–33.6 microns (23.9 microns); posterior end to kinetoplast 6.0–12.0 microns (9.8 microns); posterior end to middle of nucleus 15.0–24.0 microns (18.2 microns); anterior end to middle of nucleus 39.0–60.0 microns (51.4 microns); nucleus round and its diameter 1.8–3.6 microns (2.3 microns); clear space or vacuole present in anterior end of nucleus; kinetoplast marginal; anterior part of kinetoplast sometimes vacuolated; pre-nuclear portion of body sometimes striated with fine granules; the kinetoplast index= 1.6–3.5 (2.2); the nuclear index=0.3–0.6 (0.4).

Type smear: Holotype and paratype smears are in the collection of the author in the Department of Epidemiology, Institute for Tropical Medicine, Nagasaki University. Smear No. 1976-6-19-25, No. 1976-6-20-24, No. 1976-7-1-1, No. 1976-6-20-29, No. 1976-6-22-8, and No. 1976-6-20-31.

Type host: *Rana namiyei* Stejneger, 1901.

Type locality: Yona, Kunigamison, northern part of Okinawa Island.

Remarks: This trypanosome somewhat resembles *Trypanosoma neveulemairei* Brumpt, 1928, described from the blood of Corsican frog, *Rana esculenta*, however in *Trypanosoma tsukamotoi* n. sp., the distance between posterior end and kinetoplast is shorter and length of free flagellum is longer than those of *Trypanosoma neveulemairei*. *Trypanosoma karyozeukton* Dutton and Todd, 1903, (Host: *Bufo regularis*, *Rana oxyrhynchus*. Loclaity: Africa) has

Table 10. Size (in microns) of *Trypanosoma tsunezomiyatai* n. sp.

	Longest Length	Shortest Length	NL
	25.2	21.0	9.6
	18.6	15.0	7.2
	28.2	18.6	6.6
	22.2	20.4	6.6
	19.8	19.2	5.4
	28.2	15.6	5.4
	23.4	16.8	4.8
	21.0	18.0	4.2
	25.2	15.0	5.4
	26.4	21.6	7.2
	25.8	22.8	8.4
	25.2	19.2	4.8
	25.2	22.8	8.4
	27.6	22.8	5.4
	14.4	13.8	4.8
	22.2	14.4	4.8
	23.4	20.4	6.0
Average	23.6	18.7	6.2
Minimum	14.4	13.8	4.2
Maximum	28.2	22.8	9.6

