HELMINTHS OF VERTEBRATES IN MAE SA STREAM, CHIANG MAI, THAILAND

C Wongsawad[1], J Rojtinnakorn[2], P Wongsawad[1], A Rojanapaibul[1], T Marayong[1], S Suwattanacoupt[1], P Sirikanchana[3], O Sey[4] and BV Jadhav[5]

[1]Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai; [2]Department of Fishery Technology, Faculty of Agricultural Production, Maejo University, Chiang Mai; [3]Department of Fishery Biology, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand; [4]Department of Biology, University of Pecs, Pecs, Hungary; [5] Marathwada University, Aurangabad, India

Abstract. Freshwater vertebrates from Mae Sa Stream, Doi Suthep-Pui National Park, Chiang Mai, were collected from January 1997 to June 1999. They comprised 3,900 fishes of 32 species; 149 amphibians of 9 species; and 3 reptiles of 3 species. Fifty-six species of helminths were recovered: the 5 monogenea were *Dactylogyrus* sp I, *Trianchoratus* sp, *Gyrodactylus* sp, *Diplozoon* sp and *Dactylogyrus* sp II; the 27 trematodes were *Allocreadium* sp I, II, *Haplorchoides* sp (metacercaria; meta), *Posthodiplostomum* sp, *Gauhatiana* sp, *Plagiophorus* sp, *Transversotrema patialense*, *Euryhelmis* sp, (meta), *Centrocestus caninus* (meta), *Acanthostomum* sp (meta), *Genarchopsis goppo*, *Phyllodistomum* sp I, *Brevicreadium* sp, *Gorgoderina gracilis* n sp, *Pleurogenoides sphaericus*, *Stellantchasmus falcatus* (meta), *Haplorchis* sp (meta), *Urotrema* sp, *Haplorchoides* sp (adult), *Encyclometra bungara*, *Pleurogenes chiangmaiensis*, *Telorchis* sp, *Mantereilla* sp, *Genarchopsis* sp (meta), *Phyllodistomum* sp II, *Phyllodistomum* sp III, and *Ganeo tigrinus*; the 6 species of cestodes were *Senga chiangmaiensis* n sp, *Ptychobothrium mystacoleucusi* n sp, *P. rojanapaibuli* n sp, *P. discusae* n sp, *Circumoncobothrium baimaii* n sp and *Ptychobothrium maesae* n sp; the 3 species of acanthocephala were cystacanth, *Pallisentis* sp, and *Acanthocephalus lucidus*; the 15 species of nematodes were *Spinitectus* sp (cyst), *Rhabdochona* sp, *Rhabdochona* sp I, *Camallanus* sp, *Zanclophorus* sp, *Spinitectus* sp (adult), *Anisakis* sp, Unknown I, II, *Rhabdochona* sp II, III, *Proleptus* sp, *Cosmocerca* sp, *Ascaridia* sp, and *Camallanus anabantis*. Specimens were surveyed one of each season for the first year. The prevalence (%) and intensity of infection were recorded. The second year and half of the third year, they were collected every two months. Parasite distribution, relationships between host and parasite, and classification, were analyzed by cluster analysis.

INTRODUCTION

Helminths (Platyhelminthes, Nematoda and Acanthocephala) were collected from Mae Sa Stream, Doi Suthep-Pui National Park, Chiang Mai, Thailand. The stream is 26 km long, 300-1,200 m above sea level and flows pass communities and agricultural farms. Parasitic infection may occur with man and animals. From this study, the results will be the key for identifying the helminths and epidemiology and controlling of helminthes infecting fish, amphibians, and reptiles.

MATERIALS AND METHODS

Freshwater vertebrates were collected from 12 sites along the Mae Sa Stream for 3 seasons in the first year (Fig 1). In the second year and third year, the animals were sampled from 4 sites (sites 2, 5, 9, and 10) every two months. The helminthes were collected, processed to a permanent slide and identified under a compound microscope. Parasite distribution, host-parasite relationships, and classification were analyzed by cluster analysis. The prevalence (%) and intensity of helminthic infections were studied and recorded.

