A SURVEY OF HELMINTH INFECTIONS IN GRASS FROGS, *Fejervarya limnocharis* (GRAVENHORST, 1829) FROM CHIANG MAI PROVINCE

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Abstract: A total of 38 grass frogs (*Fejervarya limnocharis*) were collected from paddy field of Chiangmai province. Helminth parasite infection was investigated. It was revealed that 36 out of 38 grass frogs were infected with parasites. The prevalence and intensity were calculated. The result showed that, the total prevalence of infection was 94.74 % (36/38). Six species of adult worms and 3 larval stages were recorded and identified as follow: 3 cestodes, *Baerietta* sp., *Senga chiangmaiensis* and sparganum (plerocercoid), 3 nematodes, *Oswaldocruzia* sp., *Neyraplectana* sp. and encysted unknown larvae, 2 acanthocephala, *Acanthocephalus lucidus* and cystacanth and 1 trematode, *Ganeo tigrinus*. The prevalence of infection for each parasite was 5.26 %, 2.63 %, 2.63 %, 34.21%, 47.37 %, 2.63 %, 52.63 %, 42.10 % and 28.94%, respectively, whereas the intensity per host was 3, 1, 1, 5.8, 6.3, 1, 5.7, 8.3, and 5.9 respectively. From these, all adult worms were recovered from the intestines; the larval stages, including sparganum, encysted larvae and cystacanth, were recovered from leg muscles, body cavity and mesenteries, respectively. The highest prevalence of infection was *A. lucidus* followed by *Neyraplectana* sp. and *Oswaldocruzia* sp. respectively.

Introduction: Grass frog (*F. limnocharis*) is an amphibian species commonly found and plays the important role by acting as the general protein source for rural people in Thailand. This frog is mainly nocturnal by its dietary is consisting of various insects species that served as the intermediate host of helminth parasites. They live in natural habitat and discover in all section of Thailand.[1] Due to naturally growth and contact directly to the soil and water, grass frogs are infected with helminth parasites easily. The helminth parasites found in *F. limnocharis* are both of adult worms and larval stages. Infection of helminth parasites can affect growth rate and body weight. Moreover, human can also be infected with helminth parasites that transmitted from frogs. Previous study reported sparganum can cause sparganosis in human and sparganosis has been recognized as zoonotic dissease caused by the invasion of spargana which occasionally occurs in human. On the other hand, spargana is the plerocercoid (infective) larvae of various Diphyllobothroid tapeworm especially belonging to the genus *Spirometra*, which exploit copepods as the first intermediate host.[2,3] A wide range of amphibians, reptiles, birds, and mammals serve as second intermediate/paratenic hosts. Human infections occur mainly by ingesting raw intermediate/paratenic hosts.[3]The infection of sparganum has been reported in many countries but commonly found in eastern Asia. In Thailand and other Southeast Asian country, the infection is sporadic. In the period 1943–2010, there were 52 reported cases of sparganosis in Thailand.[3,4]

Methodology: A total 38 live Grass frog (*F. limnocharis*) were collected from paddy field and then were brought to the laboratory, autopsied, and various organs were examined for the presence of helminthes parasites**.** The gastrointestinal tract, lungs, kidneys, urinary bladder and muscles were carefully investigated for helminths under a stereomicroscope by using needle. All helminthes found were identified and counted. Trematode, cestode and

acanthocephalan were flattened and fixed in 4 % formalin for permanent slide preparation, stained with hematoxyline, dehydrated with grading alcohol series, cleared with xyline and mounted with Permount. All nematode specimens were fixed in 4 % hot formalin for semi-permanent slide, and then dehydrated with grading alcohol, cleared with glycerine and mounted with glycerine jelly. Prevalence is the percentage of host individuals infected in a sample of host species. Intensity is the number of individuals of a particular species in each infected host.

