Trypanosoma ogawai n. sp. (Protozoa: Trypanosomatidae) Detected from *Triturus pyrrhogaster ensicauda* (Hallowell, 1860) (Amphibia: Salamandridae) in Okinawa Island

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Abstract: *Trypanosoma ogawai* n. sp. was discovered from the blood smears of adults *Triturus pyrrhogaster ensicauda* (Hallowell, 1860) collected in Okinawa Island. This trypanosome is a slender monomorphic species, measuring 48.7 microns in total length including a free flagellum (19.9 microns) and 5.0 microns in the width at the widest part. This trypanosome is well distinguishable from *Trypanosoma tritonis* Ogawa, 1913, described from *Triturus pyrrhogaster pyrrhogaster* (Boie, 1826) in Kyushu, and other known trypanosomes from newts in the small body size and the longer free flagellum. Type of division in this trypanosome is unequal binary fission, and one of daughter trypanosomes is trypomastigote form, but another is epimastigote form. The land leech, *Haemadipsa zeylanica japonica*, is the most suspicious vector for this new trypanosome.

Trypanosoma tritonis Ogawa, 1913, was discovered from *Triturus pyrrhogaster pyrrhogaster* (Boie, 1826) (syn. *Tryton pyrrhogaster*) in Kyushu (type locality: Fukuoka). According to Pearse (1932), *Trypanosoma tritonis* was also detected from *Triturus p. pyrrhogaster* collected in Honshu (Kanto District). In Ryukyu Islands, another newt, *Triturus pyrrhogaster ensicauda* (Hallowell, 1860), distributes, but the blood examination of this newt has not been carried out until now. During a period of June to July in 1976, the present author had an opportunity to examine the blood smears of *Triturus p. ensicauda* (Japanese name: Shirikenimori) collected in Okinawa Island. A new species of trypanosome was detected from the smears in addition to another blood protozoa, *Haemogregarina shirikenimori* n. sp. which has been described in a separate paper of this issue (Miyata, 1977). The morphology of the species described herein as *Trypanosoma ogawai* n. sp. is quite different from that of *Trypanosoma tritonis* or any other trypanosomes known from the blood of various species of the newt. The new species is named after Dr. M. Ogawa, one of the pioneers for the studies on the parasitic porotozoa in Japan.

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Trypanosoma ogawai n. sp.

(Figs. 1–2 and Table 1)

The morphological features of trypanosome are based on the Giemsa stained smears as described below. The size in parentheses is expressed as the average of 15 trypanosomes shown in Table 1; monomorphic trypanosome with slender body; 48.7 microns in total length including a free flagellum and 5.0 microns in width at the widest point including the undulating membrane; the free flagellum rather long (19.9 microns); pale pinkish cytoplasm usually with fine granules and several vacuoles; rod-shaped and dark pink kinetoplast lying at a posterior part of the body and a vacuole or a clear area surrounding the kinetoplast; shape of nucleus usually round or ellipsoidal and the colour dark pink; the distance from posterior end to middle of nucleus (16.7 microns) longer than that from anterior end to middle of nucleus (11. 8 microns); the distance of kinetoplast to middle of nucleus 8.8 microns and that of posterior end to middle of nucleus 6.3 microns; the nuclear index (NI) 1.6, and the kinetoplast index (KI) 2.1.

Multiplication of *Trypanosoma ogawai* n. sp. in the peripheral blood of *Triturus pyrrhogaster ensicauda* was observed in some smears. Division of this trypanosome is due to unequal binary fission, and before division, kinetoplast moves to lateral part of nucleus as shown in Fig. 2, l. After division, each daughter trypanosome has a different size and shape (Fig. 1, c): one of them is the trypomastigote form (Fig. 2, k), and another is the epimastigote form. Both these forms might grow to the slender mature trypanosome.

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 will be deposited in the collection of the Wellcome Museum of Medical Science, London.

Type host: *Triturus pyrrhogaster ensicauda* (Hallowell, 1860) (Amphibia: Salamandridae)

Type locality: Yona, Kunigami-son, northern part of Okinawa Island, Japan. The details on the type locality and survey method will be reported in a separate paper by Miyata, Miyagi, and Tsukamoto (1977). *Trypanosoma ogawai* n. sp. was detected from 22 out of 71 newts (31%), collected in June to July, 1976.

