Tropical Medicine 20 (2), 97−112, June, 1978

Haemoprotozoa Detected from the Cold-blooded Animals in Ryukyu Islands

Akira MIYATA[1], Ichiro MIYAGI[2], and Masuhisa TSUKAMOTO[1]*

Department of Epidemiology, Institute for Tropical Medicine, Nagasaki University[1], Nagasaki 852

Laboratory of Medical Zoology, College of Health Sciences, University of the Ryukyus[2], Naha 902

Abstract: A total of 23 species of haemoparasites were reported from 11 species of amphibians and reptiles captured in Ryukyu Islands, Japan, during a period between 1974 and 1976. In the present paper, all results obtained from the survey in Ryukyu Islands were combined as a checklist together with some other related results from surveys in main islands of Japan.
1) *Triturus pyrrhogaster ensicauda*: *Trypanosoma ogawai* and *Haemogregarina shirikenimori*. 2) *Rana namiyei*: *Trypanosoma miyagii*, *Trypanosoma tsukamotoi*, and *Lankesterella* sp. 3) *Rana holsti*: *Trypanosoma loricatum*, *Trypanosoma miyagii*, *Trypanosoma chattoni*, *Trypanosoma* sp. A, *Trypanosoma* sp. E, haemogregarine A, haemogregarine B, *Toddia*, and microfilaria. 4) *Rana narina*: *Trypanosoma loricatum*, *Trypanosoma miyagii*, *Trypanosoma chattoni*, and haemogregarine C. 5) *Rana ishikawae*: *Trypanosoma miyagii*. 6) *Rana limnocharis limnocharis*: *Trypanosoma ishigakiense*, *Trypanosoma tsunezomiyatai*, *Trypanosoma* sp. A, *Trypanosoma* sp. D, haemogregarine D, and *Dactylosoma ranarum*. 7) *Rana okinawana*: haemogregarine E. 8) *Rhacophorus japonicus*: *Trypanosoma rotatorium*. 9) *Eublepharis kuroiwae kuroiwae*: *Trypanosoma ryukyuense*. 10) *Japalura polygonata*: microfilaria. 11) *Dinodon semicarinatus*: haemogregarine.

Ryukyu Islands extend from 24°N 123°E to 29°N 130°E. Climatically the islands belong to sub-tropic zone, and the average temperature is 22.1°C in Naha City. From a view point of the animal distributions Oriental fauna in the island are in a special situation as compared with Palaearctic fauna in other main islands of Japan. Excluding sea snakes and sea turtles a total of 66 species and subspecies of amphibians and reptiles are known from the islands, and most of their distributions are quite limited as relics. In spite of detailed information about these animals, little attention has been paid to their protozoon parasites by many zoologists who visited these islands.

In 1974 the study on insect-borne diseases of man and various animals in the islands

Contribution No. 809 from the Institute for Tropical Medicine, Nagasaki University
Received for publication, February, 10, 1978

*Present address: Department of Medical Zoology, University of Occupational and Environmental Health, Kitakyushu 807

began to find clues to the evolution of parasitism. Actually, surveys on the blood parasites of the cold-blooded animals were carried out in Okinawa Island by two or three of the authors in 1974 and 1976. In addition to the surveys, Miyagi continued to collect many materials in Okinawa and Ishigaki Islands. As expected results obtained were very fruitful, and some parts of them have been published by Miyata (1977a, b, c, and 1978) and Miyata and Miyagi (1977). The present paper which is the sixth publication on the surveys includes combined results related to haemoprotozoa and microfilaria detected from several cold-blooded animals in the islands during a period between 1974 and 1976.

MATERIALS AND METHODS

The cold-blooded animals used in the present study were collected by hand. Duplicated thin blood smears were prepared from each animal by cutting a leg, or a leg-toe in newts and lizards, or tail in snakes, but in the case of big frogs, the heart blood was taken by using a sterillized syringe without killing the animal. In the case of small frogs, the heart blood was taken after killing the animal.

The smears were fixed with methyl alcohol and stained by a 3% Giemsa solution for 30 to 40 minutes. Before examining the stained smear, a fresh preparation was always examined to find out any moving parasite such as trypanosome or microfilaria. Microscopic fields of the stained smear were completely examined with low magnification (at 100× or 200×). After examination, most animals were released except for parasie positive animals. In some cases, organ impression smears were also prepared to find the tissue stage of parasite.

The scientific names of cold-blooded animals adopted in the present paper were based on those used by Nakamura and Uéno (1974).

Most animals were captured at Yona and nearby Kunigamison, northern part of Okinawa Island. The area belongs to the forest station of the Faculty of Agriculture, University of the Ryukyus, and there is a building for students and teachers to stay. The authors stayed there temporarily, to examine collected materials by the courtesy of the faculty.

RESULTS

A total of 23 species of haemoparasites including 2 species of microfilaria were detected

Table 1. Detection rate of each parasite in *Triturus pyrrhogaster ensicauda* in 1976

Locality (month)	No. exam.	*T. ogawai*	*H. shiri.*	Land leech
Yona (June-July)	66	20 (30.3%)	57 (86.4%)	14 (21.2%)
Yona (November)	94	6 (6.4)	86 (91.5)	3 (3.2)
Izumi (June)	14	0	2 (14.3)	0
Total	174	26 (14.9)	145 (83.3)	17 (9.8)

T. ogawai=*Trypanosoma ogawai*; *H.shiri.*=*Haemogregarina shirikenimori*; land leech=*Haemadipsa zeylanica japonica*.

from the following 11 species of cold-blooded animals in Ryukyu Islands except for Amami Island. In addition, 3 more species of haemoparasites were detected from *Rana subaspera* in Amami Island.

Beside them, blood materials were examined from several other cold-blooded animals such as *Tylototriton andersoni* Boulenger, 1892, *Gekko japonicus* (Dumeril and Bibron, 1836), *Lygosoma pellopleurum* (Hallowell, 1860), or *Trimeresurus okinavensis* Boulenger, 1892, but no haemoparasite was detected.

1) *Triturus pyrrhogaster ensicauda* (Hallowell, 1860) (Amphibia, Salamandridae)
Japanese name : SHIRIKEN-IMORI
Detected parasites : *Trypanosoma ogawai* Miyata, 1977, and *Haemogregarina shirikenimori* Miyata, 1977.

From the newt, *Triturus pyrrhogaster ensicauda*, 2 species of haemoprotozoa, *Trypanosoma ogawai* and *Haemogregarina shirikenimori*, were detected (Miyata, 1977a and 1977b). The detection rate of each parasite was shown in Table 1. *T. ogawai* was found in 30.3% of the newts collected in June-July, 1976, at Yona, but the trypanosome was detected only from 6.4% of the newts examined in November, 1976, at almost the same locality.

