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Trypanosoma ryukyuense n. sp. (Protozoa: Trypanosomatidae) Detected from *Eublepharis kuroiwae kuroiwae* (Namie, 1912) (Reptilia: Gekkonidae) in Okinawa Island

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Abstract: *Trypanosoma ryukyuense* n. sp. was discovered from the blood smears of *Eublepharis kuroiwae kuroiwae* (Namie, 1912) captured in Yona, Kunigami-son, Okinawa Island. This trypanosome is a polymorphic species, and at least two morphological types can be distinguished. Type I (typical form) : Both ends pointed; twisted on longitudinal axis; 49.1 microns in total length including a free flagellum (11.9 microns) and 10.6 microns in width. The most peculiar feature of this type is its spiral ridges which turn along the body surface several times. Typical form of this species can be distinguished from all other trypanosomes known from reptiles and other animals based on its paticular spiral ridges. Type II (broader form): The body broader; surface costate ; 33.3 microns in length excluding a free flagellum (8.9 microns) and 23.2 microns in width. This form is quite different from the typical form, but there are some examples of the intermediate form of both types. In addition to those two types, some small trypanosomes without spiral ridges were detected, and these forms might be younger than the typical form. In any form, multiplication was not observed in the peripheral blood of the host animal. No information concerning to a vector for this trypanosome was obtained.

During a period of June, 1976, the present author had an opportunity to examine the blood smears of *Eublepharis kuroiwae kuroiwae* (Namie, 1912), which is classified in the subfamily Eublepharinae of the family Gekkonidae, in Okinawa Island. From the blood smears, a new polymorphic trypanosome was detected. According to literatures, totally twelve species of trypanosomes are known from the family Gekkonidae, but the new trypanosome is quite different from those species and any other trypanosomes known from reptiles. In the present paper, therefore, the species is described as *Trypanosoma ryukyuense* n. sp. based on the Giemsa stained blood-smears. The species is the first trypanosome recorded from the subfamily Eublepharinae.

Trypanosoma ryukyuense n. sp.

(Tables 1-2, Figs. 1-4)

The trypanosome detected from the peripheral blood of *Eublepharis kuroiwae kuroiwae*

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is a polymorphic species as shown in Figs. 1-4, and at least two types can be distinguished morphologically. The description of *Trypanosoma ryukyuense* n. sp. is mainly based on Type I (or typical form), but Type II (or broader form) and other forms should be refered the forms belonging to the same single polymorphic new species.

Type I (Table 1 ; Fig. 1, c-f; Fig. 3, c-f; Fig. 4, g): The body slender than Type II ; both ends pointed ; twisted on longitudinal axis ; 49.1 microns in average total length including a free flagellum and 10.6 microns in average width at the level of nucleus including width of undulating membrane; length of free flagellum 11.9 microns; nucleus situated near kinetoplast and distance (K-N) 4.4 microns; circular kinetoplast lying marginally; shape of nucleus usually round or ellipitical, and several chromatic granules seen in nucleus ; nuclear membrane indistinct ; distance of posterior end to middle of nucleus (P-N) 18.9 microns ; distance of anterior end to middle of nucleus (A-N) 18.4 microns ; the kinetoplast index (P-N/K-N)=4.8 ; the nuclear index (P-N/A-N)=1.0.

The most peculiar feature of this type is its spiral ridges which turn along the body surface several times as a screw. In living specimen, the spiral ridges appear as a bundle of ribons, and those ridges also are undulating, then it appears as if the trypanosome has several undulating membranes. The cytoplasm stained dark purple, therefore sometimes the nucleus cannot be distinguished.

Type II (Table 2 ; Fig. 1, g-1 ; Fig. 2, m-s ; Fig. 4, h-1) : The body broader; surface costate; 33.3 microns in body length excluding a free flagellum, and 23.2 microns in width at widest part excluding undulating membrane; distance between kinetoplast and middle of nucleus (K-N) 9.4 microns ; length of free flagellum 8.9 microns ; nucleus round or elliptical and 3.2 microns in length and 2.9 microns in width; several chromatic granules seen in nucleus.

This form is quite different from Type I, but some trypanosomes (Fig. 1, g-h) are apparently intermediate form of both types. In well developed individuals, the colour of cytoplasm is pale blue, and the spiral ridges are not conspicuous.