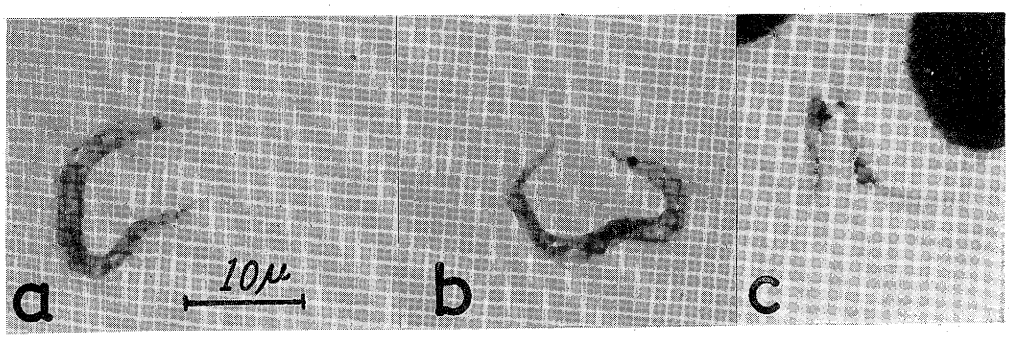


Fig. 1. *Trypanosoma ogawai* n. sp. detected from the peripheral blood of *Triturus pyrrhogaster ensicauda*.
a–b. mature trypanosomes.
c. epimastigote form (left) and trypomastigote form (right).

Vector: The natural invertebrate host of this trypanosome is not known, but a kind of land leech, *Haemadipsa zeylanica japonica*, is the most supicious vector for this trypanosome. Total 12 leeches were collected from the body of 71 newts, but unfortunately the leeches died before examination.

DISCUSSION

The following six species of trypanosomes have been known in the world from the newts:

1. *Trypanosoma diemictyli* Tobey, 1906
   Host: *Diemictylus viridescens* Vector: leech, *Placobdella parasitica* (reported by Nigrelli, 1929) and *Batrachobdella picta* (reported by Barrow, 1953) Locality: North America
2. *Trypanosoma tritonis* Ogawa, 1913
   Host: *Triturus pyrrhogaster pyrrhogaster* Vector: unknown (leech, *Hirudo nipponia* is most suspicious as a vector) Locality: Japan (Kyushu and Honshu)
3. *Trypanosoma cryptobranchi* Roudabush and Coatney, 1937
   Host: *Crytobranchus alleganiensis* Vector: unknown Locality: North America
4. *Trypanosoma barbari* Lehmann, 1952
   Host: *Triturus torosus* Vector: leech, *Actinobdella* sp. (repored by Lehmann, 1952a) Locality: North America
5. *Trypanosoma ambystomae* Lehmann, 1954
   Host: larval *Ambystoma gracile* (type host) and *Taricha granulosa* (reported by Lehmann, 1955) Vector: leech, *Erpobdella* sp. (reported by Lehmann, 1958) Locality: North America
6. *Trypanosoma granulosae* Lehmann, 1959
   Host: *Taricha granulosa twittyi* Vector: unknown Locality: North America

All those six species were shown in Fig. 3 and Table 2. The present species, *Trypanosoma ogawai* n. sp. is apparently smaller and slender than *Trypanosoma tritonis*. *Trypanosoma ogawai* has a narrow undulating membrane and a rod-shaped kinetoplast, but *Trypanosoma tritonis* has a developed undulating membrane and a circular kinetoplast. *Trypanosoma ogawai* is the smallest species distinguishable from all others on the base of the size alone.