Correspondence: Chalobol Wongsawad, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50202, Thailand.
E-mail: cwongsawd@yahoo.com

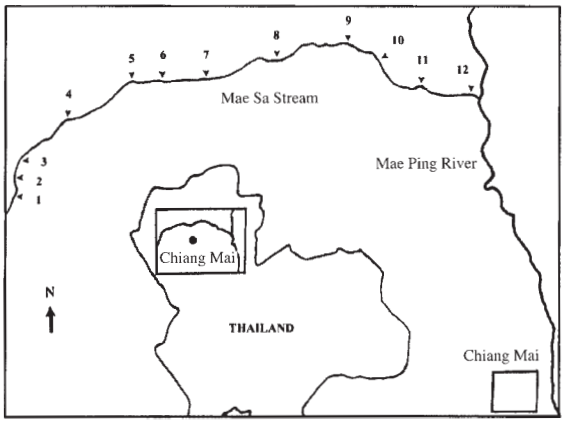

Fig 1- Map of Mae Sa Stream showing 12 sampling sites.

RESULTS

Species identification

There were 3,900 fish of 32 species, 149 amphibians of 9 species, and 3 reptiles of 3 species. Fifty-six species of helminths were recovered. The five species of monogenea were *Dactylogyrus* sp I and II, *Trianchoratus* sp, *Gyrodactylus* sp, and *Diplozoon* sp. The twenty-seven species of trematodes were *Allocreadium* sp I, II, *Haplorchoides* sp (metacercaria; meta), *Posthodiplostomum* sp, *Gauhatiana* sp, *Plagiophortus* sp, *Transversotrema patialense*, *Euryhelmis* sp (meta), *Centrocestus caninus* (meta), *Acanthostomum* sp (meta), *Genarchopsis goppo*, *Phyllodistomum* sp I, *Brevicreadium* sp, *Gorgoderina gracilis* n sp, *Pleurogenoides sphaericus*, *Stellantchasmus falcatus* (meta), *Haplorchis* sp (meta), *Urotrema* sp, *Haplorchoides* sp (adult), *Encyclometra bungara*, *Pleurogenes chiangmaiensis*, *Telorchis* sp, *Mantereilla* sp, *Genarchopsis* sp (meta), *Phyllodistomum* sp II, *Phyllodistomum* sp III and *Ganeo tigrinus*. The six species of cestodes were *Senga chiangmaiensis* n sp, *Ptychobothrium mystacoleucusi* n sp, *P. rojanapaibuli* n sp, *Circumoncobothrium baimaii* n sp and *Ptychobothrium maesae* n sp, and *P. discusae* n sp. The three species of acanthocephala were

Table 1
Species of helminths investigated and hosts found.

Helminths	Host
Monogenea	
Dactylogyrus sp I	F8, F10, F13, F16, F25, F27
Dactylogyrus sp II	F13, F28
Diplozoon sp	F10
Gyrodactylus sp	F8, F16, F25
Trianchoratus sp	F2, F11, F13, F16, F17, F20, F25
Trematoda	
Acanthostomum sp (meta)	F3, F5, F6, F7, F8, F11, F12, F16, F18,F20,F25
Allocreadium sp I	F5, F6, F7, F12, F13, F25
Allocreadium sp II	F5, F15, F21
Brevicreadium sp	Am3, Am4, Am5, Am6, Am7, Am9
Centrocestus caninus (meta)	F3, F4, F5, F6, F7, F8, F10, F12, F13, F14, F16, F20, F25, F26, F32
Encyclometra bungara	R1
Euryhelmis sp (meta)	Am2
Ganeo tigrinus	
Gauhatiana sp	F12, F13, F25, F26
Genarchopsis goppo	F16
Genarchopsis sp (meta)	F22
Gorgoderina gracilis n.sp[a]	Am3, Am9
Haplorchis sp (meta)	F8, F25
Haplorchoides sp (meta)	F3, F5, F6, F7, F8, F9, F10, F12, F13,F14, F26
Haplorchoides sp (adult)	F28
Mantereilla sp	Am3, Am5, Am6, Am8
Phyllodistomum sp I	F16, F20
Phyllodistomum sp II	F22
Phyllodistomum sp III	F8
Plagiophorus sp	F13
Pleurogenes chiangmaiensis n.sp[a]	Am3, Am5, Am6, Am8
Pleurogenoides sphaericus	Am3, Am5
Posthodiplostomum sp (meta)	F14, F16
Stellantchasmus falcatus (meta)	F2, F4, F13, F14, F32
Telorchis sp	Am3, Am5, Am6, Am8
Transversotrema patialense	F10, F11, F12, F13, F16, F18, F19, F25,F26
Urotrema sp	F30

Table 1 (Continued)