Other forms (Fig. 1, b; Fig. 3, a-b) : Some small trypanosomes without spiral ridges were detected, and these forms are apparently younger than typical forms, and Fig. 1, b and Fig. 3, a, are the youngest. Those forms are very rare in the blood specimens.

At present, the author believes that those types of trypanosome detected from *Eublepharis kuroiwae kuroiwae* belong to a single species, because there are distinct intermediate forms between typical form and broader form, and both forms have similar nuclear structure. Multiplication was not observed.

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

Type host: *Eublepharis kuroiwae kuroiwae* (Namie, 1912) (Reptilia: Gekkonidae). Totally four individuals of the host animal were examined, and from all of them *Trypanosoma ryukyuense* n. sp. was detected. Type smears were taken from one of the host animals (No.

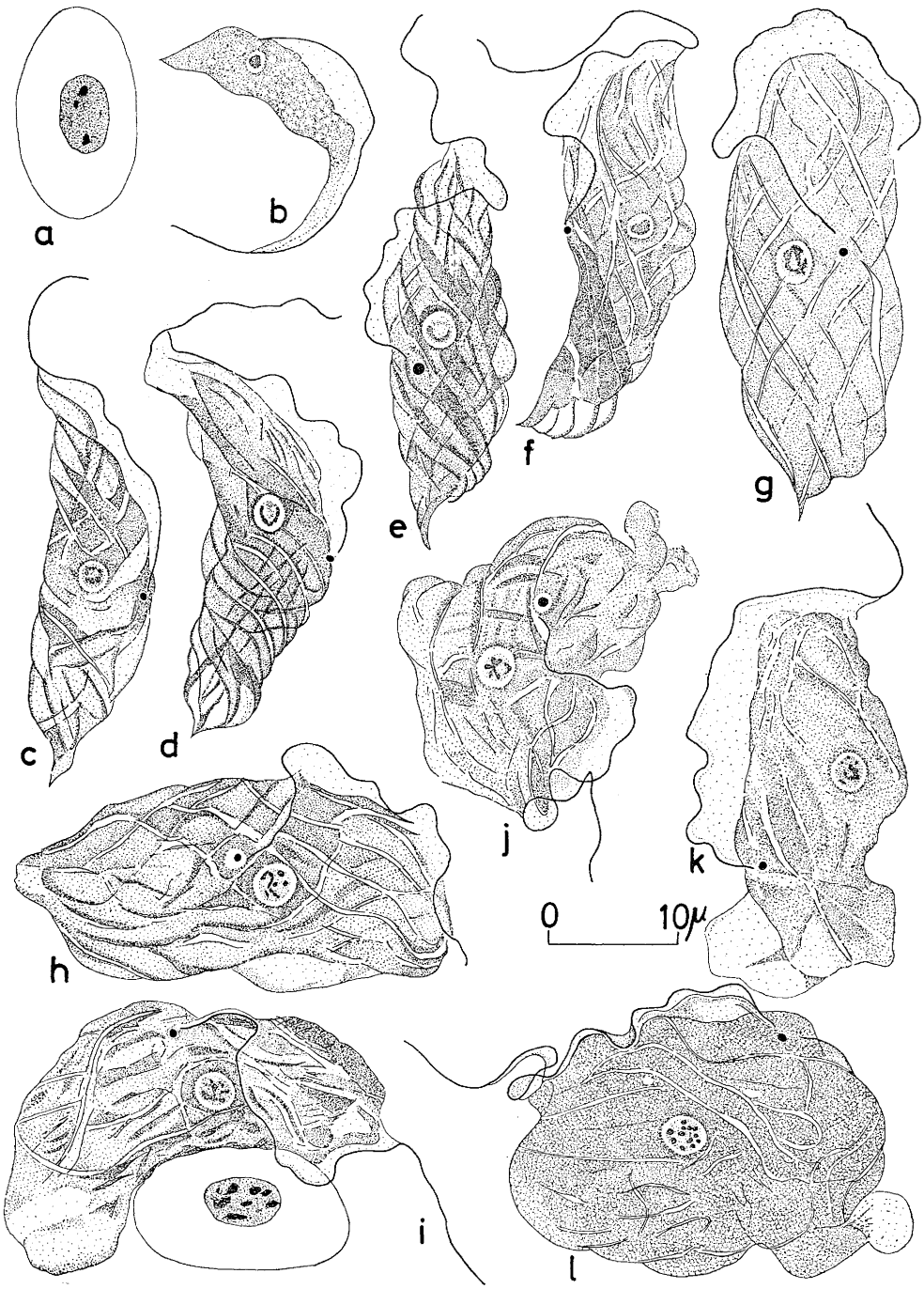


Fig. 1. *Trypanosoma ryukyuense* n. sp.
a. normal erythrocyte of *Eublepharis kuroiwae kuroiwae*
b. immature trypanosome
c–f. typical form (Type I)
g–h. intermediate form
i–l. broader form (Type II)