Some authors (for example, Nakamura and Uéno, 1974) pointed out that the genera *Diemyctylus*, *Triturus*, *Taricha*, and *Cynops* might be treated as subgenera of the genus *Triturus*, and *Triturus pyrrhogaster* belongs to the subgenus *Cynops*. Three species of *Cynops* are known from Japan proper, Ryukyu, and East China; and Japanese species, *Triturus* (*Cynops*) *pyrrhogaster* is devided two subspecies, *pyrrhogaster* and *ensicauda* (Fig. 4). Each subspecies is parasitized by different trypanosome. The most suspicious vector of *Trypanosoma ogawai* is the land leech, *Haemadipsa zeylanica japonica*, because from 17% of the host examined the leech was found, and other kind of leech was not seen in survey area. Among seven known newt trypanosomes, three species are transmitted by the leech belonging to different genera mentioned above. The vector of *Trypanosoma tritonis* is not known, but

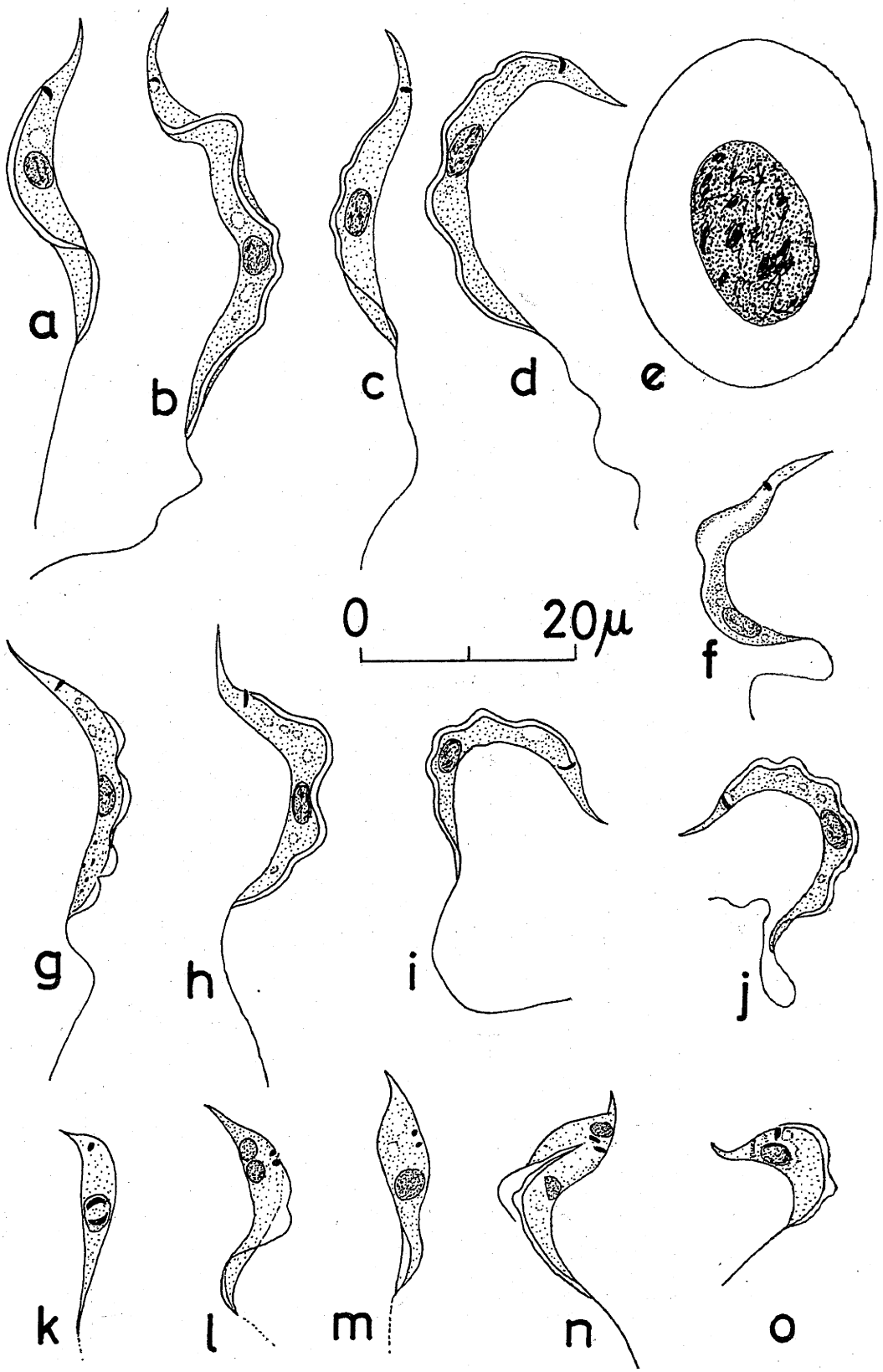
Table 1. Size (in microns) of *Trypanosoma ogawai* n. sp.

