### TREMATODES FROM AMPHIBIANS AND REPTILES OF THAILAND

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#### **ABSTRACT**

Five species of amphibians (Amolops afahanus, Ichthyophis supachaii, Kaloula pulchra, Mycrohyla sp., Rana sp.) and 6 species of reptiles (Amphiesma deschauensis, Cosymbotus platyurus, Liolepis belliana, Cuora amboiensis, Hemidactylus frenatus, Xenochrophis piscator) collected in Maesa stream (Doi Suthep-Pui National Park) and in the suburban areas of Chiang Mai, Thailand were examined for helminths. Out of the helminth parasites 11 species of trematodes (Diplodiscus amphichrus, Encyclometra bungara, Glyphthelmins staffordi, Pleurogenoides sphaericus, Ganeo tigrinus, Postochigenes majeedi, Mesocoelium sociale, Acanthostomum (Atrophecaecum) burminis, Paradistomum geckonum, Gogatea serpentium, Euryhelmis sp. have been reported in this paper. All of the species represent new locality records.

#### INTRODUCTION

A survey of the available literature shows the paucity of information on the helminth parasites of amphibians and reptiles of Thailand although this country has a reach and variable herpetofauna and their parasites might have a special interest from taxonomic and zoogeographical points of view. The authors intention was to give a preliminary report on these parasites of the hosts in question thinking that it will generate further such investigations especially in Thailand. Additional trematodes, cestodes, nematodes and acanthocephalans will be reported in other papers.

#### MATERIALS AND METHODS

A total of 137 specimens of amphibians comprising 5 species; Amolops afahanus: 3 specimens, Ichthyophis supachaii: 5 specimens (Maesa stream, Doi Suthep-Pui National Park, 10-12 January 1997); Kaloula pulchra: 59 specimens (suburban areas of Chiang Mai, September-October 1996); Mycrohyla sp.: 23 specimens (suburban areas of Chiang Mai, October 1993 – May 1995); Rana sp.: 47 specimens (suburban areas of Chiang Mai, October-September 1994) and 53 specimens of reptiles, comprising 5 species: Amphiesma deschauensis, 1 specimen, Xenochrophis piscator, 1 specimen (Maesa stream, Doi Suthep-Pui National Park, 10 May 1977 and 10-12 June 1997 respectively); Cuora amboiensis, 1 specimen, Liolepis belliana, 10 specimens (local market, 5-18 July 1997), Cosymbotus platyurus, 25 specimens, Hemidactylus frenatus, 15 specimens (vicinity of Chiang Mai), 10-26 October 1994 and 15-19 March 1994 respectively have been examined. Trematodes after washing in saline were fixed in Bouin's fixative under

slight coverglass pressure, transfered in graded series of ethyl alcohol, stored in 70% ethanol. Later stained in Borax carmine and Delafield's haematoxyline, destained in diluted HCl and after dehydration they were mounted in permount. All measurements are in millimeters unless otherwise stated. Drawings of the worms were prepared by microprojection and details were filled in through microscopic observations. All specimens are deposited in the helminthological collection, Parasitology Research Laboratory, Department of Biology, Chiang Mai University, Chiang Mai, Thailand.

#### RESULTS

# Survey of the species

# Diplodiscidae Skrjabin, 1949

Diplodiscus amphichrus Tubangui, 1949 (Fig. 1.)