Results, Discussion and Conclusion: The results showed that helminth parasites were collected from 36 of 38 grass frog (*F. limnocharis*). The total prevalence of infection was 94.74 %. Six species of adults and 3 larval stages were recorded and identified as followings; (Figure 1) 3 cestodes, *Baerietta* sp., *S. chiangmaiensis* and sparganum (plerocercoid), 3 nematodes, *Oswaldocruzia* sp., *Neyraplectana* sp. and encyst larvae, 2 acanthocephala, *A. lucidus* and cystacanth and 1 of trematode, *G. tigrinus*, with the individual prevalence of 5.26 %, 2.63 %, 2.63 %, 34.21%, 47.37 %, 2.63 %, 52.63 %, 42.10 % and 28.94% respectively whereas the individual intensity were 3, 1, 1, 5.8, 6.3, 1, 5.7, 8.3, and 5.9 per host respectively (Table 1). From these, the adults were recovered from intestine, the larval stages including sparganum, encyst larvae and cystacanth were recovered from leg muscles, body cavity and mesenteries respectively. In this study, the highest prevalent was observed in *A. lucidus* followed by *Neyraplectana* sp., Cystacanth, *Oswaldocruzia* sp. and *G. tigrinus* whereas *Baerietta* sp., *S. chiangmaiensis*, Sparganum and encysted larval stage of nematode were less. The individual prevalence of helminth species was different but the individual intensity was closely (Figure 2, 3). Contrastingly, some helminth species were rather low prevalence while their intensities were quiet high. It may due to, host abundance can cause the lower infection rate and numbers of hosts infected were limited by the narrow population of host's dietary i.e. insect species that harboring parasite's larvae. Previous study have been reported in Chiang Mai, Thailand, Chaowalit (1980) reported 15 species of helminth parasites found in *Rana* spp. which comprising of 5 trematodes, 2 cestodes, 6 nematodes and 2 acanthocephala.[5] In this study, the same species found were *Oswaldocruzia* sp. and *A. lucidus* because previous study have been studied 30 years ago, therefore the environment and climatic features may changed. The number of helminth species found in this study was lower than, which it may dues to the different number of frogs investigated and season conducted. Phattanasertthanon (1983) reported 14 parasite genera from *Kaloula pulchra pulchra* including 8 nematodes, 1 cestode, 1 trematode and 1 acanthocephala and larval stages of nematode, 2 rhabditiform and 1 of filariform.[6] In this study, the same species found were *Oswaldocruzia* sp., *Neyraplectana* sp. and *A. lucidus* respectively. The different results obtained may depending on the different host species. Sirwattanapong (1983) reported 18 species found in *Rana tigerrina cantor* from markets in Bangkok including 4 trematodes, 2 cestodes, 8 namatodes and 4 acanthocephala.[7] In this study, number of helminths species found was less than previous study but the total prevalence and individual prevalence were opposite, suggest that different results found are probably depend on seasonal variation and number of grass frog investigated. Additionally, some foreign previous studies have been reported, Duzen *et.al.* (2009) reported 9 species of helminthes found in *R. dalmatina* in Turkey including 4 nematodes, 3 trematodes, 1 monogenea and 1 acanthocephalan.[8] From these, all helminths represent new host records for *R. dalmatina.* Rizvi and Bhutia. (2010) reported 12 genera found in anuran amphibians which comprising of 3 trematodes, 7 nematodes, 1 cestode and 1 acanthocephalan.[9] Pulis *et.al.* (2011) reported 11 species of helminthes found in wood frogs, *Lithobates sylvaticus* including 7 trematodes, 3 nematodes and 1 of cestode.[10] The diversity of helminthes found in previous reports mentioned above were more than in this

study which may dues to the different species of frogs investigated, the most preferring habitat of each frog and the different of geographical distribution. However, this study confirm the situation of parasitic infection in Grass frog from Chiang Mai, Thailand which demonstrate the risk of infection to human due to this frog acting as the preferred protein source for rural people in Thailand.

Table 1 Prevalence and intensity of helminth parasites in Grass frog.