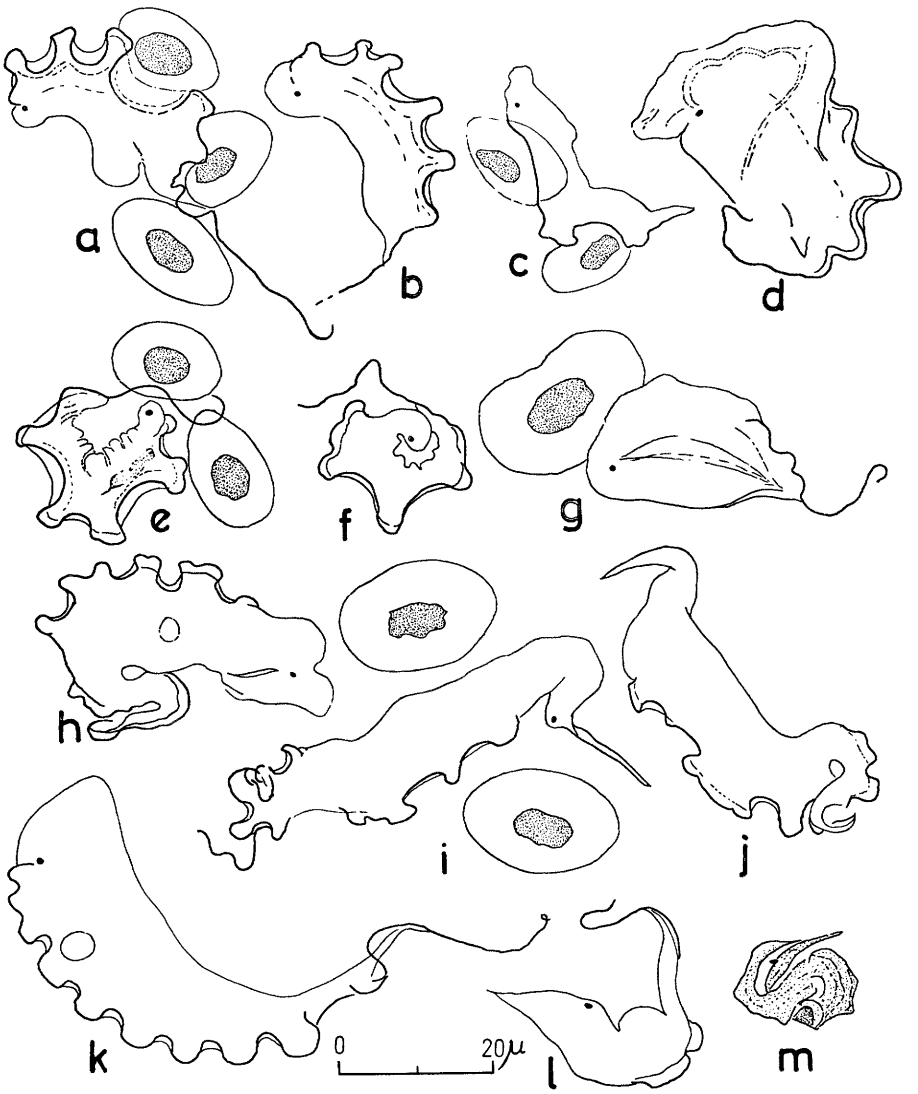

Fig. 10. *Trypanosoma rotatorium* (Mayer, 1843) (Host: a–c. *Rhacophorus japonicus*; d. *Rana nigromaculata*), *Trypanosoma* sp. B (Host: e, f. *Hyla arborea japonica*), *Trypanosoma* sp. C (Host: g. *Rana holsti*), *Trypanosoma miyagii* n. sp. (Host: h–j. *Rana ishikawae*; k, l. *Rana subaspera*), and *Trypanosoma* sp. D (Host: m. *Rana limnocharis limnocharis*)