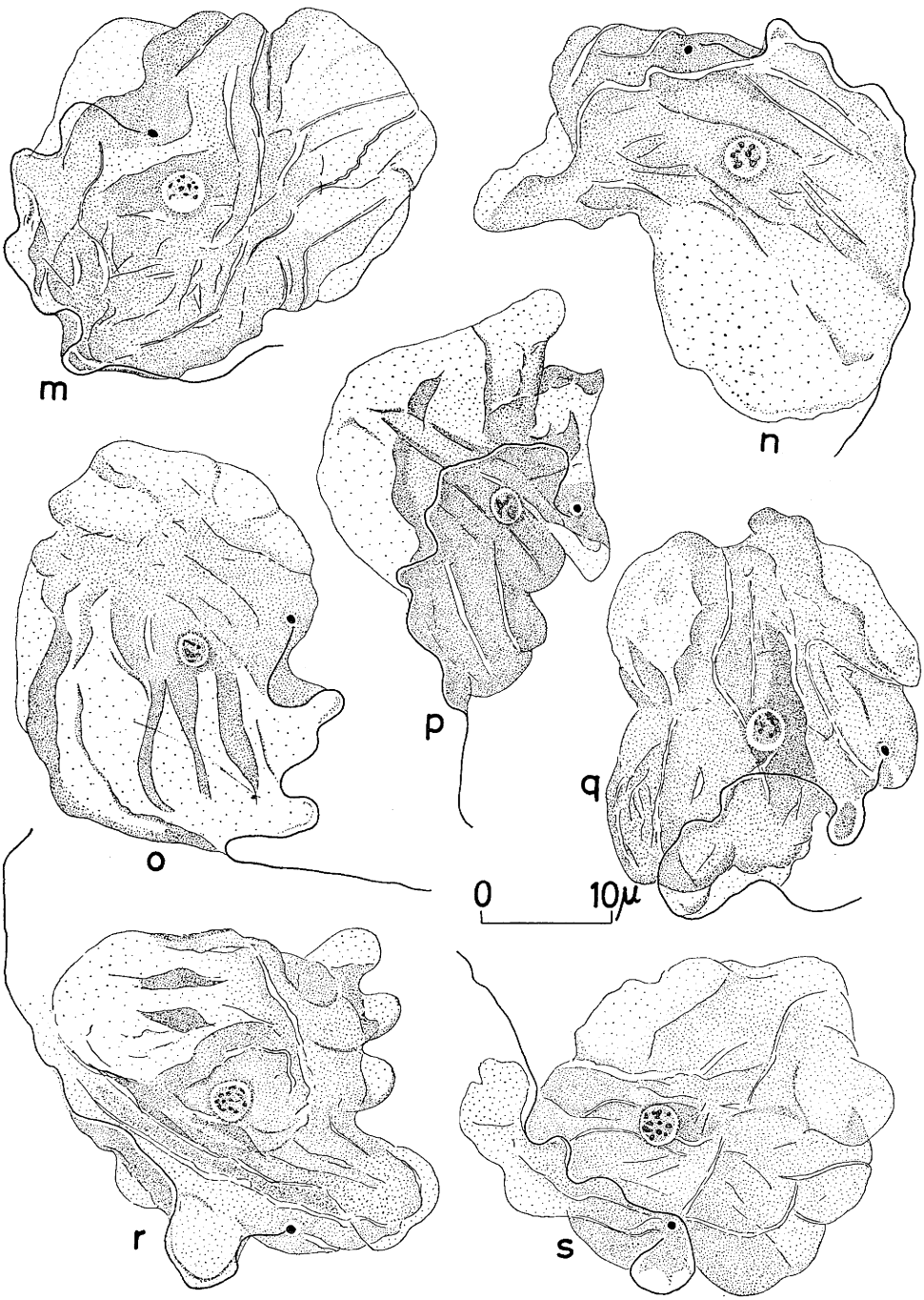


Fig. 2. *Trypanosoma ryukyuense* n. sp. (broader form).

