

SEX, ORGANAL AND SEASONAL DIFFERENCES OF HELMINTHOFAUNA OF TOAD, *BUFO MELANOSTICTUS* (SCHNEIDER, 1799)

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Abstract: A total of 75 toads (39 males and 36 females), *Bufo melanostictus*, collected from different areas of Dhaka city from May 2009 to April 2010 was examined for helminth parasites. The results showed that seven parasite species were collected, and prevalence of infection was 100%. The parasite fauna was comprised of Trematoda: 8% was the prevalence of *Ganeo kumaonensis*, 8% of *Halipegus eccentricus*; Nematoda: 100% of *Oswaldocruzia indica*, 68% of *Neopharyngodon* sp., 40% of *Rhabdias bufonis*; Acanthocephala: 28% of *Pseudoacanthocephalus bufonis*; and Pentastomida: 4% of *Raillietiella indica*. Serious damage was observed in intestine (53.94%) then rectum (24.55%), stomach (12.73%) and lungs (8.78%). The males had a higher intensity (77.92) than the females (44.58). Seasonally, the highest intensity of infection was found in the rainy season (81.21) and lowest during the winter (39.87).

Key words: Helminth parasites, toad, prevalence, intensity, season.

INTRODUCTION

Amphibian parasitism has been used as a model for understanding very important issues pertaining to the evolution of parasites and their hosts, life cycles, host-parasite relationships, etc. (Nworah and Olorunfemi 2011). Caudated amphibians are the final hosts for a large number of parasites. These are the source of infection of many wild and domestic animals (birds and mammals, for example) because they are osculant hosts for the larval stages of development of many nematode species (Jamdar *et al.* 2010).

Amphibians particularly anurans have a rich parasite fauna (Prudhoe and Bray 1982) perhaps due to the typical life style of amphibians favouring vulnerability to microbial and parasitic infection. The damp habitats preferred by amphibians favour the survival of infective stages of parasites. Moreover, most amphibians are associated with both aquatic and terrestrial habitats and hence with two different range of parasitic species. According to Cheng (1964) toad and frogs are infected by a considerable range of parasites which may occur in large numbers. He also reported that the normal growth of toad was inhibited if they had been heavily infected with endoparasites.

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Frogs and toads are abundant in Bangladesh for the obvious warm and moist climate. The potential factors determining the transmission of parasites include environmental conditions that affect the viability and behaviour of parasite propagules, and feeding, movement, and defecation patterns of the host, which determine the parasites encountered.

There are few studies on the parasites of Bangladeshi anurans (Begum *et al.* 1996, Ahmed and Begum 2006a,b). Elsewhere, Jamdar *et al.* (2010) investigated the parasitic fauna of two species of anurans from India; Chandra and Gupta (2007) described the habitat preference and seasonal fluctuations in the helminthofauna of amphibian in India. Wahab *et al.* (2008) reported the parasitic fauna of two species of anurans from Malaysia. Baylis (1998) noted the presence of various species of helminths being present from the frogs of the British Isles. The blood parasites of frogs were also reported from the frogs of Canada (John and Sherwin 1984) and Japan (Miyata *et al.* 1978), whilst parasites from various species of European and American frogs were extensively investigated by many workers, namely Prudhoe and Bray (1982), Vojtkova and Roca (1993), Naciye and Ismail (2000).

In this study, we examine the effect of sex, organal and seasonal differences on the intestinal parasite loads of *Bufo melanostictus*.

MATERIAL AND METHODS

Bufo melanostictus, the common toad, was collected from its natural habitat-damp, moist paddy fields and gardens in different areas of Dhaka city. A total of 75 toads (39 males and 36 females) was randomly collected from the areas under study over one year period from May 2009 to April 2010.

Parasitological examinations: The hosts were brought from wild conditions and maintained in the laboratory under normal conditions of food, water and aeration. The weight and sex of the collected specimens were recorded. The hosts were pitched and dissected, and the body cavity, peritoneum, muscles, lungs, stomach, intestine, rectum, urinary bladder and liver of the hosts were examined for helminth infection. The parasites were taken out in separate petri dishes, containing (0.9%) NaCl solution, and live helminths were washed 2-3 times in normal saline. They were further processed and permanent slides were made according to Gibson (1984). The parasites were identified according to the description of Yamaguti (1958 1961). Prevalence defined, as the percentage of infected hosts per examined host, and intensity, as the number of worms per infected host (Margolis *et al.* 1982), were calculated accordingly.