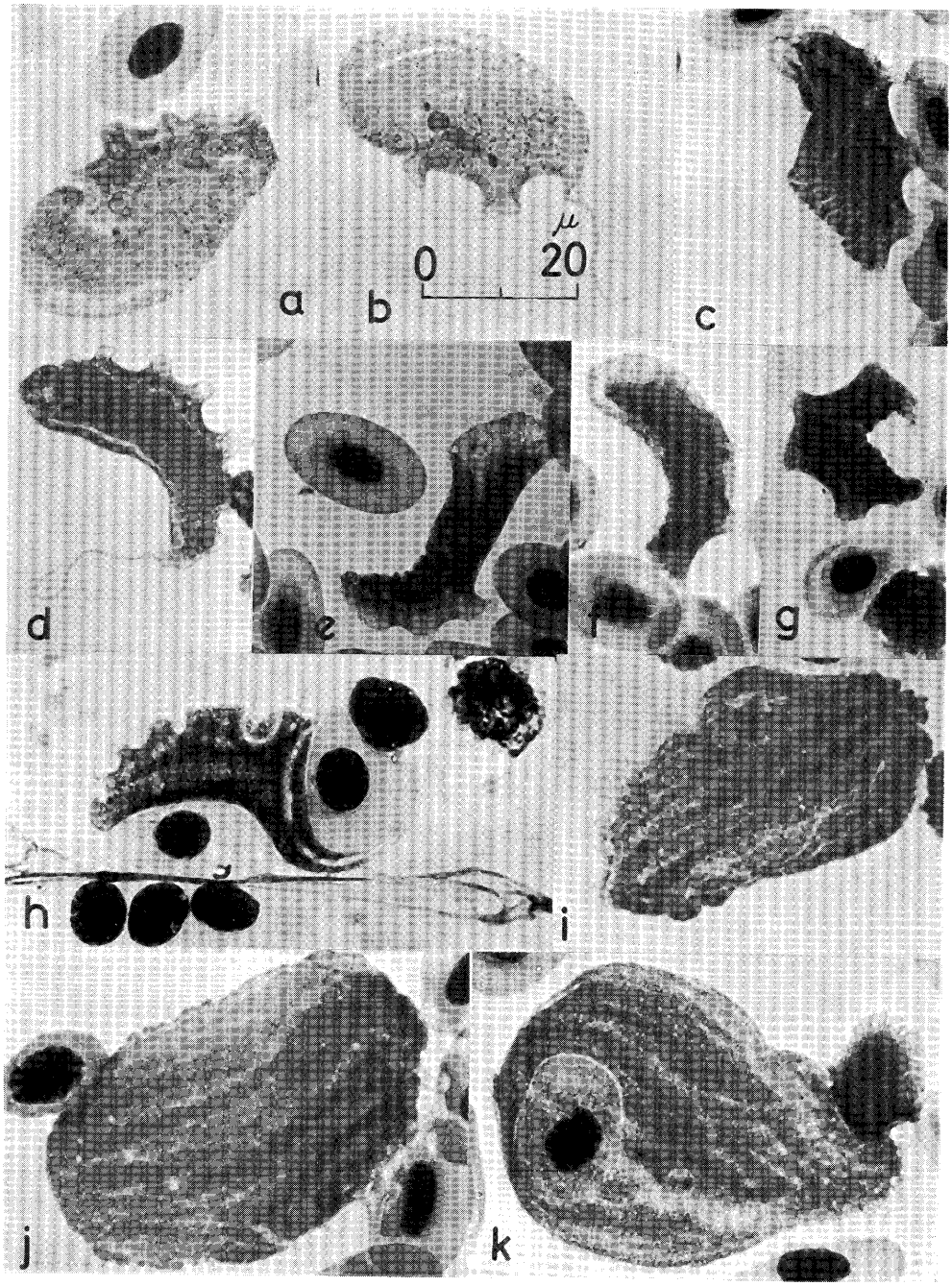

Fig. 11. *Trypanosoma nagasakiense* n. sp. (Host: a, b. *Hyla arborea japonica*)
Trypanosoma rotatorium (Mayer, 1843) (Host: c, d. *Rana rugosa*)
Trypanosoma sp. A (Host: e. *Rana rugosa*; f. *Rana limnocharis limnocharis*)
Trypanosoma sp. B (Host: g, h. *Hyla arborea japonica*)
Trypanosoma loricatum (Mayer, 1843) (Host: i. *Rana nigromaculata*; j, k. *Rana holsti*)

