Blood Parasites Detected from Small Mammals in Palawan Island, the Philippines

Akira MIYATA and Masuhisa TSUKAMOTO

Department of Epidemiology, Institute for Tropical Medicine, Nagasaki University

(Chief: Prof. Toshio NAKABAYASHI)

ABSTRACT: A total of 217 individuals of various small mammals and one varanid lizard were examined for blood parasites in the Iwahig Penal Colony, Palawan Island of the Philippines, from 1970 to 1972. The following parasites were detected: (1) *Trypanosoma lewisi* (Kent). This species was found from 21 (46%) out of 46 house rats in January, 1970, and 2 (13%) out of 15 in the same season of 1971. (2) *Trypanosoma palawanense* Miyata. This trypanosome was described as a new species in a separate paper of this issue by the senior author (1975), and detected from 1 out of 20 *Rattus panglima* in 1971, and also 1 out of 19 of the same rat species in 1972. (3) *Trypanosoma* sp. The undescribed trypanosome was detected from 1 out of 46 squirrels, *Callosciurus juvencus*, from 1971 to 1972. This parasite is about 40 microns in total length including a short free flagellum (5 microns). (4) *Hepatocystis vassali* (Laveran). Out of 46 squirrels examined, 4 cases of *H. vassali* were detected, and the parasite was not identical with the Malayan subspecies but rather similar to the Taiwan subspecies, *H. v. yokogawai* Wu. (5) *Hepatocystis pteropi* (Breinl). In all the six individuals of fruit bats, *Pteropus* spp., *H. pteropi* was detected. (6) Haemogregarine. From a varanid lizard, *Varanus salvator*, an unidentified haemogregarine was found. (7) Microfilaria from rats. From 2 out of 5 rats of *Rattus mulleri balabagensis*, a microfilaria was detected. The filaria seems to be identical with *Breinlia booliati* Singh and Ho. (8) Microfilaria from squirrels. From 2 out of 46 squirrels, an unidentified microfilaria was detected. (9) Microfilaria from the varanid lizard. From the varanid lizard, an unidentified but sheathed microfilaria was found. (10) Nematode worm was detected from the peripheral blood of a squirrel. The parasite is apparently different from microfilaria because the nerve ring or other internal organ of the worm were not seen.

Surveys on blood parasites of small wild mammals were carried out by various authors in southeast Asia, for instance, in Thailand by Coatney *et al.* (1960), in Taiwan by Manwell *et al.* (1966), in South Vietnam by Van Peenen *et al.* (1968), in Malaysia by Dunn *et al.* (1968), and others. From the Philippines, however, only few informations, if any, are available con-

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cerning to blood parasites of small wild animals except monkeys.

From 1969 to 1973 investigations on human malaria were carried out in Palawan Island by members of a team of the Nagasaki University and of the Malaria Eradication Service of the Philippines (Nakabayashi *et al.*, 1973 and 1974). During this period we have also had opportunities to examine blood parasites from small wild mammals. In the present paper, therefore, blood parasites detected from small mammals, such as rats, squirrels, and fruit bats, are reported with brief description, detection rate, and parasitaemia for each parasite species. Blood smears taken from Philippine monkeys were also examined, but the results will be published elsewhere.

GEOGRAPHICAL AND NATURAL CONDITIONS OF THE SURVEY AREA

The survey area is situated at the Montible Subcolony, Iwahig Penal Colony, Palawan Island of the Philippines, and lies at a central part of the Island on 10°N and 119°E. The Montible area was opened several decades ago, and the survey area is composed of cultivated lands, savannah-like secondary grass fields with shrubs, secondary forests which occupied between cultivated area and virgin forests, and real virgin tropical forests. The virgin forest has a physiognomic feature as a result of the adaptation of different plant species to a similar ecological condition. Tree trunks are usually tall and straight. Many of the trees have brent roots at the base to support additionally. Branches of the trees are seen near the top, and form a continuous canopy together with branches of other tree. The undergrowth of the forest consists of shrubs, small palms, and many saplings of the trees. The forest is arranged in several layers of tree crown.

The climatic condition of the area was briefly described by Nakabayashi *et al.* (1973). There are two clear seasons, dry and wet, and the surveys were carried out in the dry season, from December to February, in 1970~1971 and 1971~1972.

Palawan Island is situated very near to Borneo Island, and shallow sea less than 100 meters depth lies between these two islands (Fig. 1). A majority of the fauna and flora in these islands are common to each other, because at least in the last glacial age, they are considered to have been connected by a land bridge. Thus, Palawan and its neighbouring islands are in a special situation from a viewpoint of the animal distributions as compared with other islands of the Philippines, for instance, wild boar, *Sus barbatus*, scaly anteater, *Manis javanica*, mouse deer, *Tragulus nigricans*, and bird-wing butterfly, *Trogonoptera brookiana*, distribute in Palawan and/or its neighbouring islands and Borneo, although these species are never found in other Philippine islands. From these reasons, Palawan and its neighbouring islands are separated, by the Wallace's Line, from other Philippine islands, and belong to the Malayan Subregion of the Oriental Zoogeographic Region as well as Borneo Island.