	TL	P-N	A-N	K-N	P-K	FF	W	NL	KI	NI
	47	13.5	16.5	4	8	17	4	3	3.4	0.8
	55	20.5	11.5	13	6	23	3	3	1.6	1.8
	45	15.5	11.5	7	7	18	4	3	2.2	1.3
	50	18	12	10	6	20	2.5	4	1.8	1.5
	67	25.5	17.5	16	8	24	4	3	1.6	1.5
	47	20.5	6.5	13	6	20	3	3	1.6	3.2
	34	12.5	6.5	6	5	15	2.5	3	2.1	1.9
	46	14.5	13.5	6	7	18	3	3	2.4	1.1
	41	14.5	9.5	8	5	17	3	3	1.6	1.5
	59	19	19	10	7	21	4	4	1.9	1.0
	55	17	12.5	9.5	6	21	4	3	1.8	1.4
	39.5	17	5.5	9.5	6	17	4	3	1.8	3.1
	46	16.5	9.5	10	5	20	3	3	1.7	1.7
	49	12.5	12.5	4	7	24	3	3	3.1	1.0
	50	13.5	13.5	6	6	23	4	3	2.3	1.0
Average	48.7	16.7	11.8	8.8	6.3	19.9	3.4	3.2	2.1	1.6
Minimum	34	12.5	5.5	4	5	15	2.5	3	1.6	0.8
Maximum	67	25.5	19	16	8	24	4	4	3.4	3.2

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
see Miyata (1976)

in the distribution area of this trypanosome a water leech, *Hirudo nipponia*, is only seen in paddy field. This leech is also possible vector of an anuran trypanosome, *Trypanosoma rotatorium* (Mayer, 1843) (see Miyata, 1976). *Trypanosoma tritonis* and *Trypanosoma ogawai* were discovered from different subspecies of *Triturus pyrrhogaster*. These two subspecies are not so different, but their blood parasites are apparently different. *Triturus pyrrhogaster ensicauda* has two kinds of blood parasite, *Trypanosoma ogawai* and *Haemogregarina shirikenimori*, but *Triturus pyrrhogaster pyrrhogaster* has *Trypanosoma tritonis* only (see Pearse, 1932). *Trypanosoma tritonis* and *Trypanosoma ogawai* are quite distinct species from the morphological and ecological aspects. Those findings show that *Triturus p.*