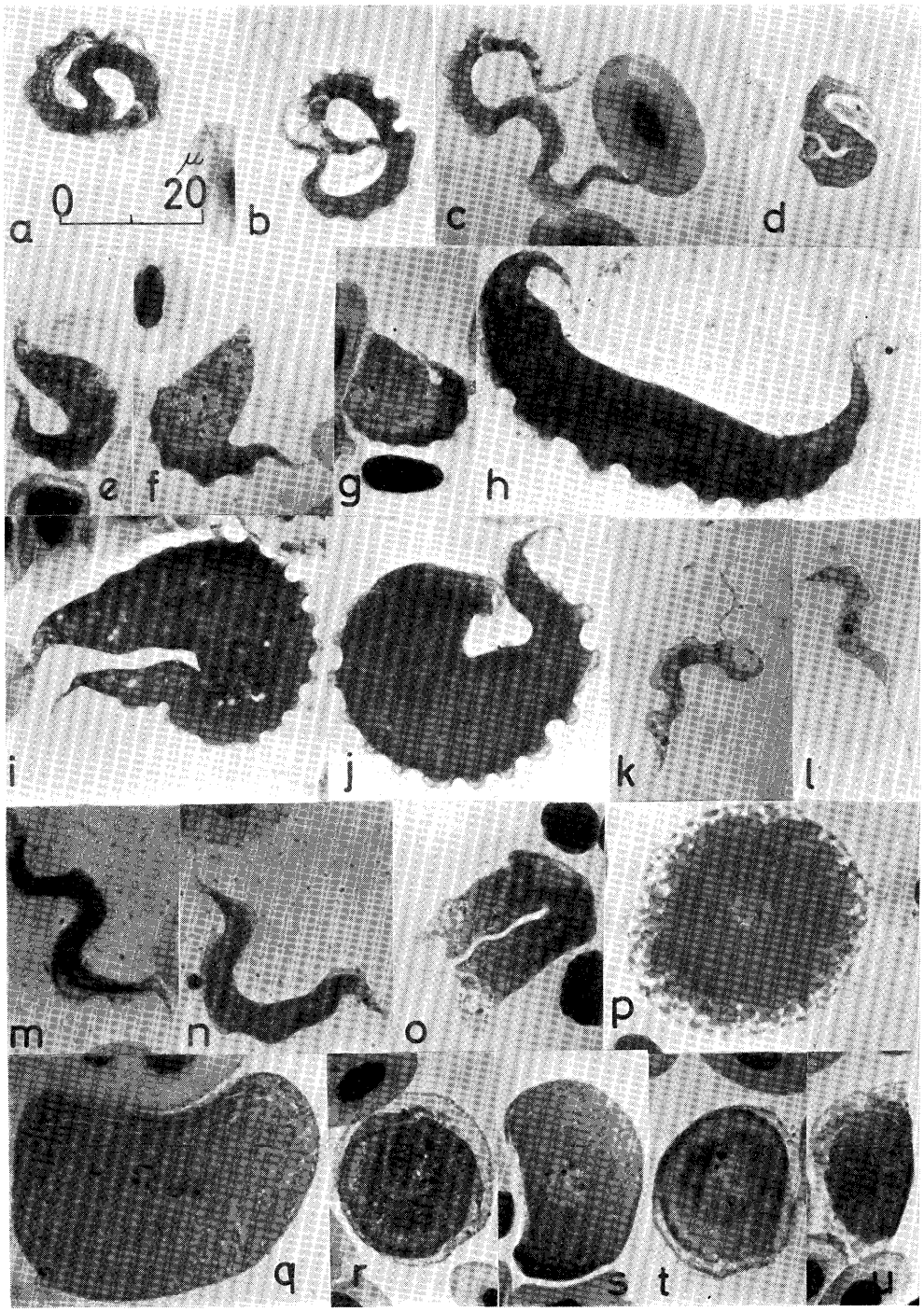

Fig. 12. *Trypanosoma tsukamotoi* n. sp. (Host: a–c. *Rana namiyei*)
Trypanosoma sp. D (Host: d. *Rana limnocharis limnocharis*)
Trypanosoma ishigakiense n. sp. (Host: e–g. *Rana limnocharis limnocharis*)
Trypanosoma miyagii n. sp. (Host: h–j. *Rana namiyei*)
Trypanosoma sp. E (Host: k–l. *Rana rugosa*, larva)
Trypanosoma rugosae n. sp. (Host: m–o. *Rana rugosa*, larva)
Trypanosoma chattoni Mathis and Leger, 1911 (Host: p–q. *Rana rugosa*)
Trypanosoma tsunezomiyatai n. sp. (Host: p–u. *Rana rugosa*)

shorter distance between posterior end and kinetoplast, and pre-nuclear portion of the trypanosome stains darkly, and post-nuclear portion stains lightly. *Trypanosoma montezumae* Pérez-Reyes, Hashimoto, Montesinos, and Valencia, 1960, (Host: *Rana pustulosa*, *Rana montezumae*, and *Rana palmipes*. Locality: Mexico) and *Trypanosoma leptodactyli* Carini, 1910 (Host: Brazilian frog, *Leptodactylus ocellatus*) are smaller species as compared with *Trypanosoma tsukamotoi* n. sp.

This species is named after Dr. Masuhisa Tsukamoto. This study was carried out with his co-operation in Okinawa and Nagasaki.

10) *Trypanosoma* sp. D (Fig. 10, m. Fig. 12, d)

This slender trypanosome was detected from *Rana limnocharis limnocharis* captured in Ishigaki Island. In this case, only one individual of the trypanosome was detected, and unfortunately free flagellum and undulating membrane were invisible. The following description is based on the individual stained with Giemsa.

Body slender; pre-nuclear portion lightly stained; post-nuclear portion darkly stained and striated with granules; body length 53.0 microns; posterior end to kinetoplast 9 microns; kinetoplast to middle of nucleus 10.2 microns; posterior end to middle of nucleus 19.2 microns; anterior end to middle of nucleus 34.8 microns; width at the widest part of body 3.6 microns; the kinetoplast index=1.9; the nuclear index=0.6; nucleus round and pinkish in colour; nucleus surrounded with clear space; vacuole present in post-kinetoplast.

Host and Locality: *Rana limnocharis limnocharis* Wiegmam, 1835, which was captured in Ishigaki Island. Smear No. 1976-6-24-5.

Remarks: This species is apparently different from all other trypanosomes detected from *Rana limnocharis limnocharis* captured in Ishigaki Island. *Trypanosoma tsukamotoi* n. sp. resembles *Trypanosoma* sp. D, but the latter is somewhat smaller than *Trypanosoma tsukamotoi* n. sp. Mathis and Leger (1911b) were figured a trypanosome similar to *Trypanosoma* sp. D under the name of *Trypanosoma elegans* (Pl. V, Fig. 8 in their paper), but their trypanosome is not identical with *Trypanosoma inopinatum* Sergent and Sergent, 1904 (=*Trypanosoma elegans*). More materials are needed to determine the systematic position of *Trypanosoma* sp. D.

11) *Trypanosoma* sp. E (Fig. 7, a-p, Fig. 12, k-l)

This small slender trypanosome is commonly observed in the blood smear of tadopole of *Rana rugosa*. The trypanosome was shown as an immature form of *Trypanosoma rotatorium* in Miyata (1976). At present, however, the author can not determine whether the trypanosome belong to a new species or an immature stage of already known species.

Host and Locality: The trypanosome was detected from larvae and an adult frog (Smear No. 1974-33) of *Rana rugosa* Schlegel, 1938.

12) *Trypanosoma rugosae* n. sp. (Fig. 7, q-G, Fig. 12, m-o, and Table 8)

This trypanosome was found from tadopoles of *Rana rugosa* captured in paddy field at Nagasaki. The following descripition is based on the blood smears stained with Giemsa and

the size in parentheses is expressed as the average of 10 trypanosomes shown in Table 8.