Helminths	Host
Cestoda	
Circumoncobothrium baimaii n.sp[a]	F21
Ptychobothrium discusae n sp[a]	F8, F11
P. maesae n sp[a]	F19
P. mystacoleucusi n sp[a]	F5, F8, F19, F22
P. rojanapaibuli n sp[a]	F8, F10, F19
Senga chiangmaiensis n sp[a]	F3, F8, F21, F22
Acanthocephala	
Acanthocephalus lucidus	Am4, Am6
Cystacanth	Am4, F2, F8, F10, F16, F17, F18
Pallisentis sp	F2, F8, F10, F16, F17, F20, F28
Nematoda	
Anisakis sp	R1
Ascaridia sp	F25, F28
Camallanus anabantis	F2
Camallanus sp	F2, F5, F6, F7, F8, F10, F13, F16
Cosmocerca sp	Am3, Am6, Am7
Proleptus sp	F16
Rhabdochona sp	F3, F5, F6, F7, F8, F10, F15, F16, F25
Rhabdochona sp I	F5, F6, F7, F8, F10, F12, F13, F16, F20
Rhabdochona sp II	F12, F13
Rhabdochona sp III	F21
Spinitectus sp (adult)	F5, F6, F7, F10, F20
Spinitectus sp (cyst)	F26
Zanclophorus sp	Am1, Am3, Am6, Am16
Unknown I	F21
Unknown II	F20

[a]new species from this study

F = fish; F1 = *Homaloptera leonardi*, F2 = *Anabas testudineus*, F3 = *Lepidocephalichthys burmanicus*, F4 = *Acantopsis theimmedhi*, F5 = *Schistura buccalentus*, F6 = *S. breviceps*, F7 = *S. poculi*, F8 = *Mystacoleucus marginatus*, F9 = *Poropuntius deauratus*, F10 = *Systomus orphoides*, F11 = *S. stoliezkae*, F12 = *Macrognathus siamensis*, F16 = *Channa gachua*, F17 = *Trichogaster trichopterus*, F18 = *Trichopsis vittatus*, F19 = *Gambusia striatus*, F20 = *Channa striatus*, F21 = *Mastacembelus armatus*, F22 = *Monopterus albus*, F23 = *Oxyeleotris marmoratus*, F24 = *Garra cambodgiensis*, F25 = *Rasbora argyrotaenia*, F26 = *Discherodontus ashmeadi*, F27 = *Cyprinius carpio*, F28 = *Mystus nemurus*, F29 = *Tilapia nilotica*, F30 = *Gyptothorax trilineatus*, F31 = *Clarias betrachus*, F32 = *Xenentodon cancila*.

Am = amphibian; Am1 = tadpode *Leptobrachium pullum*, Am2 = tadpole *Amolops afahanus*, Am3 = adult *Rana kuhlii*, Am4 = adult *Amolops afahanus*, Am5 = adult *Rana nigrovittata*, Am6 = adult *Limnonectus kuhlii*, Am7 = adult *L. pileata*, Am8 = adult *L. limnocharis*, Am9 = adult *Ichthyopsis supachaii*.

R = reptile; R1 = *Xenochrophis piscator*, R2 = *Amphiesma deschauensei*, R3 = *Tropidophorus berdmorei*.

cystacanth, *Pallisentis* sp and *Acanthocephalus lucidus*. The fifteen species of nematodes were *Spinitectus* sp (cyst), *Rhabdochona* sp, *Rhabdochona* sp I, *Camallanus* sp, *Zanclophorus* sp, *Spinitectus* sp (adult), *Anisakis* sp, Unknown I and II, *Rhabdochona* sp II, III, *Proleptus* sp, *Cosmocerca* sp, *Ascaridia* sp and *Camallanus anabantis*. All worms this study are shown in Table 1 with hosts.

Prevalence and intensity

The prevalence results (%) are shown in Table 2. In the first year, the prevalence of infected fish was similar in every season. Amphibians and reptiles differed, and were highest in the rainy season. In the second year, the prevalence in every two months was similar except in March 1998, in the summer. However, in March 1999, the highest prevalence was shown.

Table 2
Total numbers and total prevalence (%) of infected vertebrates in Mae Sa Stream.

Vertebrates	1997			1998						1999		
	Winter	Summer	Rainy	Jan	Mar	Jun	July	Sep	Dec	Jan	Mar	Jun
Fish												
Collected	539	687	633	188	180	197	269	215	283	235	190	284
Infected	227	305	243	75	41	63	103	79	86	47	58	79
% prevalence	42.12	44.40	38.39	39.89	22.78	31.98	38.29	36.74	30.39	20.00	30.53	27.87
Amphibians												
Collected	13	13	3	6	6	2	4	16	25	7	28	15
Infected	1	7	2	3	4	1	0	2	10	3	15	3
% prevalence	7.70	53.85	66.67	50	66.67	50	0	12.50	28.57	42.86	53.57	20.00
Reptiles												
Collected	2	-	1	-	-	-	-	-	1	-	-	-
Infected	1	-	1	-	-	-	-	-	0	-	-	-
% prevalence	50	-	100	-	-	-	-	-	0	-	-	-

(-) host not found

Table 3
The highest intensity of adult helminths, 1997-1999.