RESULTS AND DISCUSSION

Of the 75 toads examined, seven parasite species were collected, and prevalence of infection was 100%. The helminth parasite comprised Trematoda: 8% was the prevalence of *Ganeo kumaonensis*, 8% of *Halipegus eccentricus*; Nematoda: 100% of *Oswaldocruzia indica*, 68% of *Neopharyngodon* sp. 40% of *Rhabdias bufonis*; Acanthocephala: 28% of *Pseudoacanthocephalus bufonis* and Pentastomida: 4% of *Raillietiella indica* (Table 1).

Table 1. Infectivity of helminth parasites in *Bufo melanostictus* (n = 75).

Name of parasites	No. of host infected	Prevalence (%)	Total no. of parasites collected	Intensity \pm SE
Trematoda				
<i>Ganeo kumaonensis</i>	6	8.00	21	3.50 \pm 0.037
<i>Halipegus eccentricus</i>	6	8.00	24	4.00 \pm 0.056
Nematoda				
<i>Oswaldocruzia indica</i>	75	100.0	2796	37.28 \pm 1.051
<i>Neopharyngodon</i> sp.	51	68.00	1239	24.29 \pm 0.715
<i>Rhabdias bufonis</i>	30	40.00	402	13.40 \pm 0.451
Acanthocephala				
<i>Pseudoacanthocephalus bufonis</i>	21	28.00	156	7.43 \pm 0.107
Pentastomida				
<i>Raillietiella indica</i>	3	4.00	6	2.00 \pm 0.030

n = No. of host examined.

The number of parasites found in the various organs of *B. melanostictus* is shown in Table 2. A total of 4644 parasites was collected from 75 *B. melanostictus*. 53.94% of the parasites resided in the intestine, which comprised *G. kumaonensis*, *H. eccentricus*, *O. indica*, *Neopharyngodon* sp. and *P. bufonis*. However, as regards to intestinal parasite, *G. kumaonensis* and *H. eccentricus*

Table 2. Organwise distribution of helminth parasites in *B. melanostictus*.

Name of parasites	Lung	Stomach	Intestine	Rectum	Total no. of parasite
<i>Ganeo kumaonensis</i>	-	-	21 (100%)	-	21
<i>Halipegus eccentricus</i>	-	-	24 (100%)	-	24
<i>Oswaldocruzia indica</i>	-	570 (20.39%)	1470 (52.57%)	756 (27.04%)	2796
<i>Neopharyngodon</i> sp.	-	-	882 (71.19%)	357 (28.81%)	1239
<i>Rhabdias bufonis</i>	402 (100%)	-	-	-	402
<i>Pseudoacanthocephalus bufonis</i>	-	21 (13.46%)	108 (69.23%)	27 (17.31)	156
<i>Raillietiella indica</i>	6 (100%)	-	-	-	6
Total no. of parasites	408	591	2505	1140	4644
(% of parasites in different organ)	(8.78)	(12.73)	(53.94)	(24.55)	

were more dominant than others. Subsequently, the rectum (24.55%), stomach (12.73%) and the lungs (8.78%) were also infected organs. The nematode, *O. indica* was the main parasite recorded in stomach. The nematode, *Neopharyngodon* sp. was the most frequent parasites found in the rectum. *Rhabdias bufonis* and *R. indica* were nematode and pentastomida parasites commonly found in the lungs of toads.

Out of 75 toads, 39 were males and 36 were females. The overall prevalence of infestation was 100% in both sexes and the intensity of males was higher (77.92) than the females (44.58). *Oswaldocruzia indica* showed 100% prevalence and highest intensity both in male and female toads. Lowest prevalence and intensity were observed in *R. indica* also in male and female hosts (Table 3).

Table 3. Prevalence of endoparasites according to sex in *B. melanostictus*.

Name of parasites	No. of toads infested		Prevalence (%)		Total no. of endoparasites recovered		Mean intensity \pm SE	
	M	F	M	F	M	F	M	F
<i>Ganeo kumaonensis</i>	3	2	7.69	5.56	15	6	5.00 \pm 0.070	3.00 \pm 0.056
<i>Halipegus eccentricus</i>	3	2	7.69	5.56	17	7	5.67 \pm 0.082	3.50 \pm 0.037
<i>Oswaldocruzia indica</i>	39	36	100.0	100	1865	931	47.82 \pm 2.233	25.86 \pm 1.754
<i>Neopharyngodon</i> sp.	30	21	76.92	58.33	737	502	24.57 \pm 1.375	23.90 \pm 1.317
<i>Rhabdias bufonis</i>	18	12	46.15	33.33	295	107	16.39 \pm 1.117	8.92 \pm 0.117
<i>Pseudoacanthocephalus bufonis</i>	15	6	38.46	16.67	106	50	7.07 \pm 0.246	8.33 \pm 0.184
<i>Raillietiella indica</i>	2	1	5.13	2.78	4	2	2.00 \pm 0.205	2.00 \pm 0.205

No. of female specimens = 36; No. of male specimens = 39.