Body slender; both ends pointed; undulating membrane narrow; total length 45.0-56.4 microns (49.4 microns); body length 37.8-49.2 microns (42.4 microns); posterior end to kinetoplast 3.6-5.4 microns (4.0 microns); posterior end to middle of nucleus 18.0-25.2 microns (22.4 microns); anterior end to middle of nucleus 17.4-24.6 microns (20.0 microns); free flagellum 6.0-9.0 microns (7.0 microns); width at the widest part of body including undulating membrane 4.8-6.0 microns (5.4 microns); nucleus round and 2.4-3.0 microns (2.5 microns) in diameter; posterior part of kinetoplast lightly stained; usually cytoplasm stained darkly and in some individuals, nucleus indistinguishable; the kinetoplast index = 1.2-1.3 (1.2); the nuclear index=0.8-1.4 (1.1).

From a smear (No. 1974-37) taken from a tadopole of *Rana rugosa*, broader trypanosome was detected (Fig. 7, E-G). This form also might belong to this new species.

Type smear: Holotype and paratype smears are in the collection of the author in the Department of Epidemiology, Institute for Tropical Medicine, Nagasaki University. Smear No. 1974-47, No. 1974-52, and No. 1974-55.

Type host: Tadopole of *Rana rugosa* Schlegel, 1838.

Type locality: Mogi, the vicinity of Nagasaki City.

Remarks: This species was already figured by the present author as an immature form of *Trypanosoma rotatorium* (Miyata, 1976, Fig. 2, a-g, in his paper), however, it is now concluded that this is apparently a new species distinguishable from other known trypanosomes from *Rana rugosa* in Nagasaki.

Trypanosoma montrealis Fantham, Porter, and Richardson, 1942, (host: Canadian toad, *Bufo americanus*), somewhat resembles *Trypanosoma rugosae* n. sp. in its general shape, but the former has an oval nucleus, a small kinetoplast, and a short free flagellum. Cytoplasm of *Trypanosoma rugosae* n. sp. stains more darkly than that of *Trypanosoma montrealis*.

The relationship between *Trypanosoma rugosae* n. sp. and *Trypanosoma* sp. D is not known, but they are easily distinguishable from each other, because the latter species has a longer free flagellum and lightly stained cytoplasm.

13) *Trypanosoma chattoni* Mathis and Leger, 1911a (Fig. 8, Fig. 12, p-q, and Table 9)

Large spherical bodies were detected from blood smears of *Rana holsti* and *Rana narina* captured in Okinawa Island. The following descripition was based on the Giemsa stained smears, and the size in parentheses was expressed as the average of 11 individuals.

Body round; nucleus present about center of body; kinetoplast seen on nucleus; the longest length of body 33.0-57.0 microns (40.0 microns); the shortest length of body 27.6-36.0 microns (32.1 microns); in many individuals, body edge wrinkled; nucleus round and 4.8-8.4 microns (6.1 microns) in diameter; undulating membrane absent; short free flagellum present near kinetoplast in some individuals.

Host: This species is detected from *Rana holsti* Boulenger, 1892, and *Rana narina* Stejneger, 1901, both captured at Yona, Kunigamison, northern part of Okinawa Island in

1976. The trypanosome detected from *Rana rugosa* Schlegel, 1838, captured in the vicinities of Nagasaki City, is apparently identical with *Trypanosoma chattoni*.

Remarks: According to the description of Mathis and Leger (1911a and 1911b) and Diamond (1958 and 1965), this trypanosome belongs to *Trypanosoma chattoni* (especially Fig. vi, 1-4, in Mathis and Leger, 1911b).

14) *Trypanosoma tsunezomiyatai* n. sp. (Fig. 9, Fig, 12, r-u, and Table 10)

This trypanosome was found from the blood smears of *Rana rugosa* captured in the vicinity of Nagasaki City. The following description is based on the Giemsa stained smears, and the size in parentheses is expressed as the average of 17 trypanosomes as shown in Table 10.

Body usually round ; cytoplasm finely granular ; no undulating membrane; the longest length 14.4-28.2 microns (23.6 microns) ; the shortest length 13.8-22.8 microns (18.7 microns); round nucleus present in central part of body; diameter of nucleus 4.2-9.6 microns (6.2 microns); kinetoplast usually present peripheral part of nucleus; short free flagellum usually seen near kinetoplast.

Type smear: Holotype and paratype smears are in the collection of the author in the Department of Epidemiology, Institute for Tropical Medicine, Nagasaki University. Smear No. 1974-17, No. 1974-26, No. 1974-18, and others.

Type host: *Rana rugosa* Schlegel, 1838.

Type locality: Mogi, the vicinity of Nagasaki City.