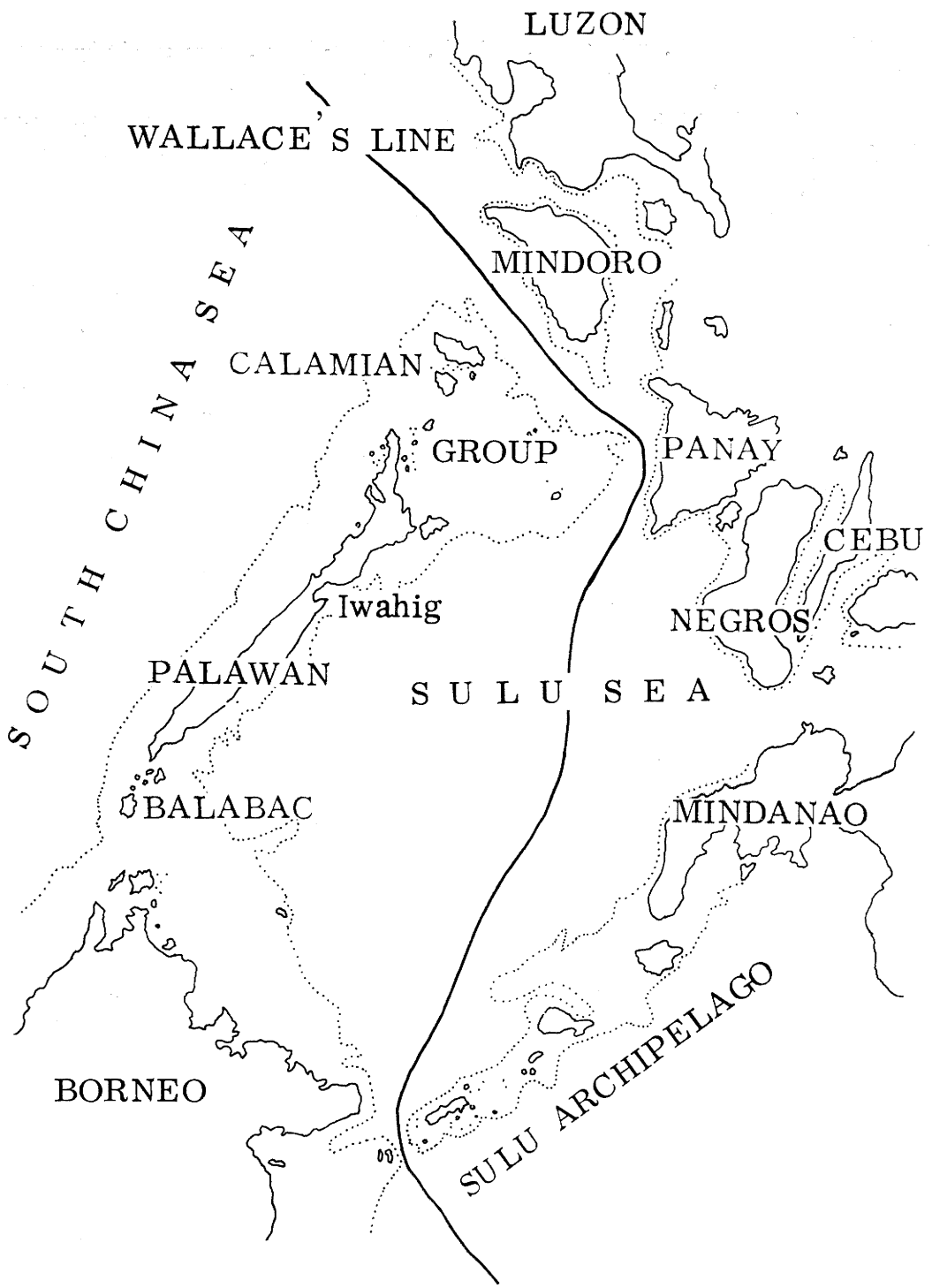

Fig 1. Map of Palawan and its neighbouring islands.
····· Shallow sea less than 100m depth

SURVEY METHODS

The small mammals, such as rats, squirrels, and tree-shrews, were caught by rat traps made of wire netting, and piecies of cassava were used for the bait.

Both thin and thick blood smears were usually prepared from each animal by pricking a leg finger, but if enough amounts of blood could not be taken, a small amounts of blood was collected, under light anesthesia, from the heart by using a sterillized and citrated syringe without the sacrifice of the animal's life. The thin smears were fixed with methyl alcohol, then both the thin and the thick smears were stained by 3% Giemsa solution for 30 minutes. The thick smear was at first examined with low magnification (at 200×) for seeking microfilaria, then with an immersion lens system (1,000×) for detecting haemoprotozoa. In the positive case the thin smear was further examined for the identification of the parasites. Except for house rats the parasite-negative animals were released from a cage to the outside of the survey area.

Every evening fruit bats used to come to eat the flower of kapok trees, *Ceiba pentandra*, planted in the colony. Prisoners of the colony sometimes shoot the fruit bat by a stone shooter made of a small tree branch and rubber band and are fond of eating the meat for their side-dish. When we had opportunities to examine a fruit bat in the colony, the blood was also taken from the heart by a syringe.

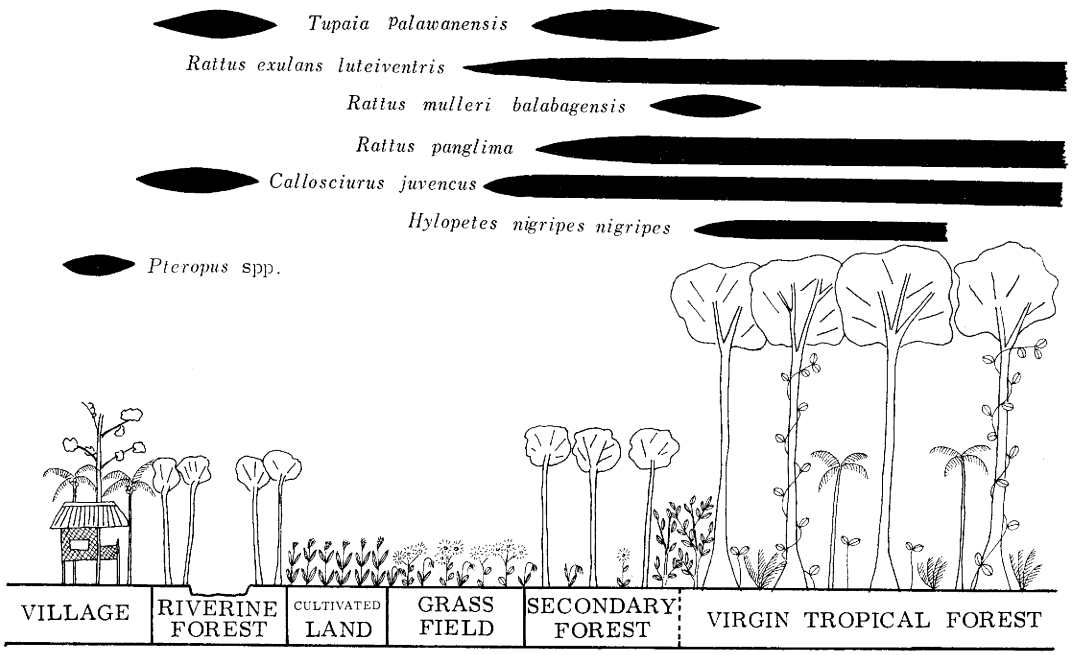

Fig. 2. Distribution pattern of each small mammal in the survey area.

Table 1. Detection of the blood parasites from small mammals in Palawan Island (1970–1972)

Mammals	Year	Total No. Examined	*Hepatocystis*	*Trypanosoma*	Microfilaria
Squirrels	1971	31	1	0	1
〃	1972	15	3	1	1+1*
R. m. balabagensis	1971	1	0	0	1
〃	1972	4	0	0	1
R. panglima	1971	20	0	1	0
〃	1972	19	0	1	0
Other forest rats	1971	18	0	0	0
〃	1972	27	0	0	0
House rats	1970	46	0	21	0
〃	1971	15	0	2	0
Flying squirrels	1971	2	0	0	0
Small bats	1972	3	0	0	0
Fruit bats	1971	1	1	0	0
〃	1972	5	5	0	0
Tree shrews	1971	10	0	0	0
Total		217	10	26	4+1

* Microfilaria-like nematode

The following animals were examined:

Callosciurus juvencus (Thomas) (squirrel; local name: *Bising*): Squirrels distribute in a wide range from the grass field with shrubs to the virgin forest.

Rattus spp. (rat; local name: *Daga*): The house rats could not be identified, but the same rat species might appear in the cultivated land or the grass field too. The three rat species in the forest were identified by Dr. Godofredo L. Alcasid, National Museum, Manila, as follows: *Rattus mulleri balabagensis* Sanborn : This large-sized species distributes in Palawan and Malay, and the subspecies was originally described from Mt. Balabag located near the Iwahig Penal Colony. Only five individuals were caught, and all of them were trapped in a limited place of the borderline between the secondary forest and the virgin forest, but never found from the grass field or the cultivated land. The average body weight of the rats was 230 g. *Rattus panglima* Robinson: This species was trapped at the grass field, the secondary forest, and the virgin forest. But the rat was never found at the cultivated land or the inside of the house, and even at the grass field the individual number was very few. Two groups of the average body weight were recognized in the collected rats, and one of them was 100~130 g, and the other was 160~170 g. Each group was consisted of the both sexes, then these groups could be thought as two generations. *Rattus exulans luteiventris* (Allen) : This species distributes to the secondary forest and the virgin forest.

Some more rat species were also trapped at the survey area, but the identification was very difficult.

Hylopetes nigripes nigripes (Thomas) (flying squirrel): Two wounded individuals of the flying

squirrel were given by a prison guard when the animals were caught by prisoners who were cutting off the forest for expanding the cultivated land.

Pteropus spp. (fruit bat; local name: *Kabog*): The fruit bats usually stay at mangrove forest near the colony during the day time, and they visit kapok trees only at night to eat the flower. At least two species of the genus *Pteropus* were examined in Iwahig area, and one specimen was identified as *Pteropus leucotis obscurus* Sanborn by Dr. Alcasid. Another individual was identified tentatively by a photo picture as *P. vampyrus lanensis* Mearns by Dr. Yoshinori Imaizumi, National Museum of Tokyo.