H. shirikenimori is a very common parasite among the newts examined in northern part of Okinawa Island, but at Izumi, central part of Okinawa Island, haemogregrarine is uncommon. In about half of positive cases of *H. shirikenimori*, 1-20 parasites per 1,000 erythrocytes were observed. In the highest parasitaemia, 182 parasites per 1,000 erythrocytes were counted. Relationship between the size of the newt and its parasitaemia is not evident, but higher parasitaemia (more than 20 parasites per 1,000 erythrocytes) was frequently observed in smaller newt.

From the newts, a land leech, *Haemadipsa zeylanica japonica*, was collected. The leech is apparently abundant in summer (detection rate 21.2%), and rare in winter (detection rate 3.2%) as shown in Table 1. The leech is a possible vector for the above two parasites.

Additionally, *Triturus pyrrhogaster pyrrhogaster* (Boie, 1826), subspecies of mainland, was collected in Omura and Isahaya, 30 to 40 km east from Nagasaki City, in June, 1977;

Table 2. Detection rate of each trypanosome in 4 species of frog living in forest zone

Host	No. exam.	*T. miyagii*	*T. tsuka.*	*T. lori.*	*T. chatt.*	others
		%	%	%	%	%
Rana namiyei	37	20 (54.1)	6 (16.2)	0	0	0
Rana holsti	11	5 (45.5)	0	8 (72.7)	7 (63.6)	5 (45.5)
Rana narina	8	3 (37.5)	0	1 (12.5)	2 (25.0)	0
Rana ishikawae	2	1 (50.0)	0	0	0	0

T. miyagii=*Trypanasoma miyagii*; *T. lori.*=*Trypanosoma loricatum*;
T. tsuka.=*Trypanosoma tsukamotoi*; *T. chatt.*=*Trypanosoma chattoni*;
others include 2 forms (*Trypanosoma* sp. A and *Trypanosoma* sp. E of Miyata, 1978).

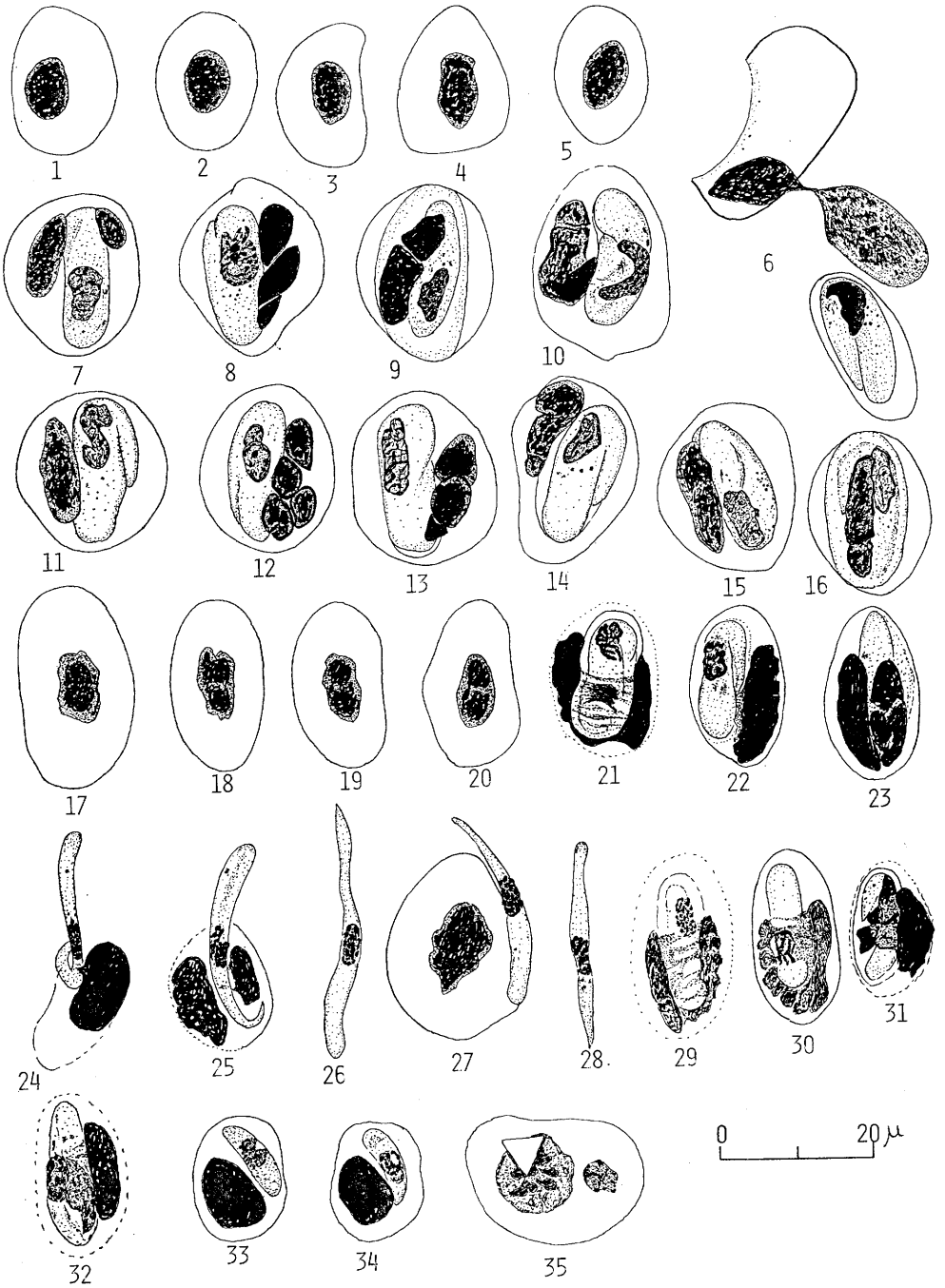

Fig.1. Haemogregarines detected from frogs.
1–16. Haemogregarine C from *Rana narina* (1–5. uninfected erythrocyte)
17–32. Haemogregarine A from *Rana holsti* (17–20. uninfected erythrocyte)
33–34. Haemogregarine B from *Rana hlosti*
35. *Toddia* from *Rana holsti*