Other host: This new species was detected from *Rana limnocharis limnocharis* Wiegmann, 1835, captured in Ishigaki Island in 1976 and at near Inbu Bay, Okinawa Island, in 1974 by Prof. Ichiro Miyagi.

Remarks: This species was already figured by Mathis and Leger (1911b, Pl. v, Figs, 15-21, in their paper) under the name *Trypanosoma borreli*, however their figures are very different from the original description of *Trypanosoma borreli* by Marchoux and Salimbeni (1907). *Trypanosoma pseudopodium* Werner and Walewski, 1976, described from North American toad, *Buof americanus* is a larger species having oval nucleus and short free flagellum.

In *Trypanosoma chattoni*, *Trypanosoma tsunezomiyatai* n. sp., and may be, *Trypanosoma pseudopodium*, trypomastigote stage is not observed, then those species can not include to the genus *Trypanosoma*, however further studies, especially on their life cycles, are needed to solve the systematic position of such species.

This new species was named after Mr. Tsunezo Miyata, father of the present author, for the memory of the 77th aniversary of his birth.

DISCUSSION

According to Diamond (1958 and 1965), 26 species of anuran trypanosome are separable as distinct species. Recently Barsley and Harmsen (1973) reviewed the literature concerning anuran trypanosome and listed many new literature after Diamond's papers. From those

Table 11. Trypanosome species and other haemoparasites detected from each frog examined

Host No. & Locality	naga.	rota.	sp. A	lori.	miya.	tsuka.	sp. E	rugo.	chat.	tsune.	others
Rana namiyei											
1976-6-19-25 Y					○	○					
1976-6-20-24 Y					○	○					
1976-6-20-28 Y					○						
1976-6-20-29 Y					○	○					
1976-6-20-31 Y					○	○					
1976-6-22-7 Y					○						
1976-6-22-8 Y					○	○					
1976-6-22-9 Y					○						
1976-7-1-1 Y					○	○					
1976-7-1-2 Y					○						
1976-7-1-4 Y					○						*Lankesterella* sp.
1976-7-1-6 Y					○						
1976-7-1-8 Y					○						
1976-7-1-39 Y					○						
1976-7-3-3 Y					○						
1976-7-3-5 Y					○						
1976-7-3-9 B					○						
1976-7-3-13 B					○						
1976-7-3-16 B					○						
1976-7-3-17 B					○						
Rana holsti											
1976-6-19-24 Y					○				○		Hg.
1976-6-19-33 Y			○	○					○		
1976-6-19-37 Y				○					○		
1976-6-19-38 Y				○							sp. C
1976-6-20-23 Y					○						*Toddia*; Hg.
1976-7-1-3 Y				○	○						Mf.
1976-7-1-12 Y				○					○		
1976-7-1-13 Y			○	○	○				○		*Toddia*; Hg.
1976-7-1-37 Y			○	○	○				○		Hg.
1976-7-3-1 Y				○					○		
1976-9-17 Y			○						○		
Rana narina											
1976-6-19-27 Y					○						
1976-6-19-30 Y				○	○				○		
1976-6-19-32 Y									○		
1976-7-1-38 Y					○						
Rana ishikawae											
1976-7-3-36 Y					○						
Rana subaspera											*Lankesterella* sp.; Hg.
No. 1 Amami Isl.					○						Mf.
Racophorus japonicus											
1976-7-1-29 Y		○									
Rana l. limnocharis											
1974-63 Okinawa										○	
1976-6-24-5 Ishi.										○	ishi.; sp. D; Hg.; Dc.
1976-7-23-6 Ishi.			○								
1976-7-23-7 Ishi.										○	Hg.
1976-7-23-8 Ishi.			○							○	Dc.
1976-7-26-7 Ishi.			○							○	Dc.
1976-7-26-11 Ishi.			○							○	Dc.
Rana rugosa											
1974-4 Mogi			○							○	
1974-13 Mogi		○								○	
1974-17 Mogi		○								○	Dc.
1974-18 Mogi										○	Dc.
1974-26 Mogi										○	Dc.
1974-27 Mogi		○								○	Dc.
1974-29 Mogi			○							○	
1974-33 Mogi							○				
1974-34 Mogi									○		
1977-6-23-125 Isa.									○		
1977-6-23-127 Isa.									○		
Tadopole											
1974-35 Mogi							○				
1974-37 Mogi								○			
1974-40 Mogi							○				
1974-47 Mogi								○			
1974-51 Mogi							○				
1974-52 Mogi								○			
1974-55 Mogi							○	○			
Rana nigromaculata											
1974-14 Mogi				○							
1977-23-124 Isa.		○		○							
Hyla a. japonica											sp. B; unidentified protozoa
1977-6-23-2 Isa.											
1977-7-9-1 Isa.	○										
1977-7-9-2 Isa.	○										