Helminths	Host	1997 (Season)			1998 (Months)						1999 (Months)		
		Winter	Summer	Rainy	Jan	Mar	Jun	Jul	Sep	Dec	Jan	Mar	Jun
Anisakis sp	*Xenochropis piscator*	8.00											
Brevicreadium sp	*Ichthyopsis supachaii*		6.200										
Encyclometra bungara	*Xenochropis piscator*			145.00									
Pleurogenoides sphaericus	*Rana nigrovittata*				45.00								
Rhabdochona sp III	*Mastacembelus armatus*					6.00							
Allocreadium sp II	*Macrognathus siamensis*						8.00						
Trianchoratus sp	*Channa striatus*							11.50					
Dactylogyrus sp	*Mystus nemurus*								53.50				
Rhabdochona sp II	*Mastacembelus armatus*									13.00			
Rhabdochona sp I	*Systomus orphoides*										6.00		
Cosmocerca sp	*Limnonectus pileata*											3.25	
Dactylogyrus sp	*Systomus orphoides*												17.00

The intensity of infection was recorded as species of helminth per host. The intensity of infection was calculated by the total number of worms in each species of collected host. Table 3 shows the highest intensity every season (1st year) and every two months (2nd and 3rd years). The highest trematode intensity was *Encyclometra bungara,* with 145 worms in a snake (*Xenochrophis piscator*), while the nematodes (*Rhabdochona* spp) infected 3 times.

Cluster analysis

Distribution depended upon altitude and distance from the source of the stream; upper (sites 1-4), middle (sites 5-8), and downstream (sites 9-12), in Fig 1. The result of site distribution, which was analyzed by cluster analysis, was divided into 3 groups. According to Fig 2, the first group was sites 5, 7, 8, and 9; the second group was sites 10, 11, and 12; and the third group was sites 1, 2, 3, 4, and 6. Notably, the results of

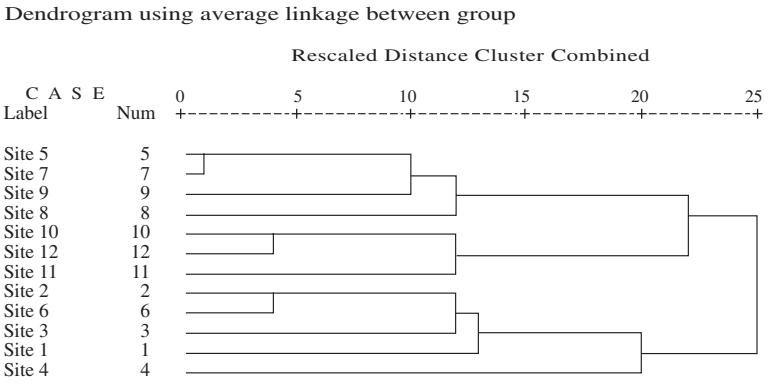

Fig 2- Cluster analysis of sites by types of the helminth at each site (1st year, 1997).

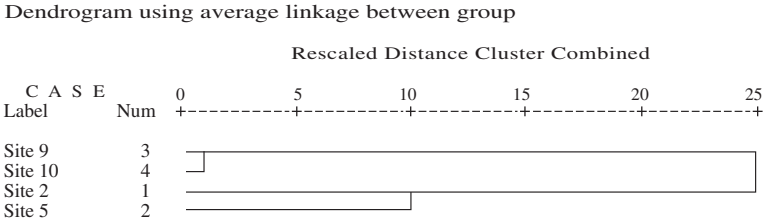

Fig 3- Cluster analysis of seasons by types of the helminth at each site (1st year, 1997).