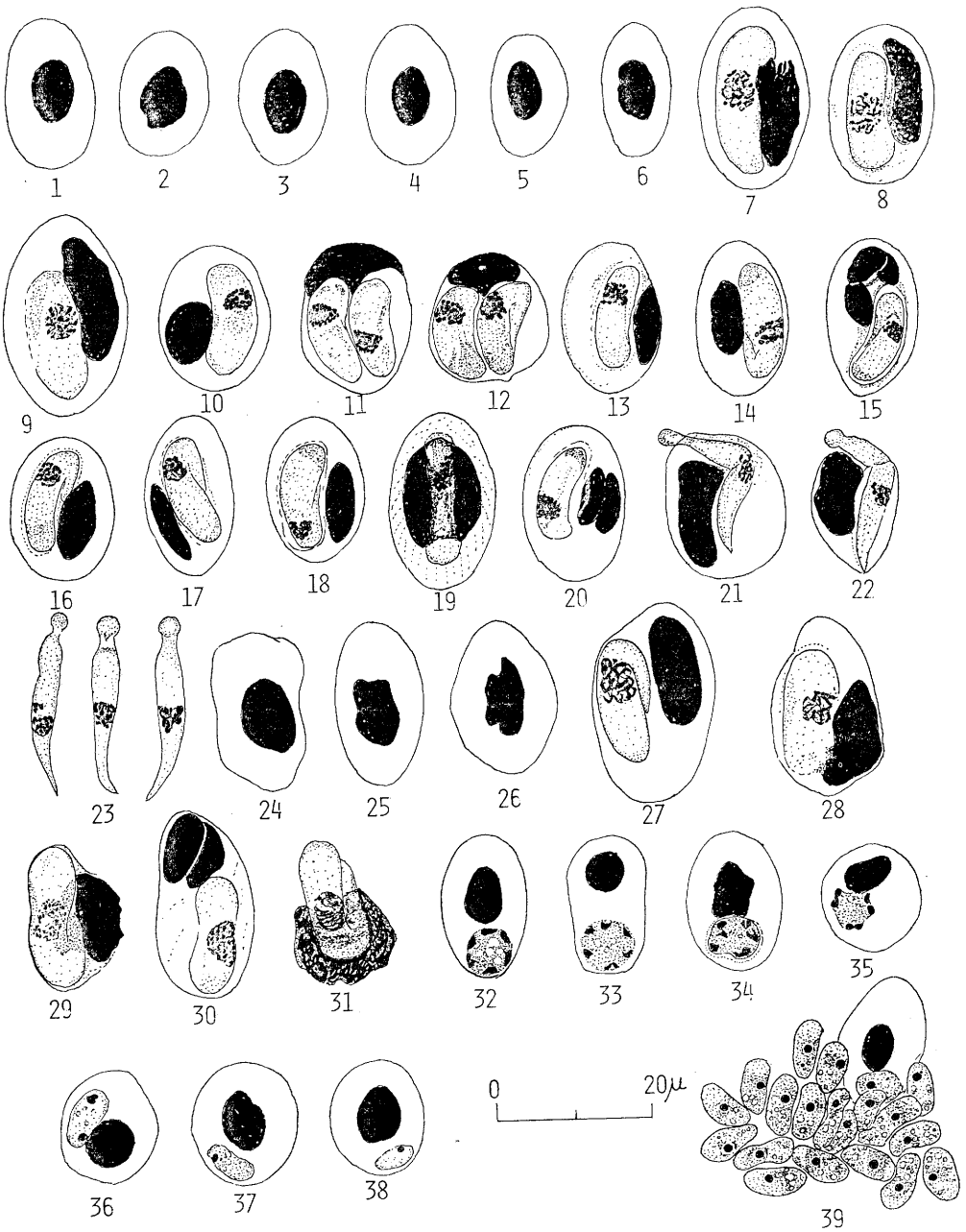

Fig. 2. Haemogregarines and *Dactylosoma ranarum* (Kruse, 1890) from frogs.
1–23. Haemogregarine D from *Rana limnocharis limnocharis* (1–6. uninfected erythrocyte)
24–31. Haemogregarine E from *Rana okinawana* (24–26. uninfected erythrocyte)
32–38. *D. ranarum* from *Rana l. limnocharis*
39. *D. ranarum* from *Rana rugosa*

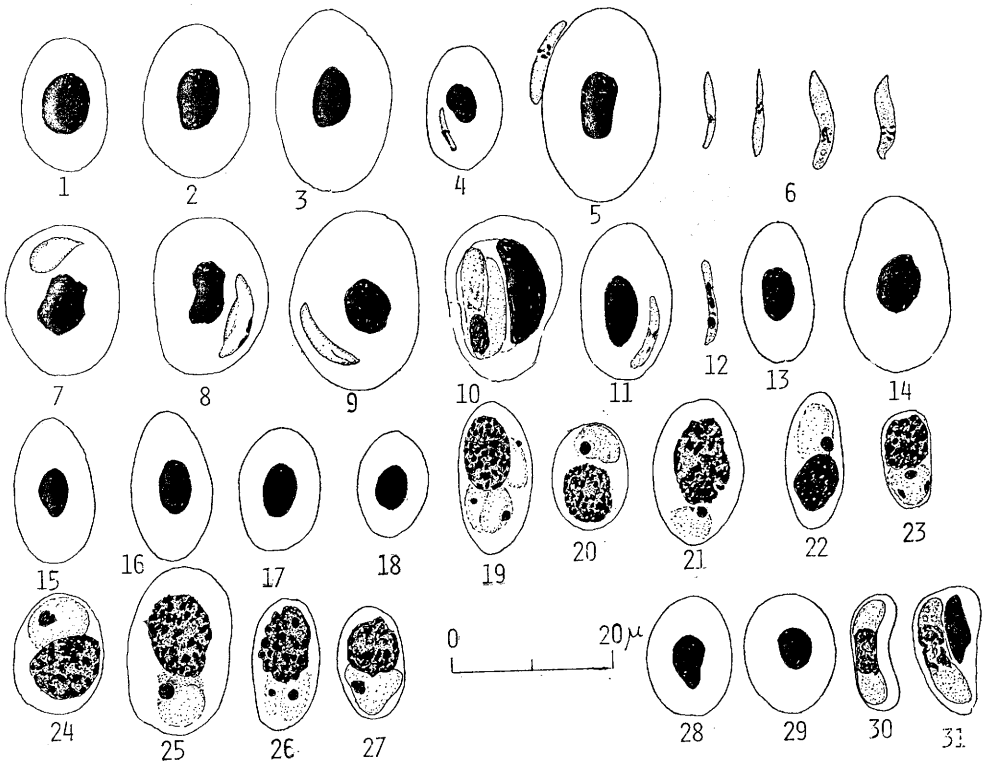

Fig. 3. Various haemoparasites detected from amphibians and reptiles.
1- 9. *Lankesterella* sp. from *Rana namiyei* (1-3. uninfected erythrocyte)
10. Haemogregarine from *Rana subaspera*
11-14. *Lankesterella* sp. from *Rana subaspera* (13-14. uninfected erythrocyte)
15-27. Unidentified haemoparasite from *Hyla arborea japonica* (15-18. uninfected erythrocyte)
28-31. Haemogregarine from *Dinodon semicarinatus* (28-29. unidentified erythrocyte)

however, no haemoparasite was detected from a total of 100 newts examined.

2) *Rana namiyei* Stejneger, 1901 (Amphibia, Ranidae)
Japanese name : NAMIYE-GAERU
Detected parasites : *Trypanosoma miyagii* Miyata, 1978, *Trypanosoma tsukamotoi* Miyata, 1978, and *Lankesterella* sp.