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

	TL	P-N	A-N	K-N	P-K	FF	W	NL	KI	NI
	55.0	21.6	22.4	3.0	19.4	11.1	11.1	3.1	7.2	1.0
	49.7	18.7	17.1	5.1	17.8	13.9	9.2	3.6	3.7	1.1
	49.7	19.9	21.6	2.1	19.4	8.3	10.6	2.5	9.5	0.9
	44.4	17.8	15.5	3.9	14.4	11.1	11.9	2.2	4.6	1.1
	48.1	18.5	18.5	3.5	18.1	11.1	10.8	3.6	5.3	1.0
	47.2	18.0	18.3	3.0	20.8	11.1	14.2	3.1	6.0	1.0
	45.0	20.3	16.7	4.2	19.7	8.1	11.4	2.8	4.8	1.2
	55.6	21.2	23.1	4.0	20.8	11.4	9.2	1.7	5.3	0.9
	54.7	21.1	23.9	5.0	19.4	9.7	8.1	3.3	4.2	0.9
	45.0	15.8	18.1	6.4	14.4	11.1	11.1	2.8	2.5	0.9
	51.1	21.0	18.8	6.3	18.9	11.4	12.2	3.1	3.3	1.1
	43.9	15.5	17.2	4.2	15.3	11.1	8.9	2.2	3.7	0.9
	47.5	18.0	15.8	6.3	16.9	13.9	10.0	3.1	2.9	1.1
	43.6	17.0	14.2	3.3	13.9	12.5	10.8	2.8	5.2	1.2
	56.1	19.1	14.9	5.7	14.4	22.2	10.0	1.9	3.4	1.3
Average	49.1	18.9	18.4	4.4	17.6	11.9	10.6	2.8	4.8	1.0
Minimum	43.6	15.5	14.2	2.1	13.9	8.1	8.1	1.7	2.5	0.9
Maximum	56.1	21.6	23.9	6.4	20.8	22.2	14.2	3.6	9.5	1.3

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 (1975)

1976-7-3-4), and all the figures and tables shown in the present paper also prepared from the type smears.

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 (1978).

Vector: unknown

DISCUSSION

According to literatures, about sixty species of trypanosomes have been known in the world from the reptiles (see Miyata, 1978). Among them the following twelve species were recorded from the family Gekkonidae.