Parasite (no. of host infected)	Prevalence %	Total no. of parasite collected	Intensity (per host)
Cestode			
Baerietta sp. (2)	5.26	6	3
S. chiangmaiensis (1)	2.63	1	1
Sparganum (1)	2.63	1	1
Nematode			
Oswaldocruzia sp. (13)	34.21	75	5.8
Neyraplectana sp. (18)	47.37	114	6.3
Larval stage of nematode (1)	2.63	1	1
Acanthocephala			
A. lucidus (20)	52.63	112	5.6
Cystacanth (16)	42.10	133	8.3
Trematode			
G. tigrinus(11)	28.94	65	5.9

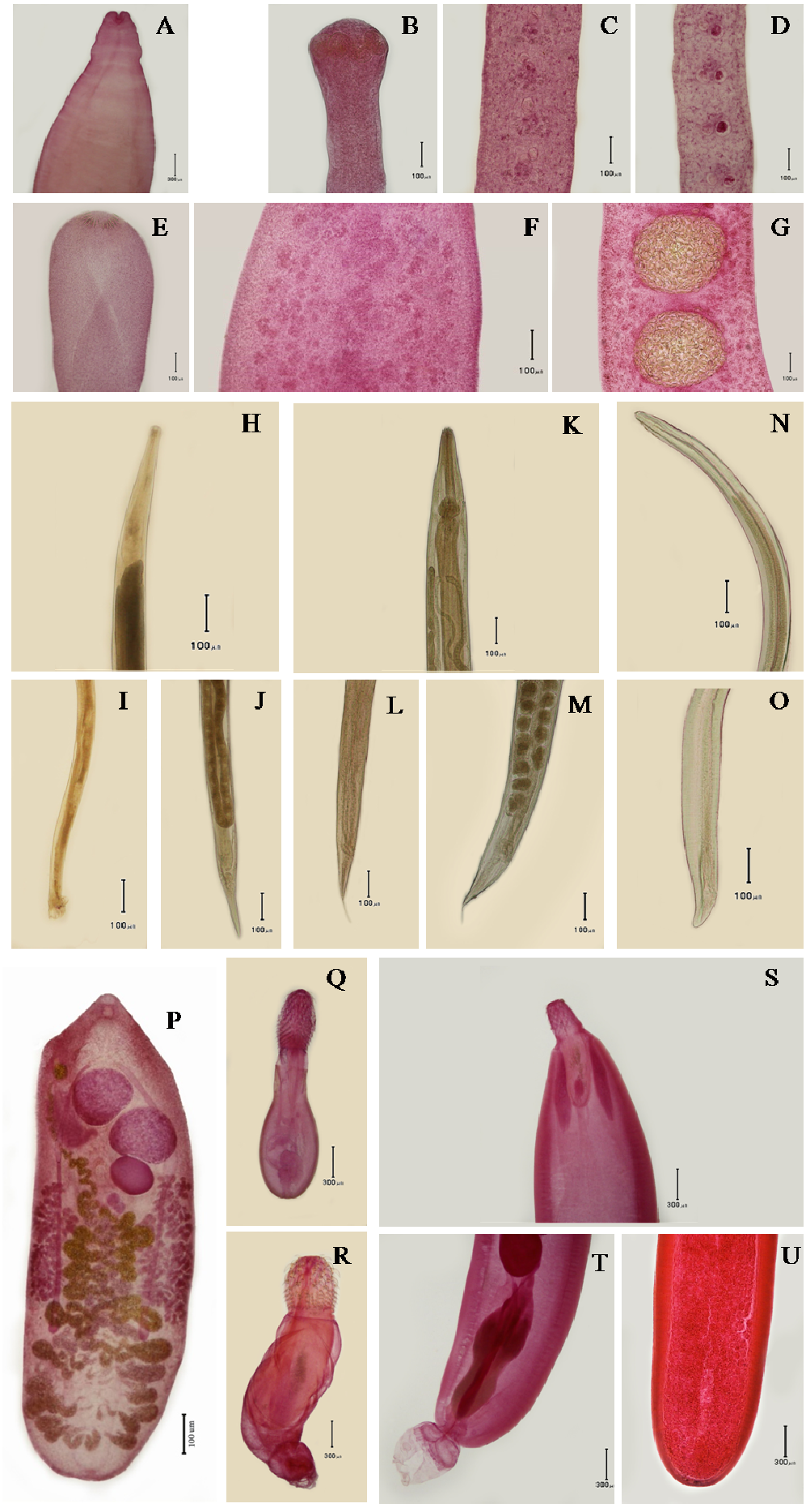
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Figure 1 Helminth parasites in *Fejervarya limnocharis*