Fig. 2. *Trypanosoma ogawai* n. sp.
a-d and f-j. mature trypanosomes.
e. normal erythrocyte of *Triturus pyrrhogaster ensicauda*.
k-n. dividing forms.
o. immature trypomastigote form.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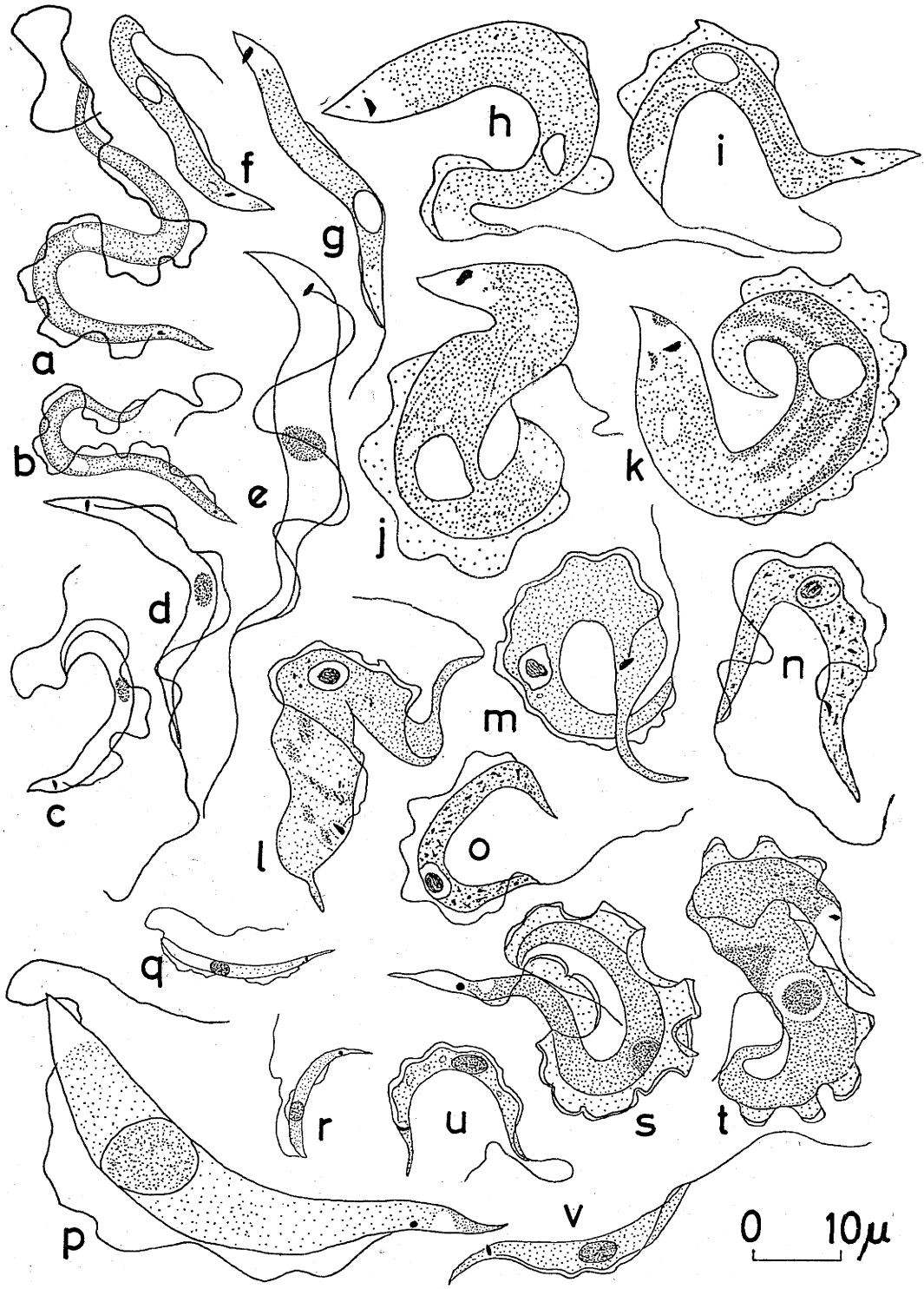
Tale 2. Morphological characters of the known species of newt trypanosomes (quoted from the original descriptions ; in microns)

	Shape of Kinetoplast	BL	P-N	A-N	K-N	P-K	FF	W	N-L
T. diemictyli[1]	circular	38.7～75.3	20.2～38.9	19～44	18～32	2.2～6.9	(25～31)	1.9～4.4	—
T. tritonis	circular	57.6～80.8	33.6～44.8	24.0～36.0	28.8～33.6	4.8～11.2	14.4～17.6	2.4～6.4	3.2～4.8
T. cryptobranchi	rod	46.8～77.4	—	19.33	13.75	6.01	21.61	1.8～5.84	—
T. barbari[2]	circular	20～30 63～81	— —	— —	— —	7～12 4～14	20～30 42～54	1～ 2.5 6～10.5	4 8
T. ambystomae	rod	28.8～43.7	16.2～24.2	12.5～20.5	3.4～7.7	—	14～19.8	1.8～7.7	2.5～7.7
T. granulosae	rod	41.1～59.0	24.8～35.1	16.3～23.9	18.0～23.8	8.4～14.0	(20～30)	3.5～10.5	5.1～5.3
T. ogwai n. sp.	rod	18～44.5	12.5～25.4	5.5～19	4～16	5～8	5～24	2.5～4	3～4

1 : quoted from Hegner (1921)
2 : two types of *T. barbari* separately shown
( ) : measured from figures
BL : Body length exclusive 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