1. *Trypanosoma pertenue* Robertson, 1908

Host: *Hemidactylus triedri* and *Hemidactylus leschenaultii* Locality: Ceylon

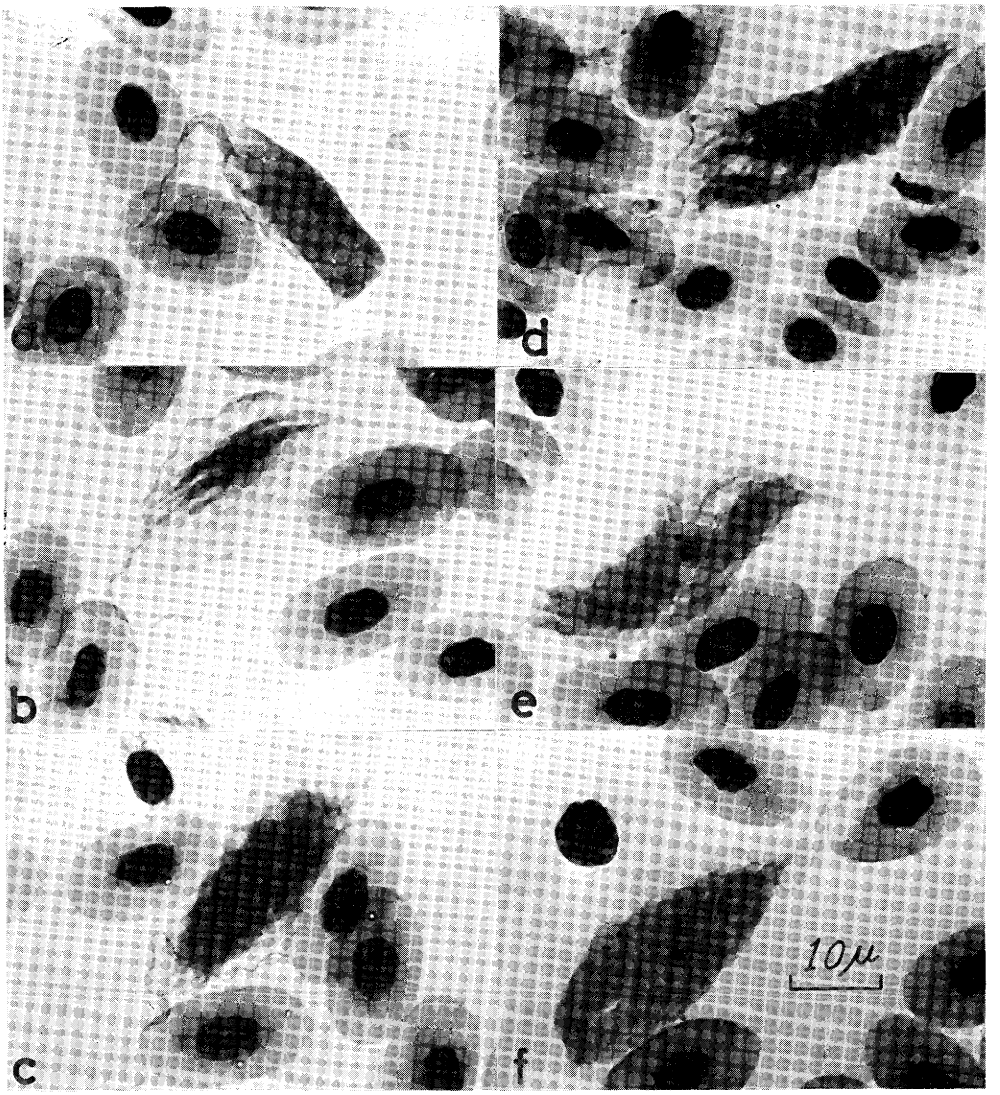


Fig. 3. *Trypanosoma ryukyuense* n. sp.
a–b. immature trypanosome
c–f. typical form (Type I)

2. *Trypanosoma leschenaultii* Robertson, 1908
Host: *Hemidactylus leschenaultii* Locality: Ceylon
3. *Trypanosoma gallayi* Bouet, 1909
Host: *Psylodactylus caudicinctus* Locality: West Africa
4. *Trypanosoma platydactyli* Catouillard, 1909
Host: *Tarentola mauritanica* (=*Platydactylus muralis*) Vector: sand fly (reported by Adler and Theodor, 1935) Locality: Africa
5. *Trypanosoma hemidactyli* Mackie, Das Gupta, and Swaminath, 1923