Diagnosis. Body 1.3-3.5 in length, 0.38-1.3 in width. Tegument aspinose. Oral sucker terminal, spherical or oval 0.12-0.36 x 0.12-0.56 in size. Pharynx 0.23-0.29 long, Subclavatus type, appendiges extramural, 0.10-0.13 in length; oesophagus 0.31-0.34 in length, oesophageal bulb present, 0.11-0.25 x 0.06-0.13 in size; intestinal caeca straight, along lateral sides, ending in front of ventral sucker. Ventral sucker 0.35-0.98 x 0.54-1.39 in size with an additional sucker in centre, Diplodiscus type; number of muscle units: d.e.c 22-24, d.i.c. 44-49, v.e.c. 14-17, v.i.c. 48-54, m.e.c. 8-10. Testis single 0.14-0.26 x 0.13-0.32 in size. Cirrus sack 0.13-0.14 x 0.07-0.12. Terminal genitalium postbifurcal, Spinolosum type. Ovary entire, measures 0.07-0.25 x 0.06-0.26. Vitellaria large, follicular, extending from appecdiges up to anterior border of ventral sucker. Uterus occupying entire intercaecal space between ootype and terminal genitalium. Eggs oval, operculated, 0.078-0.146 x 0.031-0.074 in size.

Host and locality: Rana sp. (vicinity of Chiang mai)

Site: rectum Prevalence: 6.3% Abundance: 0.14

# Plagiorchiidae Ward, 1917

Encyclometra bungara Srivastava & Ghosh, 1968 (Fig. 2.)

Diagnosis. Body 2.36-8.84 in length, 1.51-1.63 in width. Tegument aspinose. Oral sucker almost spherical, measuring 0.29-0.31 x 0.18-0.28. Prepharynx and oesophagus absent; phraynx large, size 0.28-0.32 x 0.21-0.25; intesitnal caeca long, reaching posteior extremity. Ventral sucker spherical, size 0.45-0.51 x 0.48-0.52, ratio of suckers diameter 1:1. Testes slightly lobate, oblique in posterior body half, size of right testis 0.17-0.19 x 0.22-0.27, left testis 0.16-1.19 x 0.17-0.32. Cirrus sack club-shaped, 0.61-0.80 x 0.12-0.18 in size, between intestinal bifurcation and ventral sucker. Ovary small, just behind ventral sucker, size 0.10-0.15 x 0.16-1.20. Seminal receptacle oval, at level of ovary. Vitelline follicles lateral, mainly extracaecal, from level of ovary to posterior body end. Uterus intercaecal, forming numerous convolutions between ventral sucker and posterior extremity. Genital opening lateral in front of ventral sucker. Eggs oval, containing miracidia, size 0.075-0.081 x 0.031-0.041.

Host and locality: Xenochrophis piscator (Doi Suthep-Pui National Park)

Site: intestine Prevalence: 100% Mean intensity 15 Abudance: 15 Glypthelmins staffordi Tubangui, 1928 (Fig. 3.)

Diagnosis. Body 1.32-4.03 in length, 0.51-1.3 in width. Tegument spinose, covering two-thirds of anterior body part. Oral sucker 0.17-0.30 x 0.21-0.31 in size, muscular pharynx measuring 0.06-0.21 x 0.08-0.13, oesophagus 0.04-0.09 long, caeca reaching posteroior ends of body. Ventral sucker at anterior part of body, size 0.09-0.18 x 0.09-0.21. Genital opening median. Testes almost spherical, size 0.18-0.41 x 0.18-0.43, symmetrical or slightly diagonal, near to acetabulum. Cirrus sack well developed. Size 0.16-0.48 x 0.06-0.12. Ovary spherical, size 0.15-0.32 x 0.12-0.31, just in front of first testis. Vitellaria multifollicular in two lateral groups, from ovarian level to posteriorly but not reaching ends of caeca. Eggs numerous, operculated, size 0.023-0.030 x 0.012-0.023.

Host and locality: Rana sp. (vicinity of Chiang Mai)

Site: intestine Prevalence: 8.5% Mean intensity: 1.5 Abundance: 0.12

### Lecithodendriidae (Luhe, 1901)

Pleurogenoides sphaericus (Klein, 1905) (Fig. 4)