Tupaia palawanensis palawanensis Thomas (tree-shrew): The tree-shrew was trapped in the early secondary forest, the riverine forest, and the grass field with shrubs. The most abundant place of the tree-shrew was near the virgin forest, whereas this animal was never caught from the inside of the forest.

The scientific names of the mammals used by Alcasid (undated) and Taylor (1934) were adopted in the present paper.

RESULTS

The results obtained after the microscopic examination are summarized in Table 1.

1. *Trypanosoma* spp.

(1) *Trypanosoma lewisi* (Kent, 1880) (Fig. 3 and Table 2)

In total of 21 (46%) out of 46 house rats, trypanosomes were detected in January, 1970, but this parasite was found from only two rats (13%) out of 15 at the same area and the same season in 1971. The size of the trypanosome is 30.3 microns in total length, 1.7 microns in width at the widest point of the body, and 7.1 microns in length of a free flagellum (Table 2). These trypanosomes were identified morphologically as *T. lewisi*.

(2) *Trypanosoma palawanense* Miyata, 1975

Very few of trypanosomes were also detected from *Rattus panglima*, and this flagellate was quite different from *lewisi*-type trypanosomes in the large size. This trypanosome was measured 70.4 microns in total length including a short free flagellum (6.0 microns) and 4.5 microns in width at the widest point of the body. This flagellate is easily distinguishable from other described species from various mammals by its larger size, larger Kinetoplast Index, and so on. Then the trypanosome has been described in detail as a new species, under the name of *Trypanosoma palawanense*, by Miyata (1975) in a separate paper of the same issue.

(3) *Trypanosoma* sp. (Fig. 4)

Only two individuals of unidentified trypanosomes were detected from several thin smears of a squirrel caught in forest area. Microfilaria and *Hepatocystis* were also detected from the blood smears prepared from the same individual of the squirrel. The larger individual of this trypanosome (Fig. 4, *b*) was measured approximately 40 microns in the total length including a short free flagellum (5 microns) and 4 microns in width at the widest point of the body. Undulating membrane and nucleus are difficult to see clearly because of the darkly

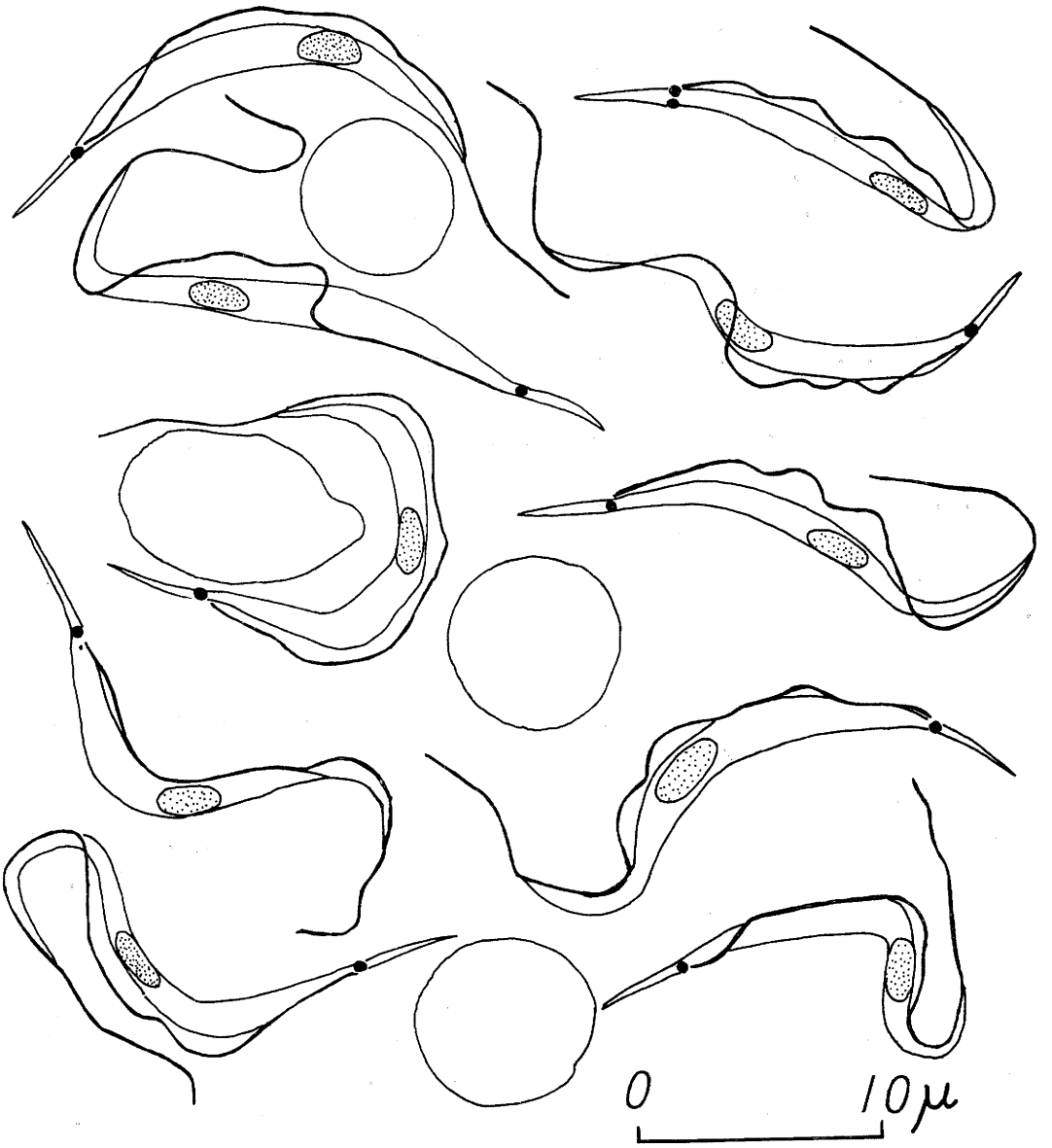

Fig. 3. *Trypanosoma lewisi* (Kent) and erythrocytes of house rats.

stained cytoplasm, then the dotted line is used to show invisible parts of the trypanosome. The other individual (Fig. 4, *a*) is rather small and pale in staining colour of the cytoplasm. No more trypanosome was found from the smears in despite of eagerly repeated examinations. This is the reason why the trypanosome has not been identified.

2. *Hepatocystis* spp.

The following two species of *Hepatocystis* were detected from squirrels and fruit bats, respectively.

(1) *Hepatocystis vassali* (Laveran, 1905) (Fig. 5)

In squirrels, 4 cases of malaria parasites were found, and the parasite was identified as *H. vassali* by the morphological feature of blood stages. The young gametocytes (Fig. 5, *a*~*f*) are ring forms, and with growth the ring becomes amoeboid (Fig. 5, *g* and *h*). The chromatin of the nucleus is assumed to be an oval or irregular form. In some cases. two chromatin dots are visible as shown in Fig. 5, *e* and *f*. The mature gametocytes become larger than the uninfected erythrocyte. The macrogametocyte (Fig. 5, *i*~*n*) is smaller in size than the microgametocyte and the colour of the cytoplasm is dark blue or purple. The nucleus is situated peripherally and chromatin dots are seen in small pink-staining area. In the microgametocyte (Fig. 5, *o*~*u*) light yellow-brown granules of pigment are seen in the cytoplasm, and the nucleus locates in a wider pale pink-staining area in which lies a single rod-like chromatin. The liver stages were not observed.