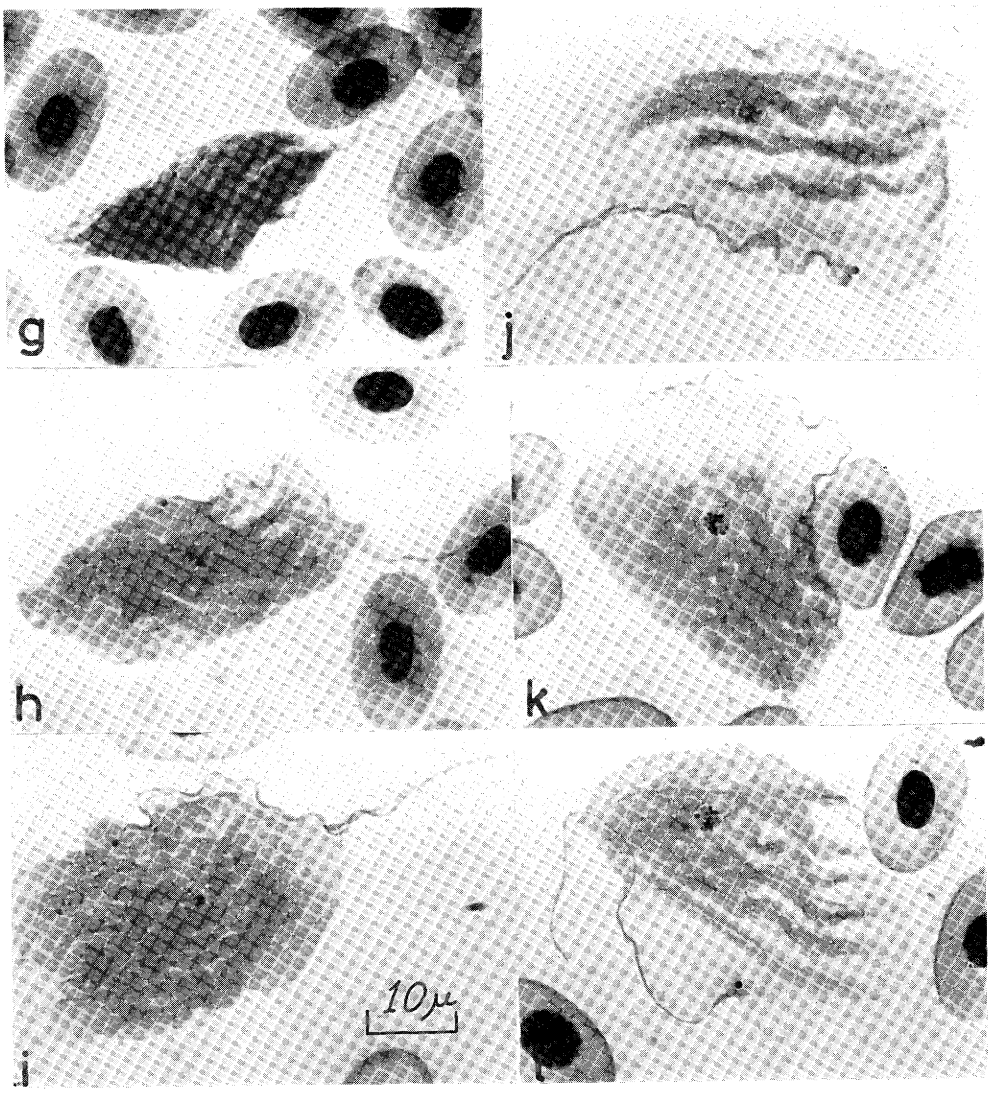


Fig. 4. *Trypanosoma ryukyuense* n. sp.
g. typical form (Type I)
h. intermediate form
i-l. broader form (Type II)

Host: *Hemidactylus fernatus* Locality: India

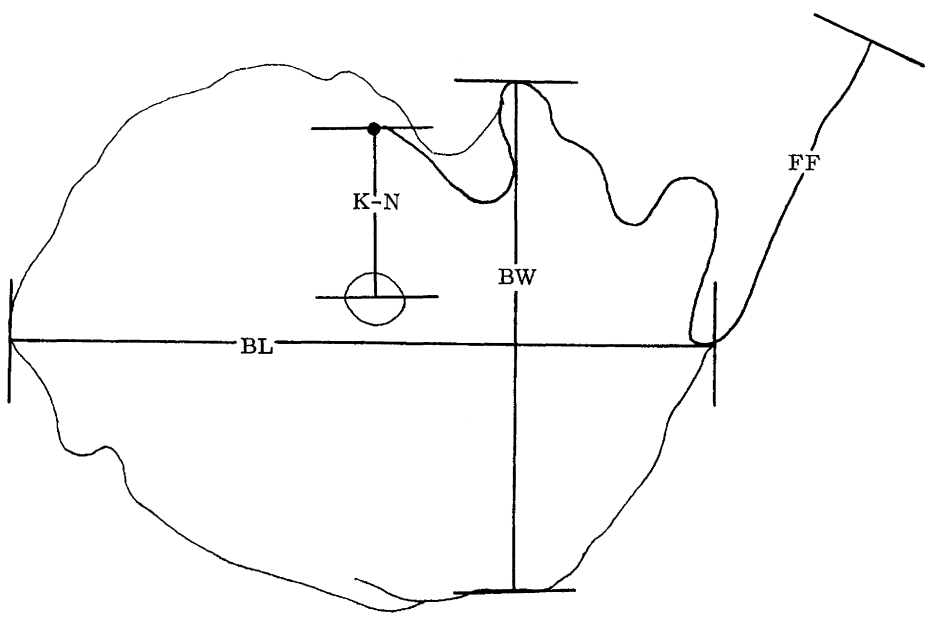
6. *Trypanosoma phlebotomi* (Mackie, 1914), Shortt and Swaminath, 1931
= *Herpetomonas phlebotomi* Mackie, 1914
Host: *Hemidactylus fernatus* Vector: sand fly, *Phlebotomus babu* var. *shortii*(reported by Shortt and Swaminath, 1931) Locality: India