From *Rana namiyei*, 2 new species of trypanosome, *Trypanosoma miyagii* and *Trypanosoma tsukamotoi*, were detected and described in a previous paper (Miyata, 1978). In total 37 individuals of the frog captured at Yona and Benoki, Kunigamison, in June-July, 1976, were examined : *T. miyagii* was detected from 20 (54.1%), and *T. tsukamotoi* was found from 6 (16.2%) as shown in Table 2. In all the detected cases of *T. tsukamotoi*, unexpectedly *T. miyagii* was also present.

Lankesterella sp. : From one of the frogs, slender and club-shaped parasites were detected

as shown in Fig. 3, 1-9, and Fig. 4, p-q. In the heart blood, the parasite which has a granular nucleus was usually seen free in the serum outside of the blood cell (Fig. 3, 5-6, and Fig. 4, p), and intraerythrocytic forms (Fig. 3, 4) were rarely observed. However, in the impression smears taken from the liver of the frog, the intraerythrocytic form (Fig. 3, 7-9, and Fig. 4, q) was very common. The length and the width of the extraerythrocytic form are 9.6-12.6 microns and 1.2-2.1 microns respectively. The intraerythrocytic form seen in the heart blood resembles the extraerythrocytic form in size and shape, but the parasite seen in impression smears of the liver is apparently broader and more crescentric. Free forms were not observed in impression smears. This parasite apparently belongs to the genus *Lankesterella* Labbé, 1899.

On one or two of frogs, a land leech, *Haemadipsa zeylanica japonica*, was found.

Remarks: The picture(Fig.4,p-q) of *Lankesterella* sp. found from *Rana namiyei* closely resembles *Lankesterella hylae* (Cleland and Johnston, 1910) figured by Mackerras and Mackerras (1961).

Another parasites of *Lankesterella* (Fig. 3, 11-12) were found from *Rana subaspera* Barbour, 1908, which was captured in Amami Island by Mr. Hiroshi Suzuki. In this case, unfortunately, parasitaemia was very low, and hence it was not determined whether or not the parasites detected from *R. subaspera* and from *R. namiyei* are the same species. Identification with *Lankesterella minima* (Chaussat, 1850) is also in question. At present the determination of specific name for these parasites is impossible without knowing the life cycle information.

3) *Rana holsti* Boulenger, 1892 (Amphibia, Ranidae)

Japanese name : HOLST-GAERU

Detected parasites : *Trypanosoma loricatum* (Mayer, 1843), *Trypanosoma miyagii* Miyata, 1978, *Trypanosoma chattoni* Mathis and Leger, 1911, *Trypanosoma* sp. A, *Trypanosoma* sp. E (both in Miyata, 1978), haemogregarine A, haemogregarine B, *Toddia*, and microfilaria.

A total of 11 individuals of *Rana holsti* were collected at Yona in June-July, 1976, and all of them were infected with trypanosomes (Miyata, 1978). As shown in Table 2, 5 types of trypanosome were detected from the frog. *Trypanosoma loricatum* and *Trypanosoma chattoni* were also abundant, and a mixed infection of 2-4 species was commonly observed (see Table 11, in Miyata, 1978).

From the frog, 2 types of haemogregarine were detected, tentatively called haemogregarine A and haemogregarine B.

Haemogregarine A (Fig. 1, 17-32, Fig. 4, e-h) : In this species both intra-and extraerythrocytic forms were detected. The extraerythrocytic form (Fig. 1, 24-28) is slender, vermiform with round and pointed extremities, 27-36 microns in length and 2.4-3.0 microns in width at nucleus. The round extremity is apparently the anterior end, because the parasite has always left the host cell by the round end, as shown in Fig. 1, 24-25. Intraerythrocytic forms are somewhat elliptical (size about 14-19×6 microns), and the parasite folds the body as shown in Fig. 1, 21-22. The parasitized erythrocyte does not increase the size, but stains more lightly as compared with the cytoplasm of normal erythocytes. The host cell nucleus enlarges, and sometimes surrounds the parasite as shown in Fig. 1, 21, and 29-30. Haemogregarine