Cent percent prevalence was recorded for *O. indica* in rainy season and summer clubbed with the highest intensity 47.85 and 30.12, respectively. Minimum infectivity of *O. indica* occurred in the winter when the prevalence declined to 60% (Table 4). *Neopharyngodon* sp. also followed a similar pattern and showed higher infectivity in rainy season, its prevalence was 58.82% and intensity was 38. On the other hand, *Neopharyngodon* sp. showed a drastic fall in infectivity in winter, the prevalence and intensity fell down to 46.67% and 20.14, respectively (Table 4). *Rhabdias bufonis* showed maximum (41.18%) infectivity in the rainy season and minimum (34.61%) in the summer (Table 4). *Raillietiella indica* showed the lowest prevalence and intensity compare to other parasites. In the rainy season, prevalence and intensity were 5.88% and 2.0, respectively, whereas this parasite was totally absent in the winter.

Table 4. Seasonal infestation of helminth parasites in *B. melanostictus*.

Name of parasites	Rainy (June to October) n=34				Winter (November to February) n=15				Summer (March to May) n=26			
	No. of host infec- ted	Preva- lence (%)	Total no. of parasites collected	Mean Intensity ± SE	No. of host infec- ted	Preva- lence (%)	Total no. of parasites collected	Mean Intensity ± SE	No. of host infec- ted	Preva- lence (%)	Total no. of para- sites collected	Mean Intensity ± SE
<i>Ganeo kumaonensis</i>	4	11.76	13	3.250±0.42	1	6.67	1	1.00±0.186	3	11.54	7	2.33±0.196
<i>Halipegus eccentricus</i>	5	14.71	15	3.00±0.043	1	6.67	1	1.00±0.186	3	11.54	8	2.67±0.277
<i>Oswaldocruzia indica</i>	34	100	1627	47.85±2.921	9	60	386	42.89±2.151	26	100	783	30.12±0.529
<i>Neopharyngodon</i> sp.	20	58.82	760	38.00±1.219	7	46.67	141	20.14±1.529	14	53.85	338	24.14±0.574
<i>Rhabdias bufonis</i>	14	41.18	248	17.71±0.129	6	40	53	8.83±0.329	9	34.61	101	11.22±0.365
<i>Pseudoacanthocephalus bufonis</i>	8	23.53	94	11.75±0.107	3	20	16	5.33±0.060	5	19.23	46	9.20±0.329
<i>Raillietiella indica</i>	2	5.88	4	2.00±0.246	0	0	0	0.00±0.00	1	3.85	2	2.00±0.246

n = No. of toad examined.

In the present investigation, the prevalence of infection was 100%, whereas, Ahmed and Begum (2006a) reported five species of helminth from 100 toads, they also found 100% prevalence. They did not find *H. eccentricus*, *R. bufonis*, *P. bufonis* and *R. indica*, but they described about *Pleurogenoides petropedatis* and *Oxysomatium srinagarensis*. They showed highest prevalence (76%) in *Neopharyngodon* sp. and intensity (5.32). Jamdar *et al.* (2010) recovered two nematode *Oswaldocruzia* sp. and *Ascaridiasp* sp., and one cestode *Proteocephalus* sp. from 37 *B. melanostictus*. Goldberge and Bursery (1991) reported the helminth parasites of three toads, *Bufo alvarias*, *B. cognatus*, and *Scaphiopus couchii* from Southern Arizona. They reported two species of cestoda and six species of nematode parasites. In the present study, the abundance of trematode parasites was very low. Prudhoe and Bray (1982) reported that most trematodes of amphibians use aquatic or semiaquatic arthropods as intermediate hosts; these observations may indicate low species richness and prevalence of adult trematodes (Bursery and Goldberg 1998, Bolek and Coggins 2000). *Raillietiella indica* represents a new parasite record for this host because this parasite has only been reported previously in India, Burma, and Taiwan and Hawaii (Barton and Riley 2004).