7. *Trypanosoma garnhami* Grewal, 1955
Host: *Hemidactylus brookii angulatus* and *Lacerta viridis* (experimental)
Vector: *Rhodnius prolixus* (experimental) and *Ornithodorus parkeri* (experimental)

Table 2. Size (in microns) of *Trypanosoma ryukyuense* n. sp. (broad form)

	BL	BW	FF	K-N	NL	NW
	37.8	25.0	5.6	17.3	3.3	3.1
	40.0	23.1	9.7	18.9	2.8	2.5
	33.3	26.9	7.8	8.9	3.3	2.8
	38.9	22.2	10.6	9.8	3.3	2.8
	33.9	26.9	8.3	5.0	3.3	3.1
	33.9	21.9	6.9	9.7	3.1	3.1
	32.2	23.6	15.3	8.1	2.8	2.5
	32.5	20.8	8.3	5.6	2.8	2.2
	28.6	25.0	6.1	9.6	3.3	3.1
	32.8	30.0	13.9	10.6	3.3	3.1
	32.5	26.1	11.7	7.4	3.1	2.8
	32.5	22.8	6.9	11.0	3.6	3.1
	25.8	17.8	11.7	6.1	3.3	2.8
	33.6	19.2	2.8	3.4	3.3	3.3
	31.7	16.4	8.3	9.9	3.1	2.8
Average	33.3	23.2	8.9	9.4	3.2	2.9
Minimum	25.8	16.4	2.8	3.4	2.8	2.2
Maximum	40.0	26.9	15.3	18.9	3.6	3.3

BL : Body length at the longest part as shown in text figure (below)
BW : Body width at the widest part
FF : Length of free flagellum
K-N : Kinetoplast to middle of nucleus
NL : Nuclear length at the longest point
NW : Nuclear width



text figure

Locality: Africa

8. *Trypanosoma ocumarensis* Scorza and Dagert, 1955
   Host: *Thecadactylus rapicaudus* Locality: Venezuela
9. *Trypanosoma phylluri* Mackerras, 1961
   Host: *Phyllurus platurus* Locality: Australia
10. *Trypanosoma thecadactyli* Christensen and Telford, 1972
    Host: *Thecadactylus rapicaudus* Vector: sand fly, *Lutzomyia trinidadensis*
    Locality: Panama
11. *Trypanosoma petteri* Brygoo, 1966
    Host: *Phelsuma madagascariensis* Locality: Madagascar
12. *Trypanosoma loricatum* (Mayer, 1843), França and Athias, 1906
    =*Paramecium loricatum* Mayer, 1843
    Host: frogs (*Rana* spp.); lizard, *Agama colonrum* and *Lygosoma* sp. (reported by Todd and Wolbach, 1912); gekko, *Hemidactylus fasciatus* (reported by Garnham and Duke, 1953, from Gambia, Africa)

Those twelve species were detected from the blood of lizard belonging to the subfamily Gekkoninae of the family Gekkonidae, which is devided into two subfamilies, Gekkoninae and Eublepharinae. The present species, *Trypanosoma ryukyuense* n. sp., is the first trypanosome recorded from the subfamily Eublepharinae. Typical form of *T. ryukyuense* can be distinguished from all other trypanosomes known from reptiles or other animals based on its peculiar morphology due to spiral ridges. *T. ryukyuense* apparently differes from *T. hemidactyli*, *T. platydactyli*, *T. leschenaultii*, *T. pertenue*, and *T. gallayi*, in appearence, all of which have long and narrow bodies. *T. ryukyuense* is different from *T. phlebotomi*, because which has no free flagellum. *T. garnhami* is somewhat similar to the broader form of *T. ryukyuense*, but the former has small body and no spiral ridges. *T. ocumarensis*, *T. phylluri*, and *T. thecadactyli* are distingushed from *T. ryukyuense* by the absence of spiral ridges. The broader form of *T. ryukyuense* is similar to *T. loricatum*, which was originally described from frogs. *T. loricatum* has ridges on the body surface, but this species has a very short free flagellum. From reasons mentioned above, it is concluded that *T. ryukyuense* is a well established species distinguishable from all the known trypanosomes, and is named after the type locality "Ryukyu", another name of Okinawa.