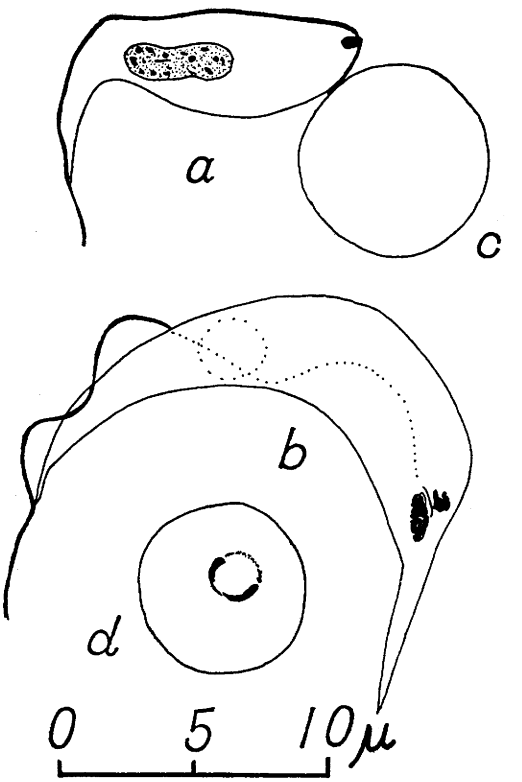

Fig. 4. *Trypanosoma* sp. detected from a squirrel, No. 1972–42. *a* & *b*. *Trypanosoma* sp.; *c*. Normal erythrocyte; *d*. Erythrocyte infected with *Hebatocystis vassali*

(2) *Hepatocystis pteropi* (Breinl, 1913) (Fig. 6)

In all the six individuals of fruit bats examined, *H. pteropi* was detected. In young gametocyte (Fig. 6, *a*~*f* and *A*~*C*), the shape of chromatin is rather large and comma- or sickle-like, and band form was also occationally found (Fig. 6, *g*). The cytoplasm of the mature microgametocyte (Fig. 6, *o*~*s* and *E*~*H*) stains lightly with yellow-brown granules. The macrogametocyte (Fig. 6, *i*~*n* and *D*) shows dark blue or purple colour and the size is smaller than that of the microgametocyte. The infected erythrocyte shows no change in size when compared with the uninfected erythrocyte. Though the detection rate is very high, parasitaemia is comparatively low and even in the highest case about 30 erythrocytes were infected with *H. pteropi* in 1,000 erythrocytes. From the bat, a species of Nycteribiidae (Diptera) was taken.

3. Haemogregarine (Fig. 7)

In addition to mammalian materials, blood smears of a big lizard, *Varanus salvator*, were also obtained by chance in January, 1972. Unexpectedly microfilaria and haemogregarine

Table 2. Size in microns of *Trypanosoma lewisi* (Kent) from house rats

	TL	P N	A-N	K-N	P K	FF	W	NL	KI	NI
Rat No.1970−13										
	32.5	14.5	10	4	10.5	8	2	2.5	1.4	1.5
	28	13	8	4	9	7	1.2	2.5	1.4	1.6
	30.5	13.5	9	3.5	10	8	1.5	2.5	1.4	1.5
	29	15	7	4	11	7	2	2.5	1.4	2.1
	29.5	14	9	4	10	6.5	2	2.5	1.4	1.6
	28	14	9	4	10	5	1	2.5	1.4	1.6
Rat No. 1970−8										
	30.5	13.5	11	3.5	10	6	2	3	1.4	1.2
	33	14	11	4	10	8	1.5	2	1.4	1.3
	30	15	9	5	10	6	2	2	1.5	1.7
	32	16	7	4	12	9	1.5	2.5	1.3	2.3
Average	30.3	14.3	9	4	10.3	7.1	1.7	2.5	1.4	1.6
Maximum	33	16	11	5	12	9	2	3	1.5	2.3
Minimum	28	13	7	3.5	9	5	1	2	1.3	1.2

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 of the body
NL : Nuclear length at the longest point
KI : Kinetoplast Index (=P-N/K-N)
NI : Nuclear Index (=P-N/A-N)

Table 3. Infection rate of erythrocytes with *Hepatocystis* in fruit bats and squirrels

Individual No. of Hosts	Infection Rate in 1,000 Erythrocytes	No. (%) of Gametocytes			Total
		Immature	Mature	Double Infection	
Fruit bat		*Hepatocystis pteropi*			
1971−3	< 1	34 (64.2)	19 (35.8)	−	53
1972−56	< 1	101 (92.7)	8 (7.3)	−	109
1972−57	12.8	103 (45.4)	122 (53.7)***	2 (0.9)	227
1972−58	32.9	109 (52.4)	99 (47.6)	−	208
1972−63	14.6	108 (56.5)	83 (43.5)	−	191
1972−76	6.0	143 (75.7)	41 (21.7)	5 (2.6)	189
Squirrel		*Hepatocystils vassai*			
1971−2*	< 1	−	−	−	−
1972−42**	2.0	89 (56.7)	98 (43.3)	−	157
1972−78	< 1	−	−	−	−
1972−87	21.0	110 (55.6)	88 (44.4)	−	198

* Mixed infection with *H. vassali* and microfilaria
** Mixed infection with *H. vassali*, *Trypanosoma* sp. and microfilaria
*** Two or three nuclei were rarely seen in some gametocytes.

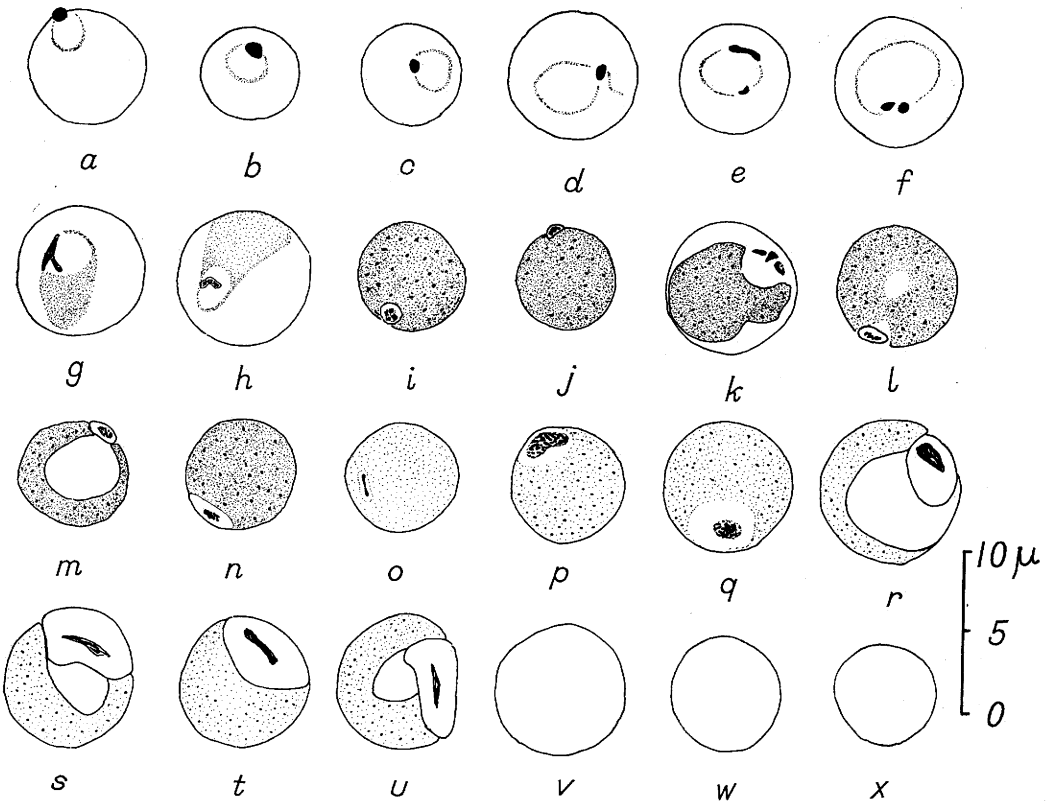

Fig. 5. *Hepatocystis vassali* (Laveran) detected from a squirrel, No. 1972–87. *a*–*f*. Young gametocytes, ring forms; *g* & *h*. Young gametocytes, amoeboid forms; *i*–*n*. Mature macrogametocytes; *o*–*u*. Mature microgametocytes; *v*–*x*. Uninfected erythrocytes