The parasites are usually found along the digestive tract, but the rectum and intestine (Noble 1931, Reichenbach-Klinke and Elken 1965) are their favourite spots and are believed to have a commensalistic relationship with its host and do not pose any threat to the host even though they are found in high abundance (Reichenbach-Klinke and Elken 1965).

The trematode, *G. kumaonensis* and *H. eccentricus* dominated the intestine over the other parasites. Prudhoe and Bray (1982) reported the result of dominance shown by trematode in the intestine of the hosts; other parasite species tend to adapt themselves by shifting to other regions of the digestive tract. In the present study, *O. indica* and *P. bufonis* were the most frequently found nematodes in the stomach, intestine and rectum. In anurans, the parasite was distributed throughout the stomach, intestine and rectum (Wahab *et al.* 2008) although Thanalechumy (1967) noted that the parasite was more confined to the rectum than elsewhere. *Rhabdias bufonis* was found only in the lungs of the toads. Similar observation is also available from Sarkar and Manna (2004), in *R. bufonis* infection in the lungs of *B. melanostictus* which causes severe damage in the lung capillary network. *Raillietiella indica* was also recovered from the lungs of *B. melanostictus*. Similarly, Barton and Riley (2004) described the occurrence of *R. indica* from the lungs of *B. regularis*.

Ahmed and Begum (2006b), Begum *et al.* (1996), Dwivedi (1975), Hollis (1972), Lees (1962) and Mazurmovich (1951) reported that the level of

parasitization of male hosts was comparatively higher than the female hosts. Hollis (1972) reported that the male frogs were more frequently parasitized than the female frogs. According to Kennedy and Lie (1974), the female hormone estrogen might inhibit the infestation by parasites. Cheng (1964) commented that the male hormones favored the growth and survival of parasites. Most of the earlier workers held that the female sex hormones might be responsible for depressing the level of parasitization. The present investigation supports the reports of Kennedy and Lie (1974), and Cheng (1964).

The parasites of amphibians are still more affected due to seasonal and climatic factors due to the reason that firstly, they are cold-blooded and secondly, they are amphibious in nature, and thus the climatic/ seasonal changes directly affect the parasite population and the amphibious mode of life provides a wider exposure to both terrestrial and aquatic conditions, thus favouring parasitism. Our investigations have indicated that *B. melanostictus* is affected by parasite invasion to the maximum during the rainy season. *Oswaldocruzia indica* occurred at the highest prevalence (100%) and intensity (49.30) in the rainy season. Parasitic infectivity of the rainy season was followed by the summer and was lowest during the winter season.

During winter, the toads hibernate and there is a reduction in metabolism resulting in hypobiosis. The influence of hibernation can be more properly considered as being the sum of the influence of several factors involving cessation of feeding. The drop in temperature retards reproduction and may lead to a reduction in the number of parasites. The sum total of the influence of hibernation may lead to its effect on parasite infectivity. The low infectivity during winter in the present work finds support from those of earlier workers when Chandra and Gupta (2007) reported seasonal fluctuations in the helminthofauna of amphibian hosts. Markov and Rogoza (1953) observed the same phenomenon in frogs from the Leningrad area and by Lees (1962) who observed a fall in helminth infestation during December and January. Plasota (1969) stressed the influence of rainfall and temperature on parasite infectivity and added that the hosts living conditions may affect the penetration of parasites like *Oswaldocruzia*. Baker (1979) observed the seasonal population changes in *Rhabdias ranae* parasitizing *Rana sylvatica* and recorded lowest infection in summer and highest in spring and early fall in winter.

The above studies on parasitic infectivity in relation to seasonal variations indicate that various authors from all over the world have recorded variance in the infectivity of the amphibian parasite fauna. As observed from the above findings, maximum infection was recorded during the rainy season when the amphibian population was abundant; the climate was moist and humid. These