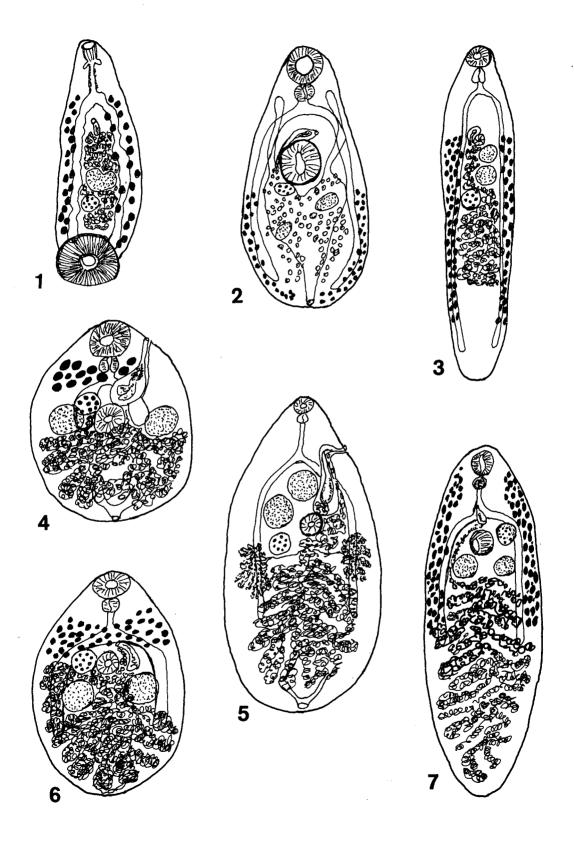
Diagnosis. Body 0.43-1.12 in length, 0.32-0.83 in width. Tegument spinose. Oral sucker measuring 0.093-0.022 x 0.099-0.25 , muscular pharynx oval, size 0.018-0.098 x 0.021-0.097, oesophagus very short terminating at level of ventral sucker, partly covered by gonads and cirrus sack. Ventral sucker centrally located, measuring 0.091-0.198 x 0.038-0.198. Testes symmetrical, lateral, at level of ventral sucker; right testis 0.182-0.295 x 0.131-0.237, left testis 0.148-0.270 x 0.128-0.237. Cirrus sack claviform, extending to ventral sucker, containing seminal vesicle and prostate cells; genital opening marginal at level of oral sucker. Ovary spherical smaller than testes, measuring 0.068-0.154 x 0.048-0.176, situating submedially near to ventral sucker. Seminal receptacle present. Vitellaria in shoulder region, either forming two grape-like bunches or follicles confluent; consisting 15-18 follicles. Uterine coils chiefly in hind body. Eggs numerous, size 0.021-0.032 x 0.013-0.017.

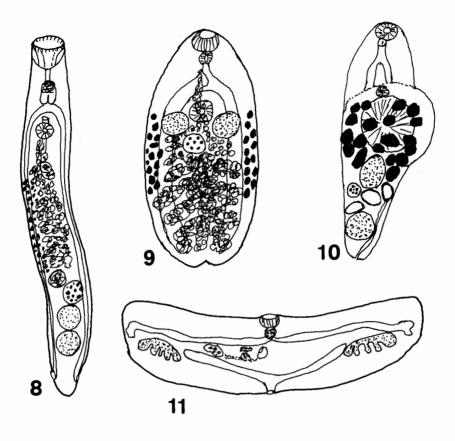
Host and locality: Rana sp. (vicinity of Chiang Mai)

Site: intestine Prevalence: 6.38% Mean intensity: 4.0 Abundance: 0.25

Ganeo tigrinus Mehra & Negi, 1928 (Fig. 5)

Diagnosis. Body 1.32-3.26 in length, 0.76-1.37 in width. Tegument covered by scale-like spines, except for posterior end of body. Oral sucker subterminal, size 0.069-0.139 x 0.079-0.23, muscular pharynx measuring 0.039-0.98 x 0.042-0.098, oesophagus 0.231-0.268 long, caeca at second third of body. Ventral sucker in anterior half of body, measuring 0.092-0.22 x 0.09-0.23. Testes near and before ventral sucker, almost spherical 0.125-0.348 x 0.158-0.347, cirrus sack elongate claviform, extending to ventral sucker, including seminal vesicle and strongly developed prostatic part; measuring 0.27-0.69 x 0.079-0.123. Genital opening lateral, marginal at level of posterior part of oesophagus. Ovary spherical, postero-lateral to ventral sucker, measuring 0.087-0.283 x 0.119-0.320. Seminal receptacle just beneath ovary. Uterine coils between ventral sucker and posterior extremity. Vitellaria along lateral sides in middle region of the body, overlapping intestinal caeca but leaving free their ends. Eggs numerous 0.023-0.031 x 0.013-0.019.