A = sparganum, B = scolex of *Baerietta* sp., C = mature of *Baerietta* sp., D = gravid of *Baerietta* sp., E = scolex of *S. chiangmaiensis*, F = mature of *S. chiangmaiensis*, G = gravid of *S. chiangmaiensis*, H = anterior part of *Oswaldocruzia* sp., I = posterior part of *Oswaldocruzia* sp. (male), J = posterior part of *Oswaldocruzia* sp. (female), K = anterior part of *Neyraplectana* sp., L = posterior part of *Neyraplectana* sp. (male), M = posterior part of *Neyraplectana* sp. (female), N = anterior part of larval stage of nematode, O = posterior part of larval stage of nematode, P = *G. tigrinus*, Q, R = excysted of cystacanth, S = anterior part of *A. lucidus*, T = posterior part of *A. lucidus* (male), U = posterior part of *A. lucidus* (female)

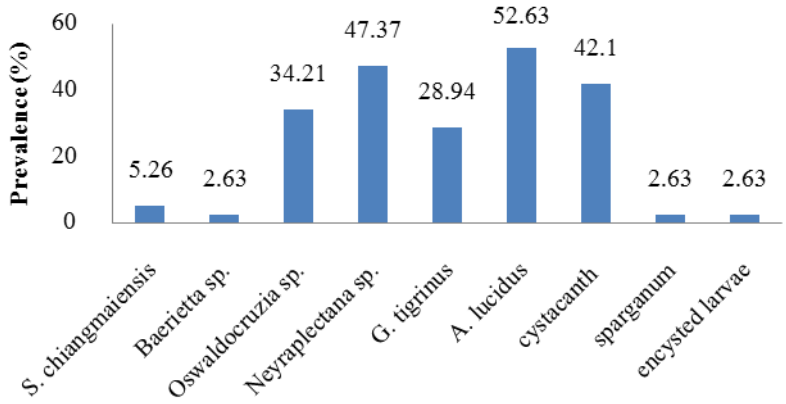

Figure 2 Diagram of individual prevalence of each helminth species

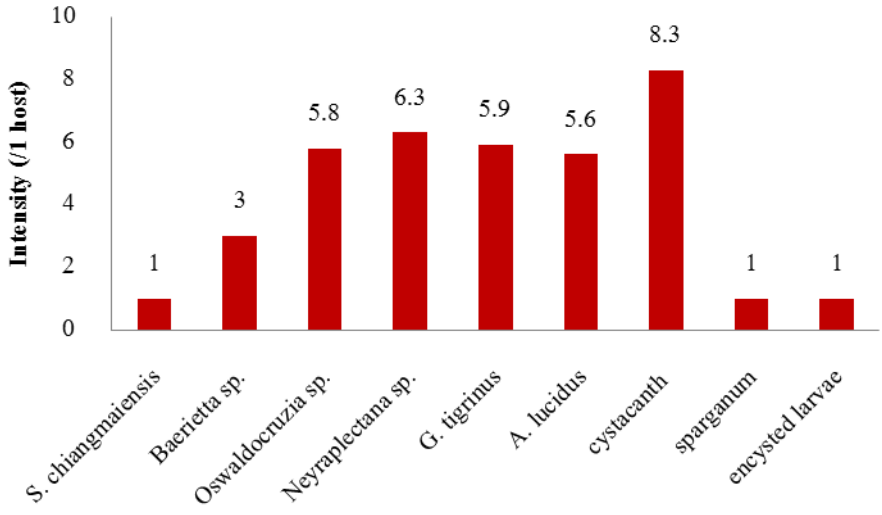

Figure 3 Diagram of individual intensity of each helminth species

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