were detected from the peripheral blood. From this species of lizard, an unidentified haemogregarine was already reported by Prowazek (1912) in Sumatra, and totally 8 species of haemogregarines were described from various varanid lizards. The present parasite found from *V. slavator* is not identical with any named species whereas only three haemogregarines were found from one smear. The size of the parasite and that of the infected erythrocyte (in parentheses) are 17×4 microns (20×7 microns), 18×4 microns (20×8 microns), and 15×3 microns (18×9 microns), respectively. The uninfected erythrocyte is 13~15 microns in length and 8~9 microns in width. The cytoplasm of the haemogregarine stains pale or pinkish and free from vacuole or granule. The large nucleus lies at the center or posterior half of the body and the colour is dark purlpe.

4. Microfilaria and other worm

In addition to haemoprotozoa, 3 species of microfilaria and one nematode worm were detected from the blood smears of animals.

(1) Microfilaria of *Rattus mulleri balabagensis* (Fig. 8, *a*~*c*)

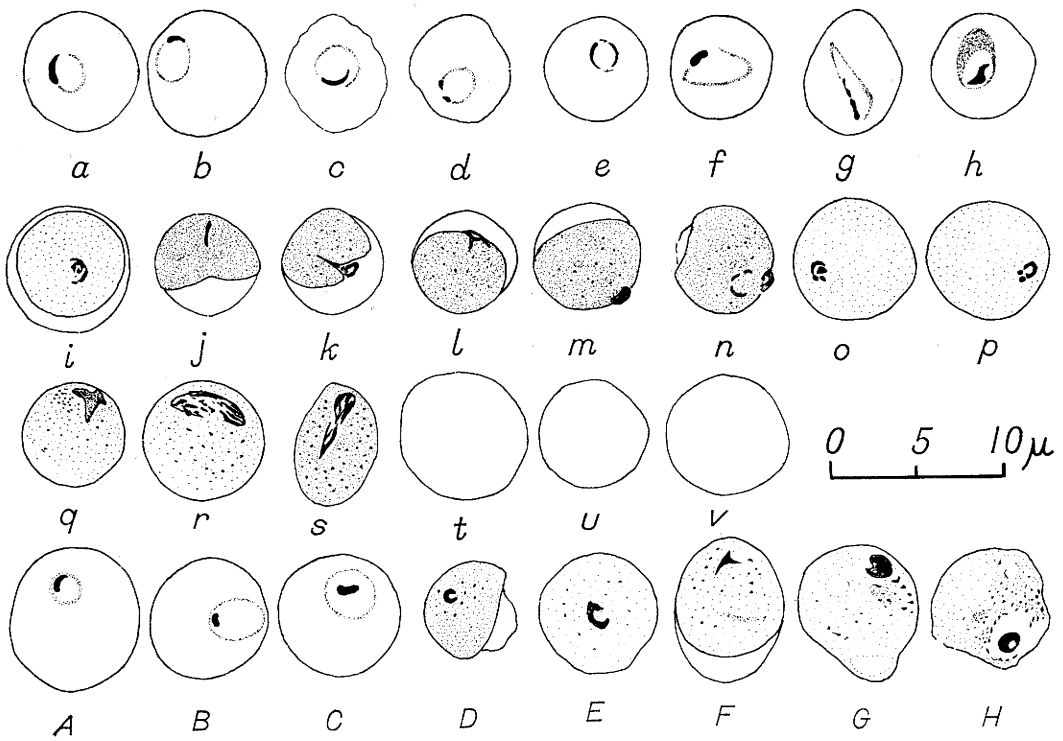

Fig. 6. *Hepatocystis pteropi* (Breinl) detected from fruit bats, No.1972–63 (*a*–*v*) and No. 1972–76 (*A*–*H*). *a*–*g* & *A*–*C*. Young gametocytes, ring forms; *h*. Young gametocyte, amoeboid form; *i*–*n* & *D*. Macrogametocytes; *o*–*s* & *E*–*H*. Microgametocytes; *t*–*v*. Uninfected erythrocytes

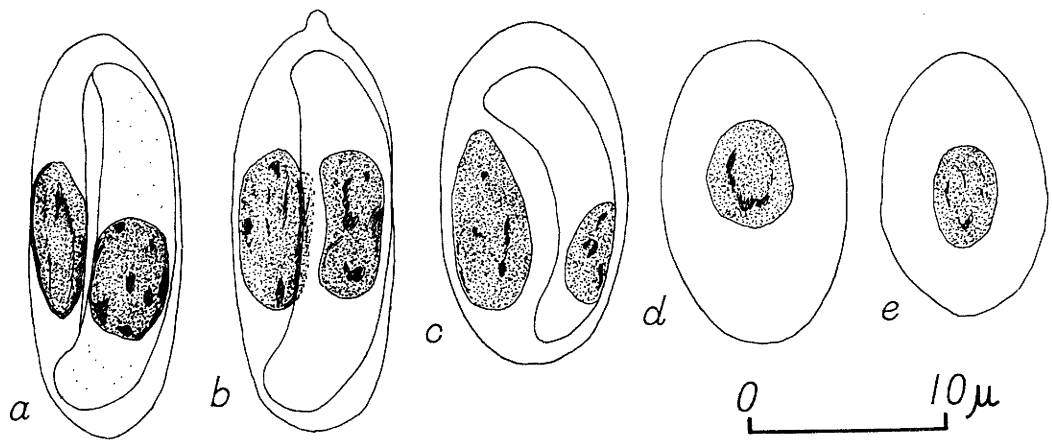

Fig 7. Haemogregarines detected from a varanid lizard, No. 1972–92. *a*–*c*. Haemogregarines; *d* & *e*. Uninfected erythrocytes

In *R. mulleri balabagensis*, microfilariae were detected from the peripheral blood, and about 2,000 microfilariae in Rat No. 1971–1 and 1,000 in Rat No. 1972–15 were counted per ml of the peripheral blood. After the inspection of organs of the infected rat, a number of

the adult worm were also observed in the thoracic cavity. The measurement of the microfilaria are as follows: total length 168～196 microns; distance from the anterior end to the first nucleus 2～7 microns, to the nerve ring 34～43 microns, to the excretory pore 50～69 microns, to the anal pore 131～139 microns, and to the last nucleus 161～189 microns. The microfilaria has no sheath. This filaria seems to be identical with *Breinlia booliati* Singh and Ho, 1973. The infected rats seemed to be healthy in appearence, and any tick or flea was not found on the body of the rats.

(2) Microfilaria of squirrels (Fig. 8, *d*～*f*)

Two cases of microfilaria were detected from squirrels. About 3,800 microfilariae in Squirrel No.1971－2 and 2,200 in Squirrel No. 1972－42 were counted per ml of the peripheral blood. The measurements of the microfilaria are as follows: total length 211～219 microns; distance from the anterior end to the first nucleus 5～9 microns, to the nerve ring 41～49 microns, to the excretory pore 66～71 microns, to the anal pore 146～183 microns, and to the last nucleus 198～213 microns. respectively; width 5～6 microns. This microfilaria is apparently distinguished from that of *B. booliati* by its larger size, longer head space, different arrangement of the column of the cells, and so on.