- 1. Diplodiscus amphichrus, ventral view
- 2. Encyclometra bungara, ventral view
- 3. Glypthelmins staffordi, ventral view
- 4. Pleurogenoides sphaericus, ventral view
- 5. Ganeo tigrinus, ventral view
- 6. Prostorchigenes majeedi, ventral view
- 7. Mesocoelium sociale, ventral view
- 8. Acanthostomum (Atrophecaecum) burminis, ventral view
- 9. Paradistomum geckonum, ventral view
- 10. Gogatea serpentium, ventral view
- 11. Euryhelmis sp. (adolescaria), ventral view

Host and locality: Rana sp. (vicinity of Chiang Mai)

Site: intestine Prevalence: 8.5% Mean intensity: 2.25 Abundance: 0.18

Prostorchigenes majeedi Simha & Hakim 1967 (Fig. 6.)

Diagnosis. Body 2.01-2.63 in length, 0.09-1.12 in width. Anterior half of the body covered by spines. Oral sucker measuring 0.16-0.22 x 0.19-0.27; pre-pharynx absent, pharynx small, size 0.069-0.075 x 0.071-0.098, oesophagus 0.18-0.32 long, caeca reaching posterior extremity or end before it. Ventral sucker small, measuring 0.11-0.16 x 0.12-0.16 in median line, before ovary. Testes oval, slightly obliquely in posterior half of the body; they measure 0.31-0.42 x 0.30-0.54; cirrus sack elongate at level of ovary, measuring 0.29-0.31 x 0.10-0.13 involving vesicula seminalis interna and prostate cells. Ovary measuring 0.19-0.24 x 0.20-0.26, at level of ventral sucker, seminal receptacle present, partle overlapping ovary. Uterine coils occupying pace between gonads and posterior extremity. Vitelline follicles numerous, small, scattered at caecal bifurcation. Eggs numerous 0.017-0.027 x 0.011-0.015.

Host and locality: Hemidactylus frenatus (vicinity of Chiang Mai)

Site: intestine Prevalence: 20.0% Mean intensity: 1.5 Abundance: 0.3

## Mesocoeliidae Dollfus, 1950

Mesocoelium sociale (Luhe, 1901) (Fig. 7)

Diagnosis. Body 2.6-3.4 in length, 0.5-0.74 in width. Tegument spinose, more conspicuous in anterior half of the body. Oral sucker measuring 0.21-0.32 x 0.27-0.31, prepharynx very short, pharynx size 0.09-0.12 x 0.062-0.091; oesophagus 0.22-0.79 long; intestinal caeca reaching posterior one-third of the body. Ventral sucker measuring 0.13-0.22 x 0.16-0.21, in anterior third of the body. Testes at sides of ventral sucker, near to caeca, right testis 0.14-0.23 x 0.11-0.22, left testis 0.14-0.22 x 0.12-0.25, vas deferens usually long cirrus sack 0.17-0.32 x 0.06-0.122 in size. Genital opening median at pharyngeal level. Ovary 0.14-0.23 x 0.17-0.24, dextral seminal receptacle 0.06-0.17 x 0.06-0.11 anteromedian to ovary. Vitellaria of large separate follicles from lateral margins of oral sucker near to tips of caeca. Eggs measuring 0.031-0.039 x 0.021-0.024.