Locality

Y: Yona, Kunigamison, northern part of Okinawa Island, Ryukyu

B: Benoki, Kunigamison, northern part of Okinawa Island, Ryukyu

Mogi: Mogi, near Nagasaki City, Kyushu

Ishi.: Ishigaki Island, Ryukyu

Trypanosome species and other haemoparasites

naga. = *Trypanosoma nagasakiense* n. sp.; rota = *Trypanosoma rotatorium*; sp. A = *Trypanosoma* sp. A; sp. B = *Trypanosoma* sp. B; sp. C = *Trypanosoma* sp. C; sp. D = *Trypanosoma* sp. D; sp. E = *Trypanosoma* sp. E; lori. = *Trypanosoma loricatum*; miya. = *Trypanosoma miyagii* n. sp.; tsuka: *Trypanosoma tsukamotoi* n. sp.; rugo = *Trypanosoma rugosae* n. sp.; chat. = *Trypanosoma chattoni*; tsune. = *Trypanosoma tsunezomiyatai* n. sp.; ishi. = *Trypanosoma ishigakiense* n. sp.; Hg. = Haemogregarine; Mf. = Microfilaria; Dc. = *Dactylosoma ranarum*; unidentified protozoa have been reported by Miyata, Miyagi, and Tsukamoto (1978).

literature, 34 species might be classified as distinct species except for *Trypanosoma aurorae* Lenmann, 1959, which is nomen nudum.

In the present study, the author described 14 types of anuran trypanosome which was detected from 10 species of frog captured in Kyushu and Ryukyu. Only three species, *Trypanosoma rotatorium*, *Trypanosoma loricatum*, and *Trypanosoma chattoni*, are already named by other authors. The following six species are described as new species, *Trypanosoma nagasakiense* n. sp., *Trypanosoma ishigakiense* n. sp., *Trypanosoma miyagii* n. sp., *Trypanosoma tsukamotoi* n. sp., *Trypanosoma rugosae* n. sp., and *Trypanosoma tsunezomiyatai* n. sp. These species are apparently different from all other known trypanosomes in shape of body and measurements of various parts of body. Other five types also are apparently different from above 9 species, but the types can not be determined whether they are distinct species or a form of already known species. Koidzumi (1911), Ogawa (1913), Ogawa and Uegaki (1927), and Tanabe (1931) published their studies on anuran trypanosome obtained in Japan and its adjacent countries. Some of the parasites figured by them are apparently different from 14 types reported in the present paper. At present, the number of known anuran trypanosomes became just 40 species by the addition of 6 new species, however much more species might be described in near future in various parts of the world, because some more trypanosomes figured in various literature under the name *Trypanosoma rotatorium* or *Trypanosoma* sp., are not identical with one of those known species morphologically.

Geographical distribution of anuran trypanosome is very wide in some species, but very narrow in other species. For example, *Trypanosoma rotatorium*, *Trypanosoma chattoni*, *Trypanosoma loricatum*, and *Trypanosoma tsunezomiyatai* have distribute in wide range, but *Trypanosoma miyagii*, *Trypanosoma tsukamotoi*, *Trypanosoma ishigakiense*, and *Trypanosoma nagasakiense* have been found in limited area. Host specificity of anuran trypanosome is also very wide in some species and very narrow in others. *Trypanosoma miyagii* is very common species among *Rana namiyei*, *Rana holsti*, *Rana narina*, *Rana ishikawae*, and *Rana subaspera*. These frogs distribute in limited areas of Ryukyu Islands, where at night they stay on the road passing stream side and across the forest. From *Rana namiyei*, a land leech, *Haemadipsa zeylanica japonica*, was detected, but any external parasite was not found from other frogs. The host of *Trypanosoma tsukamotoi* is strictly limited to *Rana namiyei*, from which the parasite often detected. *Rana holsti* was captured in same place where *Rana namiyei* was collected, but trypanosomes detected from *Rana holsti* are apparently different compared with those from *Rana namiyei*. In addition to *Trypanosoma miyagii*, three more species were seen in the blood of *Rana holsti*, of which one out of 11 trypanosome positive cases four species of trypanosome were detected at the same time. Trypanosome fauna of *Rana narina* are somewhat similar to the case of *Rana holsti*. The parasite of *Rana limnocharis limnocharis* resembles that of *Rana rugosa*, but trypanosome found from tadopole of *Rana rugosa* is somewhat different from that of adult frog. As shown in Table 11, more than one species of trypanosome are usually detected in trypanosome positive frog, and addition to this, *Dactylosoma* or haemogregarine is often found in the same blood smear (see Miyata, Miyagi, and Tsukamoto, 1978).