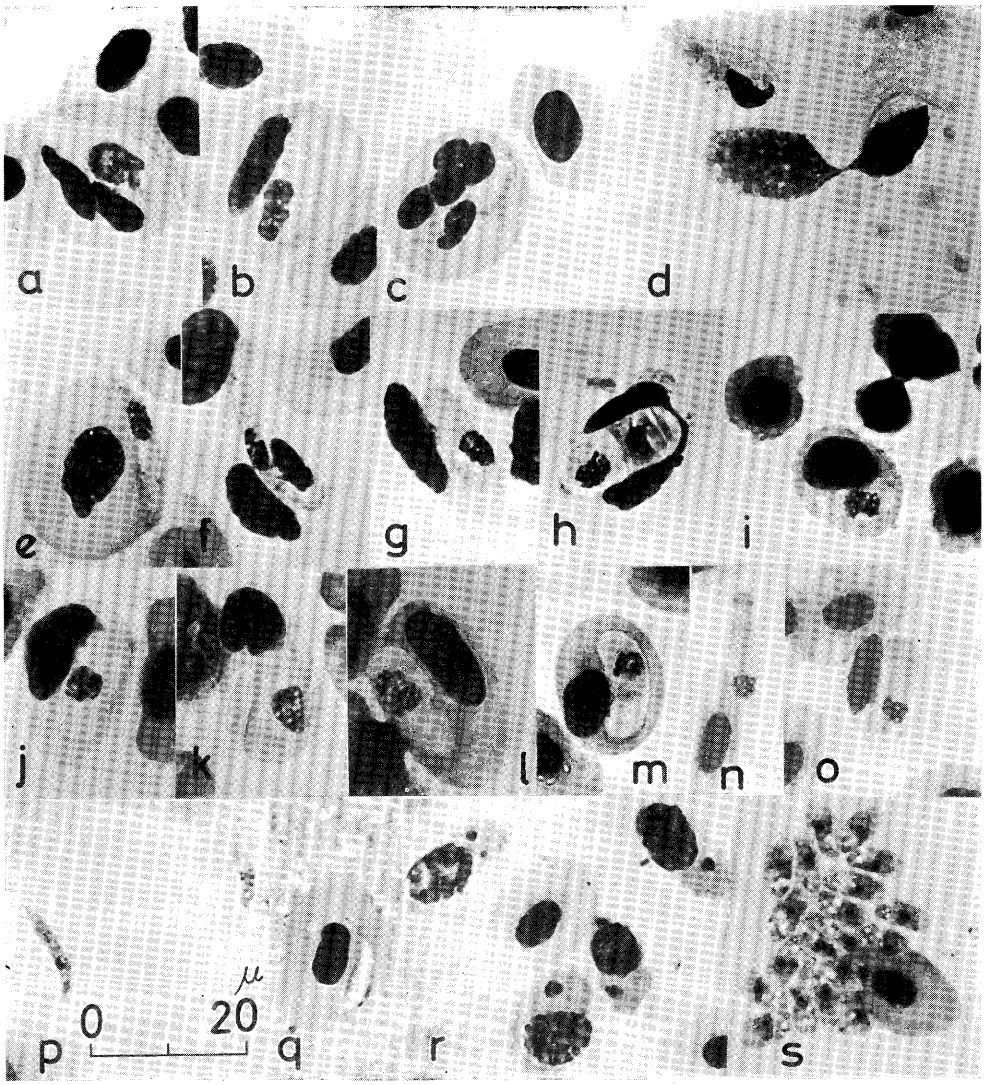

Fig. 4. Haemoprotozoa detected from frogs.
a–d. Haemogregarine C from *Rana narina*
e–h. Haemogregarine A from *Rana holsti*
i. Haemogregarine B from *Rana holsti*
j–l. Haemogregarine E from *Rana okinawana*
m–o. Haemogregarine D from *Rana limnocharis limnocharis*
p–q. *Lankesterella* sp. from *Rana namiyei*
r. Unidentified haemoparasite from *Hyla arborea japonica*
s. *Dactylosoma ranarum* (Kruse, 1890) from *Rana rugosa*

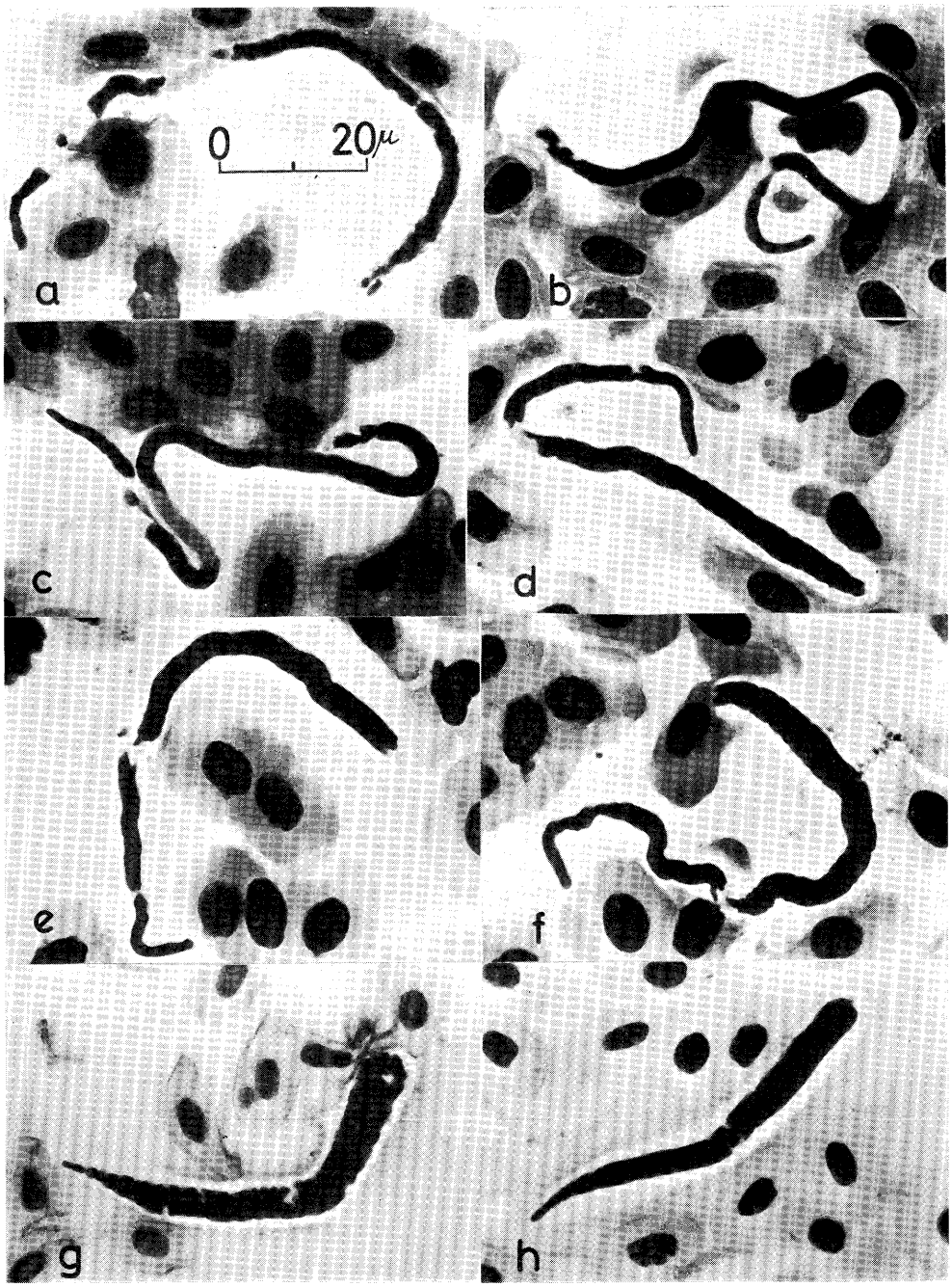

Fig. 5. Microfilaria detected from cold-blooded animals.
a-c. Microfilaria from *Rana holsti*
d-f. Microfilaria of *Icosiella sasai* Hayashi, 1960, from *Rana subaspera*
g-h. Microfilaria from *Japalura polygonata polygonata*

A was detected from 2 (18.2%) out of 11 frogs examined.

Haemogregarine B (Fig. 1, 33-34, and Fig. 4, i): This haemogregarine is smaller than haemogregarine A. Unfortunately the parasitaemia of haemogregarine B is very low and the morphological details cannot be described. This haemogregarine was detected from one (9.1%) out of 11 frogs examined.

From *R. holsti* a new type of *Toddia* was detected as reported by Miyata and Miyagi (1977). In addition to this case, one more infection of *Toddia* was found as shown in Fig. 1, 35. Thus, so far *Toddia* was detected from 2 (18.2%) out of 11 frogs examined.

Beside these above protozoan parasites, a kind of microfilaria was detected from one of *R. holsti* (Fig. 5, a-c). The size of the microfilaria is 99.0-120.0 microns in total length and about 3 microns in width. Unfortunately adult worm of this filaria was not detected.

Remarks: Two types of haemogregarine detected from *R. holsti* are apparently different from the haemogregarine found from *Rana narina* or *Rana limnocharis limnocharis* in shape, size, and reaction of host cell.

From one of *Rana subaspera* Barbour, 1908, captured in Amami Island by Mr. Hiroshi Suzuki, only one haemogregarine was observed as shown in Fig. 3, 10. This haemogregarine somewhat resembles to haemogregarine A of *R. holsti*, but much more materials from *R. subaspera* are necessary to determine their taxonomic relationship.