(3) Microfilaria of *Varanus salvator* (Fig. 9)

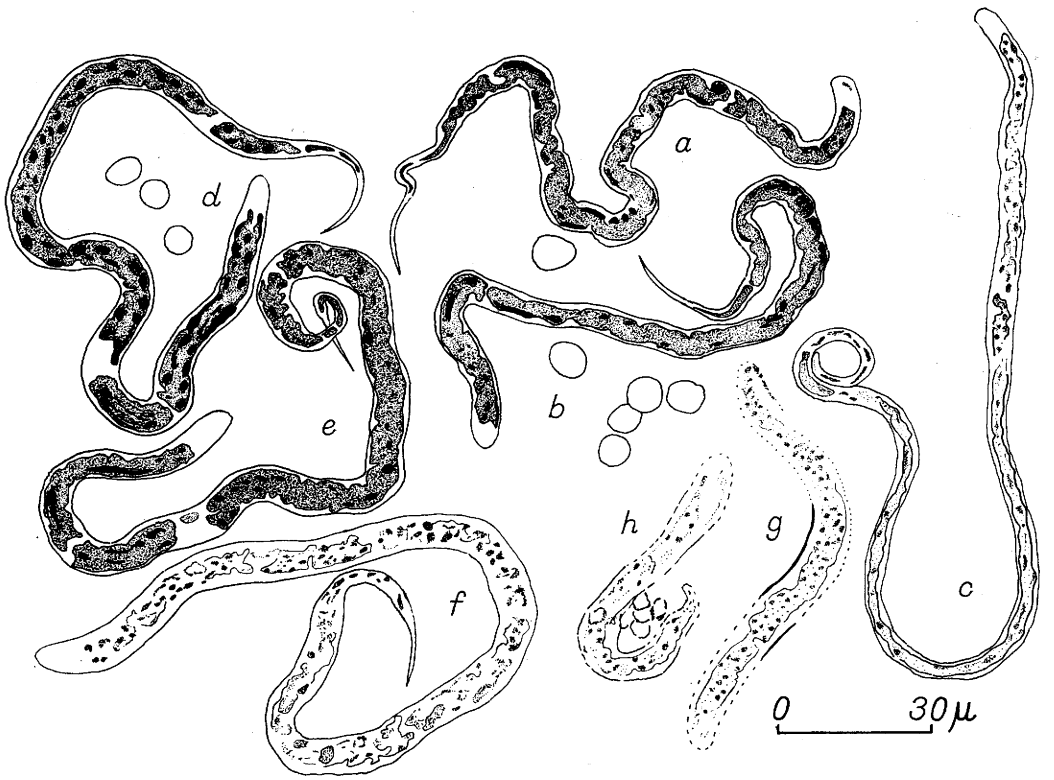

Fig. 8. Microfilariae and nematode worms detected from mammals. *a*－*c*. Microfilaria detected from *Rattus mulleri balabagensis*: *a* & *b*. No. 1971－1, thin smear; *c*. No. 1972－15, thick smear; *d*－*f*. Microfilariae detected from squirrels; *d* & *e*. No. 1971－2, thin smear; *f*. No. 1972－42, thick smear; *g* & *h*. Nematode worms detected from a squirrel, No. 1972－68, thick smear

Thin smears of only one lizard were examined from which sheathed microfilariae were detected. About 1,800 microfilariae per ml of peripheral blood were counted. The measurements of the microfilaria are as follows: total length without sheath 193~201 microns; distance from the anterior end to the nerve ring 38~40 microns, and to excretory pore 51~56 microns respectively; width at the widest point of the body 7~9 microns.

(4) Nematode worm found in the blood of a squirrel (Fig. 8, *g* and *h*).

From one of squirrels (Squirrel No. 1972 – 68), unidentified nematode worms were detected. The worm has no nerve ring, and other morphological features are also different from microfilaria as shown in Fig. 8, *g* and *h*. One end of the worm is rounded and the other end is pointed. In some cases bubble-like structures were visible at the outside of the pointed end of the body (Fig. 8, *h*). Total length of the worm is 75~80 microns, and about 700 parasites were detected per ml of the peripheral blood.

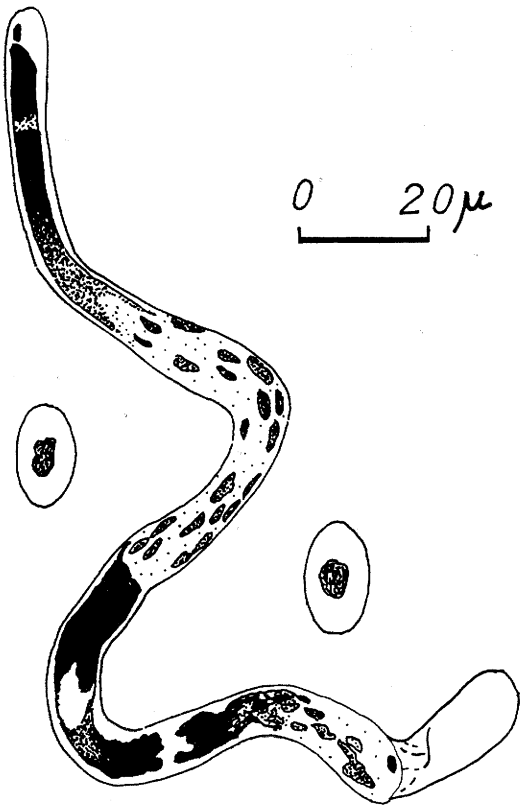

Fig. 9. Microfilaria detected from a varanid lizard, No. 1972–92.

DISCUSSION

One of the initial expectations was to find out malarial species of the genus *Plasmodium*, if any, in the survey area, and resulted in an unsuccessful attempt. On the other hand, several undescribed species, as well as known species, of other type of blood parasites have been detected from various small animals.

Two species of the genus *Hepatocystis* detected from squirrels and fruit bats are morphologically identical with *H. vassali* (Laveran, 1905) and *H. pteropi* (Breinl, 1913) respectively. Both species were recorded in various parts of southeast Asia, but the record of *H. vassali* from the Philippines has not been available yet for us up to the date. *H. vassai* has four subspecies which are mostly based on the locality of each subspecies as the criterion for identification: *vassali* s. str. from Vietnam, subsp. *malayensis* Field and Edeson, 1950, from Malaya, subsp. *ratufae* (Mulligan and Somerville. 1947) from India, and subsp. *yokogawai* Wu, 1953, from Taiwan. According to Field and Edeson (1950), the subsp. *malayensis* is different from others in the following characters, which have not been particullarly noted in other mem-

bers of the species: (1) slight enlargement of the infected erythrocyte, (2) tendency to show band forms, (3) multiple invasion to erythrocytes even with light parasitaemia, (4) agglutination of infected cells, and (5) marked 'tenue' phase. Therefore, mature gametocytes of *H. vassali* detected in Palawan is not identical with the Malayan subspecies, but rather resembles morphologically to the Taiwan subspecies, of which gametocytes are slightly larger than those of other subspecies. In the subsp. *yokogawai*, the merocyst is most distinctive in the gross enlargement of the host cell nucleus, and the localization of parasitic substance (Garnham, 1966). Due to our failure to obtain the merocyst from the infected squirrels in Palawan, we could not determine our materials whether they belong to the subsp. *yokogawai* or to a new subspecies.

