

On the parasitic fauna of two species of anurans collected from Sungai Pinang, Penang Island, Malaysia

Wahab A. Rahman, Andy Tan and Intan Sufina

School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang

Email: arawahab@usm.my

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Abstract. A total of fifty anurans, comprising of *Rana limnocharis* and *Bufo melanostictus* were collected from Sungai Pinang, Balik Pulau, Penang. The prevalence, mean intensity and distribution of parasite species along the digestive tract were reported. Seven species of parasites were recorded. Blood parasites recovered were trypanosomes and microfilariae.

INTRODUCTION

A total of 195 species of amphibian faunas from the order Anura are found in Malaysia (Inger & Stuebing, 1997). Members of anurans are frogs and toads which are further divided into 6 families and 34 genera. The rich diversity of frog species in Malaysia is due to the warm and moist climate throughout the year.

There are limited studies of parasites in Malaysian anurans (Thanaletchumy, 1976; Miyata & Yong 1990). Elsewhere, Baylis (1998) noted the presence of various species of helminths being present in frogs of the British Isles. Blood parasites of frogs had also been reported in frogs of Canada (Fantham *et al.* 1942, John & Sherwin 1984) and Japan (Miyata *et al.* 1978), whilst parasites from various species of European and American frogs had extensively been investigated by many workers (Mapletone, 1969; Prudhoe & Bary, 1982; Vojtkova, 1990; Vojtkova & Roca 1993; Naciye & Ismail, 2000).

This paper describes the parasitic fauna from two species of anurans, *Rana limnocharis* and *Bufo melanostictus* from Penang Island.

MATERIALS AND METHODS

A total of 50 anurans were collected between November 2006 until February 2007 at various sites along the Sungai Pinang river and its tributaries, located at Barat Daya District, Balik Pulau, Penang (Fig. 1). Most of the specimens were obtained at the upstream section of the river because other parts of the river were polluted and generally avoided by anurans. Most of the animals were caught using bare hand because frogs were prone to be found in the gaps and crevices of rocks. Different species of anurans were kept in different cages because mixing them in the same cage may cause death in some individuals due to the toxic secretions produced by some frogs as a self-defend mechanism (Moore, 1964; Lofts, 1976). Species of frogs collected were identified according to the descriptions given by Inger & Stuebing (1997).

In the laboratory, animals were killed using chloroform. The heart was punctured with a needle and some fresh blood were smeared onto a glass slide, then covered with a cover slips and examined under the microscope. Thick and thin blood films were made on specimens which were positive for

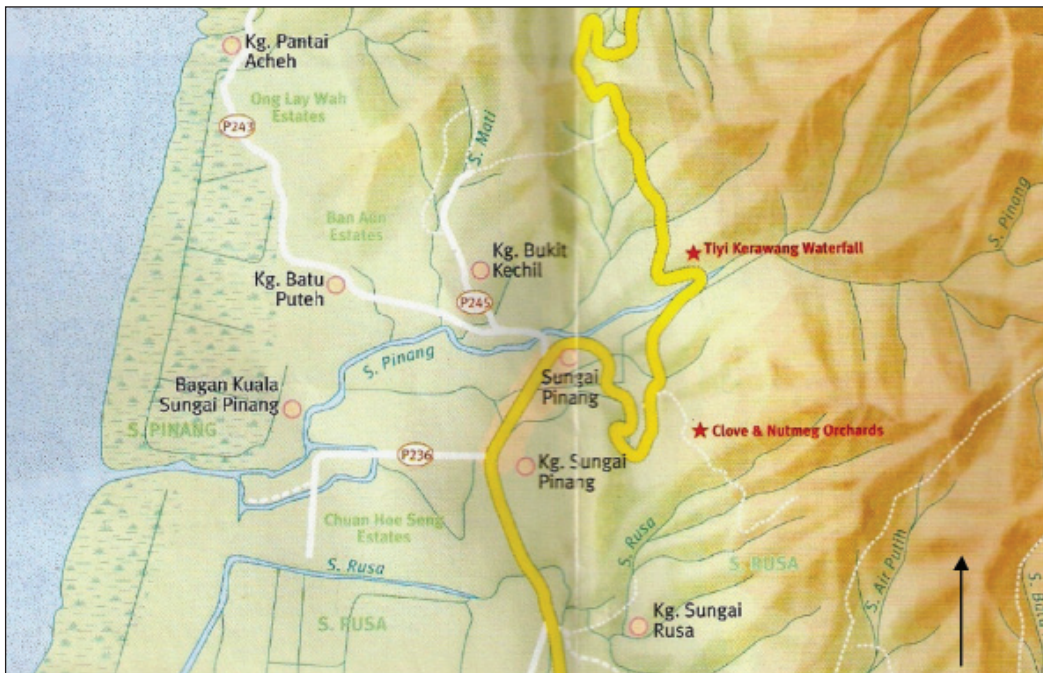


Figure 1. Sampling site along Sungai Pinang in the Balik Pulau District, Penang.

parasites. The blood slides were air-dried, fixed with methyl alcohol and stained with Giemsa.