Microfilaria of *R. holsti* was unidentified, because adult worm could not be obtained. From *R. subaspera*, *Icosiella sasai* Hayashi, 1960, was already known, of which the microfilaria is shown in Fig. 5, d-f. The microfilaria (total length 79.8-94.8 microns) apparently differs from that of *R. holsti* in size and shape.

4) *Rana narina* Stejneger, 1901 (Amphibia, Ranidae)

Japanese name: HANASAKI-GAERU

Detected parasites: *Trypanosoma loricatum* (Mayer, 1843), *Trypanosoma miyagii* Miyata, 1978, *Trypanosoma chattoni* Mathis and Leger, 1911, and haemogregarine C.

Rana narina is very active and it is somewhat difficult to capture the frog as compared with other frog species. For this reason, only 8 *R. narina* were obtained at Yona in June-July, 1976. From half of them, trypanosomes were detected as shown in Table 2. The trypanosomes consisted of three species as follows: *Trypanosoma loricatum*, *Trypanosoma miyagii*, and *Trypanosoma chattoni*. One frog has these three species in the blood, but two frogs had only *T. miyagii*. From the rest, only *T. chattoni* was detected. Trypanosomes detected from *R.narina* were already reported by Miyata (1978).

From one of the frogs, a haemogregarine was detected. This haemogregarine is apparently different from haemogregarines A and B detected from *Rana holsti* as described below, so in the present paper, this haemogregarine is tentatively called haemogregarine C of *R. narina*.

Haemogregarine C (Fig. 1, 1-16, and Fig. 4, a-d): The infected erythrocytes were hypertrophied, but the colour of cytoplasm stained more darkly as compared with normal erythrocytes. The size of normal erythrocyte is 18.0-19.2 microns in length and 12.0-13.8 microns in width. However, the size of infected cell is 20.4-25.2 microns by 16.9-19.2 microns. Nucleus of host cell is broken into 2-4 fragments. The haemogregarine is apparently enclosed with in

the capsule or cyst as shown in Fig. 1, 9, 14, and 16, and parasite leaves the host cell with the capsule. Fig. 1, 6, and Fig. 4, d, show a destroyed host cell membrane, 2 fragments of host cell nucleus, and a capsule in which parasite is involved, respectively. The parasite body is folded in the capsule, and the size of parasite (about 18–20 × 7–8 microns) is somewhat larger than that of haemogregarine A of *R. holsti*.

5) *Rana ishikawae* (Stejneger, 1901) (Amphibia, Ranidae)

Japanese name: ISHIKAWA-GAERU

Detected parasite: *Trypanosoma miyagii* Miyata, 1978.

Only two individuals of *Rana ishikawae* were obtained at Yona, and *Trypanosoma miyagii* was detected from one of them. A figure of the trypanosome was given in a separate paper by Miyata (1978).

6) *Rana limnocharis limnocharis* Wiegmann, 1855 (Amphibia, Ranidae)

Japanese name: NUMA-GAERU

Detected parasites: *Trypanosoma ishigakiense* Miyata, 1978, *Trypanosoma tsunezomiyatai* Miyata, 1978, *Trypanosoma* sp. A, *Trypanosoma* sp. D (both in Miyata, 1978), haemogregarine D, and *Dactylosoma ranarum* (Kruse, 1890).

A total of 7 *Rana limnocharis limnocharis* was captured in Ishigaki Island in 1976, and trypanosomes were detected from 6 frogs. *Trypanosoma ishigakiense* was detected from one of the frogs and was described as a new species in a separate paper by Miyata (1978). *Trypanosoma tsunezomiyatai* is very common among the frog, and this species was detected in all trypanosome positive cases. *Trypanosoma* sp. A which resembles *Trypanosoma rotatorium* also was found from 4 frogs (57.1%).

In addition to trypanosomes, a kind of haemogregarine was detected from 2 (28.6%) out of 7 frogs. The haemogregarine is apparently smaller than haemogregarine A of *Rana holsti* or haemogregarine C of *Rana narina*, and is bigger than haemogregarine B of *R. holsti*. For this reason, this haemogregarine is tentatively called haemogregarine D of *R. limnocharis limnocharis*.

Haemogregarine D (Fig. 2, 1-23, Fig. 4, m-o): The size of free from (Fig. 2, 23) is 22.2–24.0 microns in length and 3.0–3.6 microns in width at nucleus. The anterior end is globular and the posterior end is pointed. In some specimens, a capsule like structure was observed ; however, the parasite leaves the host cell as shown in Fig. 2, 21–22, and in these cases, the capsule membrane is not recognized. The infected erythrocytes were hypertrophied, and the colour of cytoplasm stained more lightly as compared with that of normal erythrocytes. The size of normal erythrocytes is 15.6–19.2 microns by 9.0–12.0 microns, but the size of infected erythrocytes is 18.6–25.8 microns by 11.4–16.2 microns.

Dactylosoma ranarum (Kruse, 1890) (Fig. 2, 32–38) : This parasite was found from 5 (71.4%) of the frogs. In 4 of them, a trypanosome was observed.

A total of 10 blood specimens taken from *R. limnocharis limnocharis* captured at a paddy field near Inbu Bay, central part of Okinawa Island, in November, 1974, was examined.

Both *T. tsunezomiyatai* and *D. ranarum* were detected from one of them.

Remarks : *Dactylosoma ranarum* detected from *Rana rugosa* captured at Mogi, near Nagasaki City, was already reported by Miyata (1976). The species detected from *R. limnocharis limnocharis* is identical with that of *R. rugosa*. In the present study, some smears taken from *R. rugosa* were reexamined for comparison with the present material, and unexpectedly a very interesting figure shown in Fig. 2, 39, and Fig. 4, t was encountered. In the figures, 20 merozites of *D. ranarum* were seen as a group. This merozoites is apparently bigger than the youngest stage reported by Miyata (1976). It may be that in *D. ranarum* two or three types of schizont might be present, then small or large merozoites are seen at the same time. More study is needed to solve this question. *Dactylosoma taiwanensis* Manwell, 1964, was described from *Rana limnochris* in Taiwan, but Miyata (1976) believed that *D. taiwanensis* is a synonym of *D. ranarum*.

7) *Rana okinawana* Boettger, 1895 (Amphibia, Ranidae)

Japanese name : RYUKYU-AKAGAERU

Detected parasite : Haemogregarine E.

Only one specimen of *Rana okinawana* Boettger, 1895, was captured in Ishigaki Island in July, 1976, and a haemogregarine was detected from the blood of the frog. The haemogregarine is apparently larger than haemogregarine D of *Rana limnocharis limnocharis* and haemogregarine B of *Rana holsti*, and is smaller than haemogregarine C of *Rana narina*. This haemogregarine resembles haemogregarine A of *R. holsti* in the size of parasite, but apparently differs in the size of infected erythrocyte. For this reason, this haemogregarine is called tentatively haemogre garine E of *R. okinawana*.