McGhee (1949) already reported *H. pteropi* which was detected from a fruit bat of the family Pteropidae in the Philippines. In the present survey all the fruit bats examined were infected with this parasite. According to observations by people of Palawan, the bats crowd during the day time in a special resting place at mangrove forest near the sea shore of Puerto Princesa, Palawan, and the place is called "Bat Island" by local people. Then, the vector, although which was not examined by us, might be abundant in the resting place during the day time. According to Garnham *et al.* (1961) *Culicoides* is a possible vector of *Hepatocystis kochi* for African monkey. Members of this genus are also abundant in Palawan.

From Taiwan flying squirrels *Hepatocystis rayi* was described as a new species by Tokura and Wu (1961), and the same species was reported from Himalayan flying squirrels by Dasgupta (1967) who described a new genus *Rayella* based on the species. More recently, *Plasmodium* sp. and *Hepatocystis* sp. have been detected from Malayan flying squirrels by Muul *et al.* (1973) and Yap *et al.* (1970). Lien and Cross (1968) also described *Plasmodium watteni* from a Formosan giant flying squirrel, *Pelaurista pelaurista grandis*. In the present survey, however, only two individuals of Palawan fly ng squirrels were available and any blood parasite could not be found out in spite of careful examinations.

Two species of trypanosomes were detected from defferent rat species. One of them found from house rats is identified with *Trypanosoma lewisi* (Kent) morphologically (Wenyon, 1926, and Hoare, 1972), and the other large-sized trypanosome detected from *Rattus panglima* has been confirmed to be a non-recorded species, which has been described as *Trypanosoma palawanense* Miyata, 1975, in the preceeding paper.

According to Hoare (1972), among the true squirrels, trypanosomes are known from four hosts: *Funambulus palmarum* of India (*Trypanosoma indicum*), *Funisciurus congicus* and *Heliosciurus gambianus* from tropical Africa (*Trypanosoma sciuri*), and *Tamiasciurus hudsonicus* from North America (*Trypanosoma* sp.). These trypanosomes belong to the subgenus *Herpetosoma*. From southeast Asian squirrels, trypasnosomes were also reported. Coatney *et al.* (1960) detected a *lewisi* type trypanosome from *Callosciurus finlaysoni* in Loei Province of Thailand, but unfortunately they did not show any figure or the detailed description on the morphology of this parasite. More recently Dunn *et al.* (1968) also reported that trypanosomes were found from 8 out of 249 *Callosciurus caniceps* and from 2 out of 486 *C. notatus* in Malayan rain forests, but again the details on the trypanosome were not described. In the present

survey, we found only one case of trypanosome from a squirrel, *Callosciurus juvencus*, but the trypanosome was different from *lewisi*-type trypanosomes in its larger size and in its shorter free flagellum. This trypanosome rather resembles to meembrs of the subgenus *Megatrypanum*, but the present material is not enough to be described in detail as an interesting new species, though it is clear that this kind of trypanosome is still undescribed from Asian spuirrels.

From a varanid lizard, *Varanus salvator*, a haemogregarine was detected. Eight species of haemogregarines of *Varanus* spp. had been described, among which 3 were recorded from Africa and all the rest from Australia (Wenyon, 1926, and Mackerras, 1961). The present haemogregarine species detected from *V. salvator* resembles *Haemogregarina varanicola* Johnston and Cleland, 1910, detected from *V. varius*, *V. tristis*, and *V. gouldii* in Australia, but this haemogregarine is rather small (14～15×2～3 microns) and no distortion of the host erythrocyte is noted, except slight enlargement of the cell (Mackerras, 1961). Therefore, this haemogregarine might be also a new species, but the material is not enough to be described. From *V. salvator*, *Haemogregarina* sp. was already reported from Sumatra, but the original paper written by Prowazek (1912) has not been available for us up to the date.

One of the most interesting findings is the detection of microfilaria from *Rattus mulleri balabagensis*, and the microfilariae are larger than those of the cotton rat filaria (*Litomosoides carini*) which is used in laboratory experimens. Dunn *et al.* (1968), Singh and Cheong (1970), and Ramachandran *et al.* (1970) also reported microfilariae from a Malayan subspecies of *R. mulleri* and other various rodents, but the detail of the parasite has not yet been published. Singh and Ho (1973) described *Breinlia booliati* as a new species from a Malayan forest rat, *Rattus sabanus*, and their attempts (1972) had been successful to transmit the filaria infection from *R. sabanus* to the laboratory albino rat by mosquitoes, *Aedes togoi* and *Armigeres subalbatus*. The same filaria species was further reported from *R. sabanus*, *R. cermoriventer* and *R. mulleri* in Sarawak, Borneo, by Joon-Wah and Lim (1974). Thus, this filaria might distribute widely in forest areas of southeast Asia. If this filaria can infect laboratory rats easily, it might be quite useful as a laboratory model in filariasis as reviewed by Schacher (1973).

Another microfilaria was also found from blood of squirrels, and the species was apparently different from *B. booliati*. There are many records of squirrel filaria from Malaya (Dunn *et al.*, 1968, and other), but the filaria is not yet described on the details. Further researches are necessary for squirrel filaria, especially on its infectivity to laboratory animals.

According to Dunn (1964), microfilaria was detected from tree-shrews. *Tupaia glis* (from Malaya and Borneo) and *T. tana* (from Borneo). Later, Orihel (1966) described *Brugia tupaiae* from *T. glis* collected in Malaya. According to Hoare (1972), there are no record of trypanosome from the genus *Tupaia*, but Dunn (1964) reported 1 case of trypanosome out of 18 *T. tana* and 5 cases out of 10 *T. glis* taken in southeastern North Borneo. The trypanosomes found by Dunn have not yet been described on the morphological details, but the trypanosomes might be one or two new species. Furthermore, Coatney *et al.* (1960) also detected 2 cases of trypanosomes out of 19 *T. belangeri* collected in Loei Province of Thailand. According to their report, these trypanosomes resemble to *lewisi*-type. In the present survey, unfortunately any kind of blood parasite was not found from the Palawan tree-shrew examined.

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REFERENCES

1) Alcasid, G. L. ( ): Checklist of Philippine mammals. Publication of the National Museum, Manila.
2) Coatney, G. R., Elbel, R. E. & Kocharatana, P. (1960): Some blood parasites found in birds and mammals from Loei Province, Thailand. J. Parasitol., 46(6), 701–702.
3) Dasgupta, B. (1967): A new malarial parasite of the flying squirrel. Parasitology, 57(3), 467–474.
4) Dunn, F.L. (1964): Blood parasites of southeast Asian primitive primates. J. Parasitol., 50(2), 214–216.
5) Dunn. F. L., Lim, B. L. & Yap, L. F. (1968): Endoparasite patterns in mammals of the Malayan rain forest. Ecology, 49(6), 1179–1184.
6) Field, J. W. & Edeson, J. F.B. (1950): A malaria parasite of the Malayan squirrel. Bull. Inst. Med. Res. Malaya, No. 2, Kuala Lumpur (cited from Garnham. 1966).
7) Garnham, P. C. C. (1966): Malaria parasites and other haemosporidia. Blackwell Scientific Publications, Oxford and Edingburgh.
8) Garnham, P. C. C., Heisch, R. B. & Minter, D. M. (1961): The vector of *Hepatocystis* (=*Plasmodium*) *kochi*; The successful conclusion of observations in many parts of tropical Africa. Trans. Roy. Soc. Trop. Med. Hyg., 55(5), 497–502.
9) Ho, B.C. & Singh, M. (1973): Observations on the development of a new filaria (*Breinlia booliati*, Singh and Ho, 1973) of a rat *Rattus sabanus* in the mosquito *Aedes togoi*. J. Helminth., 47(2), 135–140.
10) Hoare, C. A. (1972): The trypanosomes of mammals. A zoological monograph. Blackwell Scientific Publications, Oxford and Edingburgh.
11) Joon-Wah, M. & Lim, B. L. (1974): New hosts of *Breinlia booliati* in wild rats from Sarawak with further observations on its morphology. Southeast Asian J. Trop. Med. Pub. Hlth., 5(1), 22–28.
12) Lien, J. C. & Cross, J. H. (1968): *Plasmodium (Vinckeia) watteni* sp. n. from the Formosan giant flying squirrel, *Petaurista petaurista grandis*. J. Parasitol., 54(6), 1171–1174.
13) Mackerras, M. J. (1961): The haematozoa of Australian reptiles. Austral. J. Zool., 9(2), 61–122.
14) Manwell, R. D. & Kuntz, R. E. (1966): *Hepatocystis* in Formosan mammals with a description of a new species. J. Protozool., 13(4), 670–672.
15) McGhee, R. B. (1949): The occurence of bat malaria in the New Hebrides and Philippine Islands. J. Parasitol., 35(5), 545.
16) Miyata, A. (1975): *Trypanosoma* (*Megatrypanum*) *palawanense* n. sp. (Protozoa: Trypanosomatidae) detected from *Rattus panglima* Robinson in Palawan Island, the Philippines. Tropical Medicine, 16 (3), 103–111.
17) Muul, I., Yap, L.F. & Lim, B. L. (1973): Ecological distribution of blood parasites in some arboreal