The digestive tract was separated into the stomach, intestines and rectum and the organs were placed separately in individual petri dishes filled with water. Each organ was then cut open along its entire length and its linings scraped with a scalpel and the contents were examined for parasites. Parasites were identified according to the descriptions of Yamaguti (1963; 1971).

RESULTS

A total of 50 frogs were collected, of which 38 (76.6%) were the common sunda toads (*Bufo melanostictus*) and 12 (24.0%) were the grass frogs (*Rana limnocharis*). The percentage of prevalence and mean intensity within a 95% range of confidence interval for most of the identified parasites were showed in Tables 1 and 2 which represented the different parasite species found and grouped under their respective different hosts. However, parasite species of *Nyctotherus*

cordiformis and *Opalina ranarum* were found in large number and the percentages of prevalence and mean intensities were not calculated.

There were 7 different species of parasites recovered from *Rana limnocharis* (Table 1). The parasite species were *Oxysomatium* sp., microfilarial worms, *Rhabdias* sp., *Trypanosoma* sp., *Mesocoelium burti*, *Nyctotherus cordiformis* and *Opalina ranarum*. *Oxysomatium* sp. was the most abundant parasite, infecting the *Rana limnocharis*. 41.67% of the frogs were infected by this parasite species and approximately 5 to 11 of this species of nematode was recovered from one individual frog. This was followed by the microfilarial worms (41.67%), which showed the same prevalence with the nematode, *Oxysomatium* sp. There were about 4 to 8 microfilariae found in one animal. Next was *Rhabdias* sp. with a prevalence of 33.33% and a mean intensity of 3.50 ± 2.08 . *Trypanosoma* sp. and *Mesocoelium burti* also shared the same percentage of prevalence. However, the trematode, *Mesocoelium burti* showed a higher mean intensity (15.00 ± 2.83) when

Table 1. Prevalence and mean intensity \pm standard deviation of parasites found from *Rana limnocharis* (n = 12)

Parasites	Prevalence (%)	Mean intensity \pm Standard deviation	Range of Confidence interval 95%
microfilarial worms	41.67	6.60 \pm 1.95	4.89 – 8.31
<i>Trypanosoma</i> sp.	25.00	4.33 \pm 2.08	1.98 – 6.68
<i>Oxysomatium</i> sp.	41.67	8.00 \pm 2.65	0.99 – 15.01
<i>Rhabdias</i> sp.	33.33	3.50 \pm 2.08	1.46 – 5.54
<i>Mesocoelium burti</i>	25.00	15.00 \pm 2.83	11.80 – 18.20

Table 2. Prevalence and mean intensity \pm standard deviation of parasites found from *Bufo melanostictus* (n = 38)

Parasites	Prevalence (%)	Mean intensity \pm Standard deviation	Range of Confidence interval 95%
Microfilarial worms	31.58	4.75 \pm 1.14	3.62 – 5.89
<i>Trypanosoma</i> sp.	0	–	–
<i>Oxysomatium</i> sp.	71.05	4.44 \pm 1.83	3.75 – 5.13
<i>Rhabdias</i> sp.	23.68	2.44 \pm 1.01	1.78 – 3.10
<i>Mesocoelium burti</i>	47.37	12.22 \pm 3.08	10.49 – 13.95

compared to that of *Trypanosoma* sp. (4.33 \pm 2.08).

In *B. melanostictus*, the prevalence of the nematode, *Oxysomatium* sp. was the highest, which is 71.05% with a mean intensity of 4.44 \pm 1.83 (Table 2). This indicated that among all the examined 38 toads (*B. melanostictus*), 71.05% of the toads were found to be parasitised by this nematode species and it was estimated that 2 to 6 *Oxysomatium* sp. being present in an individual toad. The second highest prevalence was recorded in *Mesocoelium burti*. 47.37% of the toads studied showed the presence of this trematode with approximately 9 to 15 trematodes being present in one particular toad. Next, were the microfilarial worms (31.58%), of which about 3 to 6 microfilariae were found in the blood of an individual toad. Finally, the

lowest percentage of prevalence was observed in the *Rhabdias* sp. nematodes. There are only 1 to 3 *Rhabdias* sp. with a mean of 2.44 \pm 1.01 found in a toad. There was absence of *Trypanosoma* sp. in all toads. The other parasites recovered were, *Nyctotherus cordiformis* and *Opalina ranarum*.

The number of parasites found in the various organs of *R. limnocharis* are shown in Table 3. A total of 149 parasites were collected from 12 *R. limnocharis* (Table 3). 30.9% of the parasites resided in the blood, which comprised of *Trypanosoma* sp. and microfilarial worms. However, as regards to blood parasite, *Trypanosoma* sp. was more dominant than microfilariae. Subsequently, the stomach and the intestines were the most infected organs (37.6%), parasitized by *Oxysomatium* sp., *Rhabdias* sp., *M. burti*,

Table 3. Number of parasite found in various organs of *Rana limnocharis* (n = 12)

Parasites	Number of parasites found in various organs			Total
	Blood	Intestine + Stomach	Rectum	
A	33	0	0	33
B	13	0	0	13
C	0	17	23	40
D	0	5	9	14
E	0	34	15	49
F	0	√	√	–
G	0	√	√	–

A microfilarial worms

C *Oxysomatium* sp.