According to literatures, known vectors of trypanosome of Gekkonidae are sand flies belonging to the genera *Phlebotomus* in the Old World and *Lutzomyia* in the New World. In Okinawa Island, however, there are no record of sand fly, then other blood-sucking insect, for example a kind of mosquito, might be a possible vector for this trypanosome. *E. kuroiwae kuroiwae* (Japanese name : Kuroiwa-tokagemodoki) is a very rare animal which distributes as relics only in Okinawa Island, and two other subspecies *E. k. orientalis* (Japanese name: Madara-tokagemodoki) and *E. k. splendens* (Japanese name: Obi-tokagemodoki) distribute in other small islands located in the Ryukyu Archipelago (Nakamura and Uéno, 1974). *E. k. kuroiwae* is seen at night on a narrow path across in the forest, and the lizard usually does not escape from a spot of flash-light in such cases. This lizard has apparently a nocturnal habit, and during the day-time it seems to take rest in a small cleft of rock or holes. The vector of *T. ryukyuense* might suck the blood of *E. k. kuroiwae* in such resting places. Further studies to confirm the life cycle of *T. ryukyuense* must be carried out.

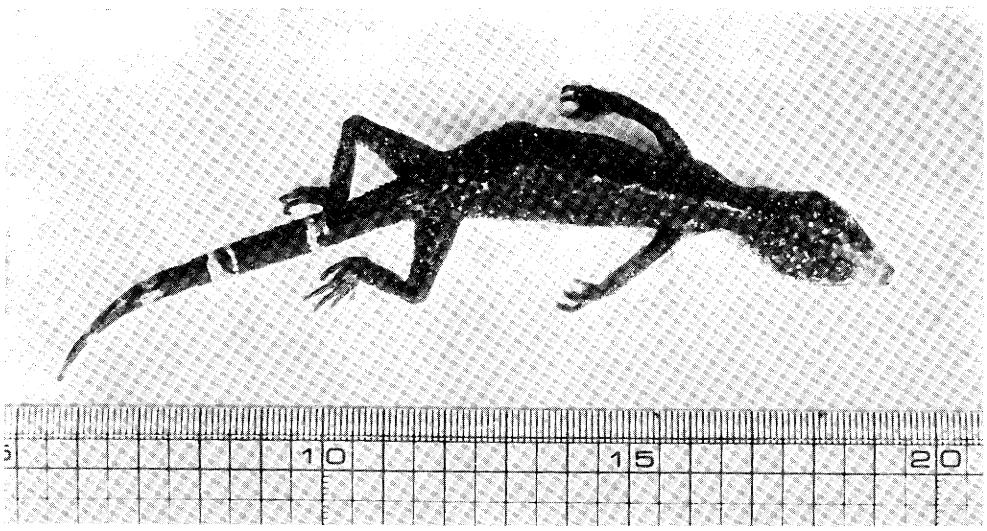


Fig. 5. *Eublepharis kuroiwae kuroiwae* (Namie, 1912), host of *Trypanosoma ryukyuense* n. sp.

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沖縄産クロイワトカゲモドキより発見されたトリパノゾーマの1新種 *Trypanosoma ryukyuense*

宮田　彬（長崎大学熱帯医学研究所疫学部門）

クロイワトカゲモドキは，沖縄本島に分布するヤモリ科トカゲモドキ亜科に属するきわめて稀れな爬虫類である。1976年7月琉球大学保健学部の宮城一郎教授と沖縄本島北部の国頭村与那を訪ねた際，この珍しい動物を入手することができ，早速採血検査したところ誠に奇妙なトリパノゾーマを発見した。生鮮標本では，丁度数本のリボンを1束にしてねじったような感じで，リボンの1つ1つが波動膜とともに波動していた。このような運動は既知のトリパノゾーマでは全く知られていないものである。染色標本では，トリパノゾーマは形態により2型にわかれ，第1型は遊離鞭毛をふくむ平均体長49.1ミクロン，体幅10.6ミクロン，遊離鞭毛の長さは11.9ミクロンで，波動膜はよく発達している。この型では虫体表面をらせん状に走る隆起線が特徴的で，このような奇妙なトリパノーマは今まで全く知られていなかった。第2型は幅広い虫体で，体表には第1型に特徴的ならせん状隆起線がみとめられる。核も既知のトリパノゾーマとは異なり，ギムザ染色では核内に多数の染色質顆粒がみられる。核の形態は第1型および第2型とも互いによく似ている。ギムザ染色した両型の虫体はきわめて美しいもので，そのカラー写真は著書「寄生原生動物――その分類・生態・進化」（B5判 1600頁，文部省研究成果刊行費申請中）の中で詳しく紹介した。

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