conditions favoured parasite occurrence and multiplication where not only the prevalence of the parasites was higher, but also the intensity reached their peak values. On the other hand, the infectivity was lowest during the winter season when the toads were hibernating and their metabolic activities were the lowest. As parasites are characterized by metabolic dependence on their hosts, the conditions during winter did not promote parasite occurrence perhaps because they could not metabolically depend on their host. During winter, the frogs hibernate and their feeding is minimum. It has also been suggested that from the end of November till the commencement of the breeding season, the blood sugar levels of frogs are low (Smith 1950). Moreover, the lack of food especially for intestinal parasites during this period may also account for their low sustenance as has also been opined by Mazurmovich (1951) that the lack of adequate food for helminths during hibernation may be a sound reason for their decline in number. This low value of infection started to increase during the spring when the hosts regained their metabolic activities and resumed feeding by the end of winter with consequent opportunities of acquiring new infections. This spring stimulation of parasites was also recorded by Markov and Rogoza (1955). The parasite infection increased further during the summer when the toads were quite active and metabolically sound. During the rainy season, the hosts were most abundant and the higher metabolic activity of the host coupled with suitable climatic and humid conditions promoted parasitic existence which may account for the highest infectivity during the rainy season.

Toads (*B. melanostictus*) were found to harbour more helminth than previously reported from Bangladesh. During the present study some new parasites are recorded while some previously reported helminthes were not found. No cestodes were recovered from the samples obtained. Maximum infection was recorded during the rainy season when the amphibian population was abundant.

LETTERATURE CITED

- AHMED, A.T.A. and BEGUM, A. 2006a. A survey of helminth parasites of toad (*Bufo melanostictus* Schneider, 1799), bull frog (*Hoplobatrachus tigerinus* Daudin, 1802) and skipper frog (*Euphlyctis cyanophlyctis* Schneider, 1799) from Dhaka and Feni districts. *Bangladesh J. Zool.* **34**(1): 79-86.
- AHMED, A.T.A. and BEGUM, A. 2006b. A distribution of helminth parasites in toads and frogs in Bangladesh. *Dhaka Univ. J. Biol. Sci.* **15**(1): 77-84.
- BAKER, M.R. 1979. Seasonal population changes in *Rhabdias ranae* Walton, 1929 (Nematoda, Rhabdiasidae) in *Rana sylvatica* of Ontario. *Can. J. Zool.* **57**: 179-183.
- BARTON, D.P. and RILEY, J. 2004. *Raillietiella indica* (Pentastomida) from the lungs of the giant toad, *Bufo marinus* (Amphibia), in Hawaii, U.S.A. *Comp. Parasitol.* **71**(2): 251-254.
- BAYLIS, H.A. 1998. The parasitic worms of British reptiles and amphibia. In: *The British Amphibians and Reptiles* by Smith, M. (edited). Collons, London. 322 pp.