At present, vector for these parasites is not known, but some of them might be transfered by the land leech or the water leech. In the previous paper (Miyata, 1977a and b) the author described *Trypanosoma ogawai* and *Haemogregarina shirikenimori* from *Triturus pyrrhogaster ensicauda* which was captured at Yona, Kunigamison, northern part of Okinawa Island. Possible vector of these two parasites is the land leech, *Haemadipsa zeylanica japonica*, because the leech was often seen on the body of the newt. In the addition to the leech, mosquitoes or some other species of blood-sucking insects might transfer some of anuran haemoparasites shown or suggested by Feng and Chung (1941), Bailey (1962), Ayala (1972), and Desser, McIver, and Rychman (1973).

At present taxonomic study of anuran trypanosome based on morphological features is very important, but in future ecological study of each trypanosome and experimental transmission with pure culture of trypanosome to laboratory rared frog are needed.

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九州および琉球列島産無尾類寄生トリパノゾーマについて （付：6新種の記載）

宮田 彬 （長崎大学熱帯医学研究所疫学部門）

カエルのトリパノゾーマは生物学および医学の分野で最も手近かな材料として広く実験に供せられ，特にトリパノゾーマ類の栄養要求性の研究や培地の改善の研究に使われている．しかし広く用いられている割合には分類学的な混乱が解決されていないようで，例えばある実験に用いられたトリパノゾーマが果して何という種であったか確定できないことが多い．これは世界中のカエルに唯1種あるいは2～3種のトリパノゾーマが寄生しているだけであるという固定観念が強く信仰されてきたことが原因であろう．しかし実際には既知カエル・トリパノゾーマは40種近く知られており，しかもまだ相当の種類が追加されそうな形勢である．カエルのトリパノゾーマはわれわれの身近の手軽な材料ではあるが，以上のような分類学上の問題を十分知っておかないと何を研究したのかわからなくなる．日本のカエルのトリパノゾーマについては，小泉丹博士らによる研究があるが，それらはきわめて簡単である．そこでこの論文では，九州および琉球列島で入手したカエル類の血液塗抹標本により，純形態学的に分類し，結局それらのトリパノゾーマが14型にわけられることを確認し詳しく図説した．14型のうち *Trypanosoma rotatorium*, *Trypanosoma loricatum*, *Trypanosoma chattoni* の3種は分布の広い既知種である．また出現頻度が高くしかも純形態学的に区別が明瞭な次の6種は新種として命名した．1. *Trypanosoma nagasakiense* n. sp. 諫早市の山口明技官宅で捕えられたアマガエルより発見された卵形の美しい種．2. *Trypanosoma ishigakiense* n. sp. 石垣島産のヌマガエルより発見された種で，近縁種はアフリカで発見されている．3. *Trypanosoma miyagii* n. sp. 沖縄本島産のナミエガエル，ホルストガエル，ハナサキガエル，イシカワガエル，および奄美大島産のオットンガエルより発見された雄大な形の種で，学名は共同研究者である畏友宮城一郎教授（琉球大学）にちなむ．4. *Trypanosoma tsukamotoi* n. sp. 沖縄本島産のナミエガエルより発見された蛇のように細長い種で，系統的には魚類のトリパノゾーマに近い．学名はこの仕事の共同研究者でありまた良き師でもある塚本増久博士にちなむ．5. *Trypanosoma rugosae* n. sp. 長崎近郊のツチガエルのオタマジャクシの体液中に発見されるトリパノゾーマでウナギのような感じの種である．学名は宿主の学名に由来する．6. *Trypanosoma tsunezomiyatai* n. sp. 長崎市のツチガエル，石垣島および沖縄本島のヌマガエルより発見された波動膜を欠くまるいトリパノゾーマである．本種はもともとヨーロッパの研究者により何度か図示されているが，独立種として命名した学者はないので，長崎産ツチガエルを模式宿主として新しく命名した．本種と *Trypanosoma chattoni* とを含む新属をつくる必要があるが，今回発見された新種の生活史を確認した上で処置したい．学名は筆者の父宮田常蔵の喜寿を記念して命名した．その他現時点では分類上の位置を決定できない5型についてはそれぞれ種A～Eとして図示した．これらのうちいくつかは将来材料を得て命名することがあると思う．なお以上のトリパノゾーマは1匹のカエルに多い例では4種も寄生しており，2～3種のトリパノゾーマが寄生している例は決して稀れではない．またパラサイテミアが低く，1枚の標本に1～2虫体がみつかる程度のことも多いので，カエル・トリパノゾーマを実験に供する際には数枚の血液標本を用意し，それぞれ全視野を検し，出現するトリパノゾーマの種を確認する必要がある．

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