Haemogregarine E (Fig. 2, 24-31, j-l) : The infected erythrocytes were hypertrophied, and the colour of cytoplasm stained lightly as compared with normal erythrocytes. The size of normal erythrocytes is 19.8-21.0 microns by 11.4-13.8 microns, and that of infected erythrocytes is 22.8-25.8 microns by 12.6-16.2 microns. The size of parasite is about 15 microns by 6-7microns.

8) *Rhacophorus japonicus* (Hallowell, 1860) (Amphibia, Rhacophoridae)

Japanese name : NIHON-KAJIKAGAERU

Detected parasite : *Trypanosoma rotatorium* (Mayer, 1843)

A total of 7 frogs belonging to *Rhacophorus japonicus* captured at Yona in June-July, 1976, were examined for blood parasites, and from one of them a species of trypanosome was detected. The trypanosome is identical with *Trypanosoma rotatorium* as shown by Miyata (1978).

9) *Eublepharis kuroiwae kuroiwae* (Namiye, 1912) (Reptilia, Gekkonidae)

Japanese name : KUROIWA-TOKAGEMODOKI

Detected parasite : *Trypanosoma ryukyuense* Miyata, 1977

Eublepharis kuroiwae kuroiwae is a very rare lizard, which belongs to the subfamily Eublepharinae of the family Gekkonidae. From the lizard, a polymorphic trypanosome, *Trypanosoma ryukyuense* was detected (Miyata, 1977c). In all 4 individuals captured at Yona and Benoki, Kunigamison in July, 1976, were examined and from all of them *T. ryukyuense* was detected.

10) *Japalura polygonata polygonata* (Hallowell, 1860) (Reptilia, Agamidae)

Japanese name : KINOBORI-TOKAGE

Detected parasite : Microfilaria.

A total of 15 *Japalura polygonata polygonata* captured in Okinawa Island in 1976 was examined, but no haemoparasites were detected.

Besides these, in January, 1976, Mr. Aizo Yamamoto caught one of these lizards in Komi, Ishigaki Island, and gave it to the authors to examine for blood parasites. From the blood smear, no protozoa was detected but a microfilaria was found (Fig. 5, g-h). The lizard died without eating, and adult worms were obtained from the body cavity just behind the lung. The specimen was sent to Dr. Sam R. Telford, Jr. According to his personal communication (23 May 1976), the filaria might belong to the genus *Conispiculum*, described from the related agamid genus *Calotes*. The size of microfilaria is 39.0-59.4 microns in total length and about 6-9 microns in width.

In July, 1976, one of the authors, Miyagi, collected three individuals of the lizard at Komi, Ishigaki Island, but no blood parasite was detected.

11) *Dinodon semicarinatus* (Cope, 1860) (Reptilia, Colubridae)

Japanese name : AKAMATA

Detected parasite : Haemogregarine.

One specimen of *Dinodon semicarinatus* was captured at Yona, in July, 1976, and a haemogregarine was detected from the snake (Fig. 3, 28-31). Unfortunately the parasitaemia is very low, and we cannot describe its morphology.

Remarks : According to Toshioka (1970), a haemogregarine was detected from *Dinodon semicarinatus* examined in Amami Island, but he did not give a specific name. The haemogregarine found in Okinawa is apparently identical with Toshioka's figure.

Additional Note : Unidentified parasite from *Hyla arborea japonica* Günther, 1858

Beside of the animals collected in Ryukyu Islands, an unidentified parasite belonging to the phylum Protozoa (Fig. 3, 15-27, Fig. 4, r) was detected in the erythrocytes of *Hyla arborea japonica* which was captured in Isahaya, 30 km east from Nagasaki City in July, 1977. The parasite is seen in the erythrocyte, and remarkable change is observed in the host cell nucleus which becomes more granular as compared with the normal erythrocyte. The parasite is round in shape and a reddish stained nucleus situates peripheral part of the body. Usually the cytoplasm of this parasite stained very lightly, then it is difficult to distinguish from the cytoplasm of host cell. The diameter of this parasite is about 7-8 microns. Double or triple infection is rarely observed, and a parasite having three nuclei, which looked as if a schizont, is seen, but exact method of multiplication is not known at present. No other stages were observed.

Remarks : This parasite is not identical with *Toddia*, because the parasite have no crystal as seen in *Toddia*. This parasite differs from *Cytamoeba bacterifera* reported by Hegner (1921) and by Lehmann (1961). *Cytamoeba* have no clear cytoplasm as seen in the present parasite. This parasite somewhat resembles those of the genus *Plasmodium*, but the parasite

has neither pigment nor vacuole. In the genus *Dactylosoma*, hypertrophy of host cell nucleus is not known. The present authors can not identify this parasite to one of known genera reported from the frog blood cell, and more materials are necessary to determine the systematic position of this parasite.

DISCUSSION

The results obtained in the present survey are very fruitful, as a total of 23 species of haemoparasite were detected from the cold-blooded animals captured in Ryukyu Islands. Five species of trypanosome and one species of haemogregarine were described as new species by one of the authors (Miyata, 1977a, b, c, and 1978). Other species of haemoparasite are not named at present, because material of those species still are insufficient to create new species.

According to Nakamura and Uéno (1974), a total of 66 species and subspecies of amphibians and reptiles are known in Ryukyu Island, but the present survey covered only 15% of those animals. This means that more haemoparasites will be discovered from the islands in near future.

Among the haemoparasites detected in Ryukyu Islands, *Trypanosoma* spp. and anuran haemogregarines are interesting from the viewpoint of geographical distribution and life cycle. From Honshu and other main islands, no amphibian haemogregarine is reported until now as far as the authors know. In Okinawa, however, amphibian haemogregarines (*Haemogregarina shirikenimori* Miyata, 1977, and anuran haemogregarines) are commonly detected.

The haemoparasite fauna of *Rana holsti* is most diverse, and from each of 2 frogs, 4 species of trypanosome were detected. In addition, *Toddia* and haemogregarines were detected from each of these frogs, respectively.

These parasites might be transfered by blood sucking invertebrate animals such as land leeches or mosquitoes. However, at present, the authors have no information concerning the life cycle of haemoparasites reported in the present paper. Some results on blood-sucking animals are already published (Miyagi, 1976). The survey of heamoparasites is still being continued by the authors, and within the near future, more interesting finding on the ecology of haemoparasites in the Ryukyu Islands will be obtained.

ACKNOWLEDGEMENTS

The authors are deeply indebted to Mr. Hiroshi Suzuki, Department of Virology, Mr. Akira Yamaguchi, Department of Epidemiology, Institute for Tropical Medicine, Nagasaki University, and Mr. Aizo Yamamoto, Nagasaki Kita High School, Nagasaki, for their co-operation in the collecting the materials used. The authors also express their sincere thanks to Dr. Sam R. Telford, Jr., Division of Malaria and Other Parasitic Diseases (Trypanosomiasis & Leishmaniases), World Health Organization, Genève, who kindly examined lizard filaria.