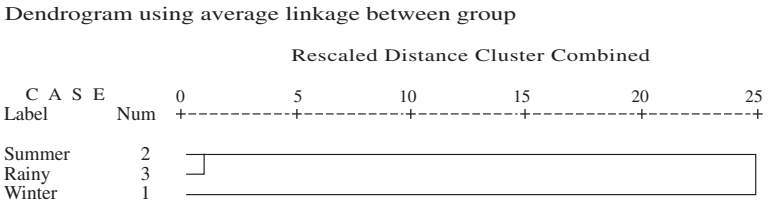

Fig 4- Cluster analysis of sites by types of the helminth at each site (2nd year, 1998-1999).

statistical cluster grouping found helminthes at sites 6 and 9 were different from expected groups at that altitude. Site 6 was lower in the stream than site 5, but the same fish were found as at sites 1-4 because of similar rocky ground. Site 9 was at a similar altitude to sites 10-12 in range 330-340 m above the sea level. However, the fish and helminths found were similar to those at sites 5, 7 and 8. Some parameters of water quality were measured, *ie* DO, pH, conductivity, and temperature. Their values were similar and in the standard range (data not shown). It is probable that other physical factors - environment and water quality- should be considered.

Seasonal analysis was divided into 3 seasons, summer, rainy, and winter (Fig 3). The sampling sites in the second and third years were divided into 2 groups, 2 and 5, and 9 and 10 (Fig 4). The numbers of species of helminths in every two months was divided into 4 groups (Fig 5).

DISCUSSION

From this investigation, 56 species of helminths, including two new species of trematodes and 5 new species of cestodes, were recovered. Some metacercarial stage trematodes were reported to infect

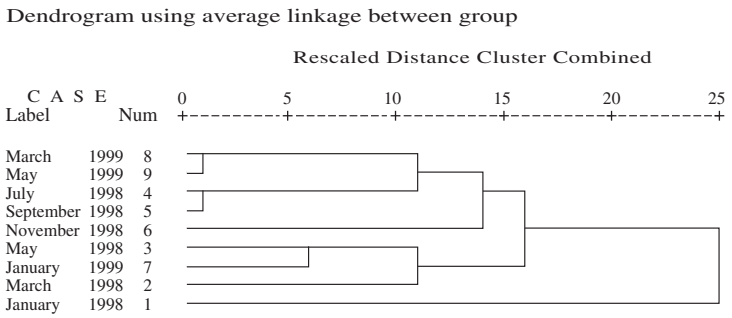

Fig 5- Cluster analysis of months by types of the helminth at each site (2nd year, 1998-1999).

humans *eg Stellantchasmus falcatus* (Tantachamrun and Kliks, 1978; Radomyos *et al*, 1990; Wongsawad *et al*, 1996), *Haplorchis taichui* (Chen, 1949), *Centrocestus caninus* (Waikagul *et al*, 1990). Some species infect only animals as *Acanthostomum* sp (Fischthal and Kuntz, 1963; Karyakart, 1963; Moravec, 1976; Brook and Holcman, 1993), *Urotrema* sp (Yamaguti, 1958), *Euryhelmis* sp (Yamaguti, 1958).

Identification of some helminths may not be to species level because of a lack of keys and reference journals, and some samples were only one specimen in the metacercarial stage such as *Euryhelmis* sp, though it would be the new species. Some unknown nematodes could not be identified because only one sex was found. However, 8 new species of helminths were found in this study. Two species of trematodes were new species: *Gorgoderina gracilis* n sp (Wongsawad *et al*, 1999) and *Pleurogenes chiangmaiensis* n sp (Sey and Wonsawad, 1999). Six species of cestodes were new species: *Senga chiangmaiensis* n sp, *Ptychobothrium mystacoleucusi* n sp, *P. rojanapaibuli* n sp, *P. discusae* n sp, *P. maesae* n sp and *Circumoncobothrium baimaii* n sp (Wongsawad, 1998; Wongsawad and Jadhav, 1998; Wongsawad *et al*, 1998 a, b).

The number of collected hosts was rare every season. It's may be that some difference occurred. The number and species of parasites differed in winter, especially the species which had many differences. The summer and rainy seasons have similar temperature and humidity affecting the number and species of parasites. Observed host size and species were different in the first, second, and third years; some hosts require a long time to grow and develop. Prevalence and intensity were used to indicate when these parasites might be found over one year. This information could be used in epidemiology and control of these parasites.

Sites 2 and 5 were at higher altitude than sites 9 and 10. The stream bed in the upper parts (sites 2 and 5) was mostly rocky, while the lower parts (sites 9 and 10) were muddy. Therefore, the difference in the streambed also affected differences in the species of hosts and helminths.

Helminth species in November 1998 and January 1998 were different from the other groups probably because the hosts that were collected in the first year were unseen in the second year, and the helminths disappeared. On the other hand, the hosts were not constant each year, so that the helminths synchronized with the hosts.

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