E *Mesocoelium burti*

G *Opalina ranarum*

B *Trypanosoma* sp.

D *Rhabdias* sp.

F *Nyctotherus cordiformis*

√ Present (but not counted because of their presence in large numbers)

Table 4. Number of parasite found in various organs of *Bufo melanostictus* (n = 38)

Parasites	Number of parasites found in various organs			Total
	Blood	Intestine + Stomach	Rectum	
A	57	0	0	57
B	0	0	0	0
C	0	47	73	120
D	0	15	7	22
E	0	157	63	220
F	0	√	√	–
G	0	√	√	–

A microfilarial worms

C *Oxysomatium* sp.

E *Mesocoelium burti*

G *Opalina ranarum*

B *Trypanosoma* sp.

D *Rhabdias* sp.

F *Nyctotherus cordiformis*

√ Present (but not counted because of their presence in large numbers)

N. cordiformis, and *O. ranarum*. The trematode, *M. burti* was the main parasite recorded in stomach and intestine. The nematode, *Oxysomatium* sp. was the most frequent parasites found in the rectum.

A total of 419 parasites were recovered from 38 *Bufo melanostictus* (Table 4). The stomach and intestines were the most

infected organs (52.3%). With the exception of *Trypanosoma* sp. and microfilaria worms, all the parasites recorded in the present study were found in these organs. However, the dominant parasite species was the trematode, *M. burti*. The rectum showed 34.1% of the total parasites recovered. The parasite species in the rectum were similar

to those found in the stomach and rectum. However, the nematode, *Oxysomatium* sp. were the most frequently found. Microfilariae worms comprised only 13.6% of the total parasites were recorded.

DISCUSSION

There were 3 species of protozoa (*Trypanosoma* sp., *N. cordiformis*, and *O. ranarum*), 3 species of nematodes (*Oxysomatium* sp., *Rhabdias* sp., microfilaria worms) and 1 species of trematode (*M. burti*) recovered from the frogs and toads in Penang Island.

Over 60 species of trypanosomes had been reported in anurans worldwide, but the taxonomy has not been fully described (Miyata *et al.*, 1992). In fact, trypanosomes had previously been recovered from different species of frogs (Fantham *et al.*, 1942; Miyata *et al.*, 1978; Martin *et al.* 1992). In the present research, *Trypanosoma* spp. were only found in *R. limnocharis* with a prevalence of 25.0% but totally absent in *B. melanostictus*. In the present study, *R. limnocharis* was generally recovered in the wetter habitats as compared to *B. melanostictus* which preferred the terrestrial habitats of Penang Island. The former showed a much higher prevalence of infection with *Trypanosoma*, which is suggestive of an aquatic vector. However, most trypanosome infections in frogs are non-pathogenic (Miyata *et al.* 1978).

Opalina ranarum and *N. cordiformis* are protozoan parasites commonly found in frogs and toads. They are usually can be found along the digestive tract but the rectum (Baylis, 1951) and intestine (Noble, 1931; Reichenbach-Klinke & Elken, 1965) are their favourite spots and are believed to have a commensalism relationship with its host and do not pose any threat to the host even though they are found in high abundance (Reichenbach-Klinke & Elken, 1965). Nyctotherean species is a common parasite in anurans. In the Cameroon, 45 species were found in 41 anuran species (Naciye 2000). In studies carried out in many countries in Europe, it was reported that at least three

species of *Nyctotherus* existed in different anuran amphibians (Vojtkova, 1990; 1993).

Filarial worms had also been reported in frogs elsewhere, when Wayne (1969) also reported an unidentified filarial worm in New jersey bullfrogs (*R. catesbeiana*). Microfilariae are capable of completing development to the infective stage in amphibian feeding mosquitoes (Crans 1964).

A total of 160 *Oxysomatium* sp. were recovered. The prevalence of the infected animals were high (41.67% for *R. limnocharis* and 71.05% for *B. melanostictus*). In both animals the parasite was distributed throughout the stomach, intestine and rectum although Thanalechumy (1967) noted that the parasite was more confined to the rectum than elsewhere. *Rhabditis* sp. is a common nematode in vertebrates, including frogs as concurred by the findings of Moravec and & Kaiser (1995).

The trematode, *M. burti* dominated the intestine over the other parasites. The trematode was found throughout the digestive tract but are most abundant in the intestine, thus bringing significant effects on other parasites. As the result of this dominance by *M. burti* in the intestine of the hosts, other parasite species tend to adapt themselves by shifting to other regions of the digestive tract (Prudhoe & Bray 1982).

In conclusion, the frogs examined were parasitized mainly by protozoans and nematodes followed by trematodes. However, no cestodes were recovered from the samples obtained. With regards to blood parasites, only trypanosomes had been recovered from the anuran population.

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