REFERENCES

1)*Chaussat, J. B. (1850) : Des hématozoaires. (Thesis for the Doctorate degree in Medicine) Paris.

2)*Cleland, J. B. & Johnston, T. H. (1910) : The haematozoa of Australian batrachians. No. 1. J. Roy. Soc. N. S. W., 44, 252-260.

3) Hayashi, S. (1960) : Description of a new frog filaria, *Icosiella sasai* n. sp. with redescription of an allied species, *Icosiella kobayashii* Yamaguchi, 1941. Japan. J. Exp. Med., 30, 1-12.

4) Hegner, R. W. (1921) : *Cytamoeba bacterifera* in the red blood cells of the frog. J. Parasitol., 7, 157-161.

5)*Kruse, W. (1890) : Über Blutparasiten. Arch. Path. Anat., 120, 541-560 ; 121, 359-372.

6)*Labbé, A. (1899) : Sporozoa. (in Schulze : Das Tierreich, 5, Friedlander und Sohn, Berlin).

7) Lehmann, D. L. (1961) : *Cytamoeba bacterifera* Labbé, 1894. I. Morphology and host incidence of the parasite in California. J. Protozool., 8, 29-33.

8) Mackerras, M. J. & Mackerras, I. M. (1961) : The haematozoa of Australian frogs and fish. Aust. J. Zool , 9, 123-139.

9) Manwell, R. D. (1964) : The genus *Dactylosoma*. J. Protozool., 11, 526-530.

10) Mathis, C. & Leger, M. (1911) : Trypanosomes des crapauds du Tonkin. (Deuxiéme note). Compt. Rend. Soc. Biol., 70, 1008-1009.

11)*Mayer, A. F. I. C. (1843) : Spicilegium observationum anatomicarum de organo electrico in Raiis anelectricis et de Haematozois. Bonnae.

12) Miyagi, I. (1976) : Description of new species of the genus *Topomyia* Leicester from the Ryukyu Islands, Japan (Diptera : Culicidae). Trop. Med., 17, 201-210.

13) Miyata, A. (1976) : Anuran haemoprotozoa found in the vicinity of Nagasaki City. 2. *Dactylosoma ranarum* (Kruse, 1890). Trop. Med., 18, 135-141.

14) Miyata, A. (1977a) : *Haemogregarina shirikenimori* n. sp. (Protozoa : Haemogregarinidae) detected from *Triturus pyrrhogaster ensicauda* (Hallowell, 1860)(Amphibia : Salamandridae) in Okinawa Island. Trop. Med., 19, 105-111.

15) Miyata, A. (1977b) : *Trypanosoma ogawai* n. sp. (Protozoa : Trypanosomatidae) detected from *Triturus pyrrhogaster ensicauda* (Hallowell, 1860) (Amphibia : Salamandridae) in Okinawa Island. Trop. Med., 19, 113-122.

15) Miyata, A. (1977c) : *Trypanosoma ryukyuense* n. sp. (Protozoa : Trypanosomatidae) detected from *Eublepharis kuroiwae kuroiwae* (Namie, 1912) (Reptilia : Gekkonidae) in Okinawa Island. Trop. Med., 19, 157-167.

17) Miyata, A. (1978) : Anuran trypanosomes in Kyushu and Ryukyu Islands, with descriptions of six new species. Trop. Med., 20, 51-80.

18) Miyata, A. & Miyagi, I. (1977) : *Toddia* detected from *Rana (Babina) holsti* Boulenger, 1892, in Okinawa Island. Trop. Med., 19, 123-128.

19) Nakamura, K. & Uéno, S-I. (1974) : Japanese reptiles and amphibians in colour. Hoikusha Publ. Co., Ltd., Osaka. (in Japanese)

20) Toshioka, S. (1970) : Natural infection of protozoal blood parasite in captive Japanese snakes (Preliminary report). The SNAKE, 2, 53-56. (in Japanese)

*cited from other literatures

琉球列島産冷血動物より検出された 住血性原虫類

宮田　彬（長崎大学熱帯医学研究所疫学部門），宮城一郎（琉球大学保健学部医動物学教室），
塚本増久*（長崎大学熱帯医学研究所疫学部門）

1974年以来，著者らは特異な動物相をもつ 琉球列島において，寄生現象の進化とその生物学的な本質の解明を目的として，冷血動物に寄生する原虫類の研究をすすめてきた．まずその第１段階として，住血性原虫類の種類について調査し，その結果琉球を模式産地とする トリパノゾーマ５種とヘモグレガリン１種を新種として記載した (Miyata, 1977a, 1977b, 1977c, および1978)．またアジアから初めて *Toddia* を報告した (Miyata and Miyagi, 1977)．以上の報告に続き，この論文では著者らが確認できた 住血性原虫および ミクロフィラリアを宿主ごとに列挙するとともに次のような新しく記録された種を図示した．1) ホルストガエルより2種，ハナサキガエルより１種，ヌマガエルより１種，リュウキュウアカガエルより1種，合計5種のヘモグレガリンをカエル類より確認した．このうち１～2種は既知種と似ている点もあるが，ヘモグレガリン類の同定についてはまだ資料が不十分であるので保留した．2) ナミエガエルからは *Lankesterella* 属の1種が発見された．これも血液内のスポロゾイトが見つかったのみであるので，同定を保留した．3) *Dactylosoma ranarum* はヌマガエルからのみ発見された．4) ホルストガエルより未同定の ミクロフィラリアが発見された．また石垣島産のキノボリトカゲより *Conispiculum* 属に属すると思われるミクロフィラリアとその親虫が見つかった．後者は WHO の Telford 博士に同定を依頼したので近く新種として命名されるものと思われる．5) 奄美大島産のオットンガエルからはミクロフィラリア，*Lankesterella* の1種およびヘモグレガリンが発見された．このうちミクロフィラリアは *Icosiella sasai* に同定された．6) トリパノゾーマ類については別に報告したが，琉球列島ではカエルより10種，シリケンイモリより1種，トカゲモドキより1種のトリパノゾーマが知られている． 7) ヘビは検査数が少ないが，アカマタよりヘモグレガリンが検出された．8) 結局調査した11種の冷血動物より23種の住血性寄生虫が発見されたことになり，これにオットンガエルより出た3種を加えると26種となる．9) これらに関連して，長崎近郊諫早産のアマガエルより発見された所属不明の原虫についても報告した． これが赤血球内に寄生すると宿主の核は著しく顆粒状となり 膨大することが特色である．10) 今回は今まで発見された種をまとめて記録したものであるが，これらの種の1つ1つの生活史の 解明については 現在検討中である．

熱帯医学　第20巻　第2号　97－112頁，1978年6月

*現所属：産業医科大学医動物学教室