- BEGUM, A., AHMED, A.T.A. and ZAMAM, Z. 1996. Incidence of helminth parasites in bullfrog *Rana tigrina* (Daudin). *Dhaka Univ. J. Biol. Sci.* **5**(2): 113-120.
- BOLEK, G.M. and COGGINS, J.R. 2000. Seasonal occurrence and community structure of helminth parasites from the Eastern American Toad, *Bufo americanus americanus*, from Southern Wisconsin, U.S.A. *Comp. Parasitol.* **67**(2): 202-209.
- BURSEY, C.R. and GOLDBERG, S.R. 1998. Helminths of the Canadian toad, *Bufo hemiophrys* (Amphibia: Anura), from Alberta, Canada. *J. Parasitol.* **84**: 617-618.
- CHANDRA, P. and GUPTA, N. 2007. Habitat Preference and Seasonal Fluctuations in the Helminthofauna of Amphibian Hosts of Rohilkhand Zone, India. *Asian J. Exp. Sci.* **21**(1): 69-78.
- CHENG, T.C. 1964. *General parasitology*. W.B. Saunders Comp. Philadelphia and London. 827 pp.
- DWIVEDI, M.P. 1975. Incidence of trematodes in a particular species of frog population. *Ind. J. Helminth.* **27**: 25-32.
- GIBSON, D.I. 1984. Technology as applied to museum collection: the collection, fixation and conservation of helminthes. *Syst. Parasitol.* **6**: 241.
- GOLDBERGE, S.R., and BURSEY, C.R. 1991. Helminths of three toads, *Bufo alvarias*, *Bufo cognatus* (Bufonidae), and *Scaphiopus couchii* (Pelobatidae), from Southern Arizona. *J. Helminth. Soc. Washington* **58**: 142-146.
- HOLLIS, P. D. 1972. Host sex influence on the seasonal incidence of *Haematolocehus medioplexus* (Trematoda: Plagiorchiidae) in *Rana pipiens*. *J. Parasitol.* **58**: 128.
- JAMDAR, S., HIWARE, C.J., PARDESHI, P. and DHONDGE, T. 2010. On the parasitic fauna of two species of anurans collected from MIDC Area of Aurangabad (Maharashtra) India. *Asian J. Exp. Biol. Sci.* **1**(3): 638-641.
- JOHN, R.B. and SHERWIN, S.D. 1984. Blood parasites of amphibians from Algonquin Park, Ontario. *J. Wild. Dis.* **20**(3): 180-189.
- KENNEDY, C.R. and LIE, S.F. 1974. The distribution and pathogenicity of strongylids (nematode) in brown trout *Salmo trutta* (L) in Feraworthy Reservoir. *Devon. J. Fish. Scot.* **8**: 293-302.
- LEES, E. 1962. The incidence of helminth parasites in a particular frog population. *Parasitol.* **52**: 95-102.
- MARGOLIS, L.G.W., ESCH, J.C., HOLMES, A.M. and SCHAD, G.A. 1982. The use of ecological terms in parasitology. *J. Parasitol.* **68**: 131-133.
- MARKOV, G.S. and ROGOZA, M.L. 1953. Seasonal and microzonal differences in parasitic fauna of grass frogs. *C. R. Acad. Sci. and U.R.S.S.* **91**(1): 169-172.
- MARKOV, G. S. and ROGOZA, M.L. 1955. Annual differences in the parasitic fauna of grass frogs (*Rana temporaria* L.). *Zool. Zh.* **34**: 1203- 1209.
- MAZURMOVICH, B. N. 1951. *Parasitic worms of amphibia. Their relationships to hosts and environment*. - Kiev: Kiev Univ. Publ., 97 p. (in Russian)
- MIYATA, A., MIYAH, I. and TSUKAMOTO, M. 1978. Haemoprotozoa detected from the cold blooded animals in Ryukyu Islands. *Trop. Med.* **20**: 97-112.
- NACIYE, G.S. and ISMAIL, Y. 2000. The ciliate fauna in the digestive system of *Rana ridibunda* (Amphibia: Anura)-II *Nyctotherus* (Nyctotheridae: Heterotrichida). *Turk. J. Zoo.* **24**: 245-252.
- NOBLE, G.K. 1931. *The Biology of the Amphibia*. Dover Publications Inc. New York, pp. 433-441.
- NWORAH D.C. and OLORUNFEMI O.J. 2011. The helminth parasitofauna of *Bufo regularis* (Reuss) in Awka, Anambra State, Nigeria. *Int. J. Parasit. Res.* **3**(2): 26-30.
- PLASOTA, K. 1969. The effect of some ecological factors on the parasitofauna of frogs. *Acta Parasitologica Polonica* **14**(6): 47-60.
- PRUDHOE, S. and BRAY, R.A. 1982. *Platyhelminth parasites of the Amphibia*. Oxford University Press, 217 pp.

- REICHENBACH-KLINKE, H. and ELKEN, E. 1965. *The Principle of Lower Vertebrates. Diseases of Amphibians*. Academic Press, London, 381 pp.
- SARKAR, M.D. and MANNA, B. 2004. On a New Species of the Genus *Rhabdias* Stiles and Hassall, 1905 (Nematoda: Rhabditida) from *Bufo melanostictus* Schneider, 1799 from Belur and Habra, West Bengal, India, With a Host-Parasite List. *Philippine J. Sci.* **133**(1): 55-69.
- SMITH, C. L. 1950. Seasonal changes in blood sugar, fat body, liver glycogen and gonads in the common frog, *Rana temporaria*. *J. Exp. Biol.* **26**: 412-429.
- THANALETCHUMY, R. 1967. *Some helminthes from five species of Malayan frogs*. Latihan Ilmiah, University Malaya, 44 pp.
- VOJTKOVA, L. and ROCA, V. 1993. Parasites of frogs and toads in Europe. Part 1: Protozoa. *Review Espanola Herpetologica* **7**: 37-45.
- WAHAB, A. R., TAN, T. and SUFINA, I. 2008. On the parasitic fauna of two species of anurans collected from Sungai Pinang, Penang Island, Malaysia. *Tropical Biomedicine* **25**(2): 160-165.
- YAMAGUTI, S. 1958. *Systema Helminthum*. Vol I. The Digenetic Trematodes of Vertebrates. Interscience Publishers Inc. New York, USA. pp. 28-225.
- YAMAGUTI, S. 1961. *Systema Helminthum*. Vol III The Nematodes of Vertebrates. Interscience Publishers Inc. New York, USA. pp. 22-108.

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