Fig. 3. Known trypanosomes from the newts.
a-b. *Trypanosoma diemictyli* Tobey, 1906
c-e. *Trypanosoma cryptobranchi* Roudabush and Coatney, 1937
f-k. *Trypanosoma ambystomae* Lehmann, 1954
l-o. *Trypanosoma granulosae* Lehmann, 1959
p-r. *Trypanosoma barbari* Lehmann, 1952
s-t. *Trypanosoma tritonis* Ogawa, 1913
u-v. *Trypanosoma ogawai* n. sp.
(after each original description except *T. diemictyli* which was redrawn from Hegner, 1921).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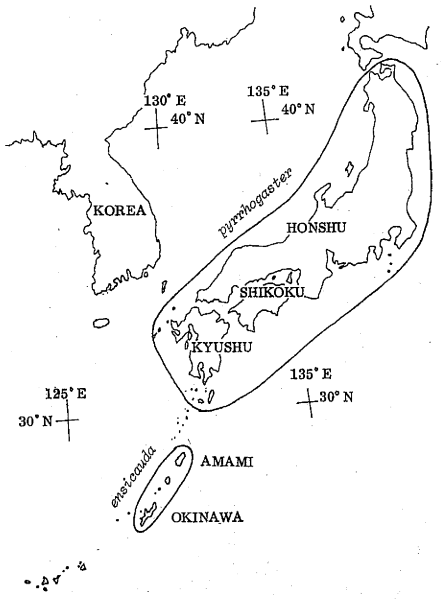

Fig. 4. Distribution map for *Triturus pyrrhogaster pyrrhogaster and Triturus pyrrhogaster ensicauda*, the known hosts of *Trypanosoma tritonis* and *Trypanosoma ogawai* n. sp., respectively.

pyrrhogaster and *Triturus p. ensicauda* might derive from different origins. The present author examined other cold-blooded animals in Okinawa Island, and he found some trypanosomes (including at least two more new species), but any trypanosome similar to *Trypanosoma ogawai* was not detected from other animals.

According to literatures, division stages of newt trypanosomes in the peripheral blood of host have not been observed until now, but in *Trypanosoma ogawai* unequal binary fission is sometimes observed in the blood smears. Similar unequal binary fission were reported in the crochodile trypanosome, *Trypanosoma grayi* Novy, 1906, reported by Hoare (1931), and avian trypanosome, *Trypanosoma bouffardi* Léger and Blanchard, 1911, reported by Molyneux (1973). The type of fission shows systematic relationship between amphibian trypanosome and reptilian and avian trypanosomes.

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沖縄産シリケンイモリより発見されたトリパノゾーマの1新種 *Trypanosoma ogawai*

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1976年6月，沖縄本島北部国頭村与郡とその近辺で採集したシリケンイモリ *Triturus pyrrhogaster ensicauda* の流血中から発見されたトリパノゾーマは，未知の新種であるので，*Trypanosoma ogawai* と命名した．このトリパノゾーマは，鞭毛をふくむ平均体長48.7ミクロン，遊離鞭毛の平均の長さは，19.9ミクロン，平均体幅は，5.0ミクロンである．日本本土産のイモリ *T. p. pyrrhogaster* からは，*Trypanosoma tritonis* Ogawa, 1913 が知られているが，*T. ogawai* は，小さく，細長い別種である．世界の有尾類のトリパノゾーマは，今まで6種知られており，今回記載した種は，7番目の種である．この論文では，既知種全種の図と虫体の測定値をしめした．*T. ogawai* の媒介者は，陸生のヒルの1種と考えられる．シリケンイモリの流血中での *T. ogawai* の不等2分裂についても報告した．この分裂により生じる娘虫体は，1方がエピマステイゴート型で，他方は，トリポマステイゴート型である．有尾類のトリパノゾーマの流血中における分裂は，今まで，全く報告されていなかった．今回観察された分裂の型は，ある種の爬虫類や鳥類のトリパノゾーマの分裂と類似しており，*T. ogawai* の系統進化を考える上で，きわめて興味深い．*T. ogawai* の和文の詳しい記載およびカラー図版は，著者が文部省成果刊行費を申請中の著書「寄生原生動物－その分類・生態・進化」（B 5判，1600頁）の中で，紹介した．

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