rodents. Southeast Asian J. Trop. Med. Pub. Hlth., 4(3), 377–381.

18) Nakabayashi, T., Tsukamoto, M., Motomura, I., Miyata, A., Tsuneda, K., Miyagi, I. & Dulay, I. S., Jr. (1973): Epidemiologic survey on malaria in some rural areas, especially in Palawan Island, of the Philippines. Tropical Medicine, 15(3), 154–168.

19) Nakabayashi, T., Tsukamoto, M., Miyata, A., Tsuneda, K., Yamaguchi, K., Miyagi, I., Dulay, I. S., Jr & Puriran, J. A. (1974): Chloroquine-resistant *Plasmodium falciparum* in the Iwahig Area of Palawan Island, the Philippines. Tropical Medicine, 16(1), 1–10.

20) Orihel, T. C. (1966): *Brugia tupaiae* sp. n. (Nematoda: Filarioidea) in tree shrews (*Tupaia glis*) from Malaysia. J. Parasitol., 52(1), 162–165.

21) Prowazek, S. von (1912): Beitrage zur Kenntnis der Protozoen und verwandter Organismen von Sumatra (Deli)-VII. Arch. Protist., 26, 250–74 (cited from Wenyon, 1926).

22) Ramachandran, C. P., Lim, B. L., Ramiah, N. & Stevens, S. (1970): Blood surveys of animals in West Malaysia for the presence of Wuchereria like infections. Southeast Asian J. Trop. Med. Pub. Hlth., 1(3), 423–424.

23) Schacher, J. F. (1973): Laboratory models in filariasis: A review of filarial life-cycle patterns. Southeast Asian J. Trop. Med. Pub. Hlth., 4(3), 336–349.

24) Singh, M. & Cheong, H.-C. (1971): On a collection of nematode parasites from Malayan rats. Southeast Asian J. Trop. Med. Pub. Hlth., 2(4), 516–522.

25) Singh, M., Ho, B.C. & Lim, B. L. (1972): Preliminary results on experimental transmission of a new filarial parasite, *Breinlia booliait* sp. n., Singh & Ho, 1972 from Malayan wild rats to laboratory albino rat. Southeast Asian J. Trop. Med. Pub. Hlth., 3(4), 622.

26) Singh, M. & Ho, B. C. (1973): *Breinlia booliati*, sp. n. (Filarioidea: Onchocercidae) a filaria of Malayan forest rat *Rattus sabanus* (Thos). J. Helminth., 47(2), 127–133.

27) Taylor, E. H. (1934): Philippine land mammals. Monograph 30. Bureau of Science. Dept. Agric. and Commerce, Manila.

28) Tokura, N. & Wu, C.-L. (1961): A malarial parasite, *Hepatocystis*, of the flying squirrel in Taiwan. Endem. Dis. Bull. Nagasaki Univ., 3(3), 174–179 (in Japanese with English summary).

29) Van Peenen, P. F. D., Hoogstraal, H., Duncan, J. F. & Ryan, P. F. (1968): Hematozoa from mammals of South Vietnam. J. Protozool., 15(3), 608–614.

30) Wenyon, C. M. (1926): Protozoology. A manual for medical men, veterinarians and zoologists. Reprint of Hafner Publishing Company in 1965, New York.

31) Wu. C.-L. (1953): Contributions to the study on the mammalian malaria in the Formosa (Taiwan). IGAKU KENKYU (Acta Medica), 23(10), 1979–1994 (in Japanese with English summary).

32) Yap, L. F., Muul, I. & Lim, B. L. (1970): A *Plasmodium* sp. from the spotted giant flying squirrel in West Malaysia. Southeast Asian J. Trop. Med. Pub. Hlth., 1(3), 418.

フィリピン・パラワン島産野生哺乳類より検出された住血寄生虫

宮田彬，塚本増久（長崎大学熱帯医学研究所疫学部門）

1970年から1972年にかけて，フィリピン・パラワン島でマラリアの研究に従事したが，その期間中に217頭の野生小哺乳類（但し霊長類を除く），および1頭のオオトカゲの血液標本を検査する機会を得た．*Plasmodium* 属のマラリアは発見できなかったが，次のような既知および未記載の住血性寄生虫を検出することができた．(1) *Trypanosoma lewisi* (Kent)：家屋内のネズミ計61頭中23頭から本種が発見された．(2) *Trypanosoma palawanense* Miyata：森林内のネズミ *Rattus panglima* 39頭のうち2頭から細長く大型の未記載のトリパノゾーマが発見され，これは新種として別の論文で記載した．

(3) *Trypanosoma* sp. : 46頭のリスのうち1頭から未記載のトリパノゾーマを発見した．体長約40ミクロンで，短い鞭毛（5ミクロン）を持つ．これも新種と考えられるが材料が不十分なので命名しなかった．(4) *Hepatocystis vassali* (Laveran) : 46頭のリスのうち4頭の血液から本種の生殖母体が検出された．これはマレイ産の亜種とは明らかに異なり，むしろ台湾産の亜種 *H. v. yokogawai* Wu に近いが，新亜種の可能性もある．組織内型が発見できなかったので，亜種の決定は保留した．(5)*Hepatocystis pteropi* (Breinl) : 検査した6頭のオオコウモリ類 *Pteropus* spp. 全部の血液標本から本種が検出された．(6) ヘモグレガリン：オオトカゲ1頭から未記載のヘモグレガリンが検出されたが，生活史が判明するまで種名の決定は保留した．(7) ミクロフィラリア：5頭の森林性のネズミ *Rattus mulleri balabagensis* のうち2頭から *Breinlia booliati* Singh and Ho と考えられるミクロフィラリアが検出された．このフィラリアは，最近マレイ半島およびサラワクで発見され，蚊によって媒介されることが判明しているので，実験室内におけるフィラリア症のモデルとして将来広く用いられる可能性がある．(8) ミクロフィラリア：46頭のリスのうち2頭からミクロフィラリアが検出された．これは形態学的に *B. breinlia* とは，明らかに異なるが，種名は確定できなかった．(9) ミクロフィラリア：種名不詳の有鞘ミクロフィラリアがオオトカゲの血液から検出された．(10) そのほか，1頭のリスの血液から形態学的にミクロフィラリアとは明らかに異なる線虫の1種を検出したが，その分類学的な位置については不明である．

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