Host and locality: Kaloula pulchra (vicinity of Chiang Mai)

Site: intestine Prevalence: 5.0% Mean intensity: 2 Abundance: 0.1

# Acanthostomatidae Poche, 1926

Acanthostomum (atrophecaecum) burminis (Bhalerao, 1926) (Fig. 8)

Diagnosis. Body elongate, slender, measuring 1.56-1.82 x 0.26-0.36. Tegument covering spines from anterior end to just past anterior vitelline follicles. Oral sucker cup-shaped, 0.15-0.19 x 0.14-0.16, armed with rod-like spines of variable size; ventral spines smaller 0.012-0.018, dorsal spines 0.018-0.025 long, prepharynx short 0.040-0.055, pharynx muscular 0.020-0.080 x 0.020-0.060 in size with strong fibres of sphincter muscles in anterior third; oesophagus very

short (0.025) or absent. Bifurcation of caeca just anterior of ventral sucker; caeca thin-walled, extending to posterior end of the body, opening to exterior through small, lateral pores. Ventral sucker 0.070-0.090 x 0.060-0.065, in anterior fourth of the body. Testes ellipsoidal, frequently contigous, anterior testis size 0.115-0.130 x 0.100-0.110; posterior testis 0.120-0.130 x 0.110-0.134, no cirrus sack; seminal vesicle voluminous lying free in parenchyma, measuring 0.50-0.70, genital opening just anterior to ventral sucker and armed with a comb-like structure. Ovary anterior to testes, spherical, 0.085-0.120 x 0.070-0.090 in size; seminal receptacle variable in size, 0.200-0.220 x 0.055-0.100. Loops of uterus extend between vitellaria from ovary to seminal vesicle. Vitellaria comprising small follicles in both lateral sides from seminal vesicle to seminal receptacle. Eggs 0.023-0.026 x 0.010-0.014, operculated.

Host and locality: Amphiesma deschauensis (Maesa stream, Doi Suthep-Pui National Park)

Site: intestine Prevalence: 100% Mean intensity: 10 Abundance: 10

### Dicrocoelidae Odhner, 1911

Paradistomum geckonum Bhalerao, 1929 (Fig. 9)

Diagnosis. Body lanceolate or broadly oval, measuring 4.16-5.34 x 2.11-2.67. Tegument aspinose. Oral sucker subterminal, size 0.31-0.41 x 0.29-0.39; muscular pharynx measuring 0.12-0.18 x 0.11-0.17; oesophagus very short or absent, intestinal caeca wide, reaching near to posterior extremity. Ventral sucker spherical, with poorly developed musculature. Testes oval, situating behind ventral sucker, right testis, size 0.17-0.25 x 0.22-0.39; left testis 0.18-0.26 x 0.24-0.43; cirrus sack oval, near to middle line of the body, 0.27-0.37 long, including seminal vesicle and prostate cells. Ovary spherical-oval, behind testes, measuring 0.19-0.31 x 0.23-0.31, seminal receptacle close to ovary. Uterine coils loosly packed, locating between ventral sucker and posterior extremity. Vitellaria in two lateral sides, comprising numerous follicles from ventral sucker to posterior third of the body. Eggs operculated, size 0.031-0.038 x 0.020-0.025.

Host and locality: Hemidactylus frenatus (vicinity of Chiang Mai)

Site: gall bladder Prevalence: 13.3% Mean intensity: 1.5 Abundance: 0.19

# Cyathocotylidae Poche, 1926

Gogatea serpentium (Gogate, 1932) (Fig. 10)

Diagnosis. Body bipartite, size 1.13-1.38 x 0.41-0.48. Forebody concave ventrally, hindbody short and cylindrical. Tegument of forebody spinose, Oral sucker measuring 0.06-0.013 x 0.08-0.13; pharynx muscular 0.028-0.054 x 0.027-0.063; oesophagus 0.028-0.138 long; intestinal caeca running dorsal to tribocytic organ and terminate near to posterior extremity. Ventral sucker 0.023-0.058 x 0.023-0.069 at anterior border of tribocytic organ. Tribocytic organ large, spherical 0.19-0.32 x 0.16-0.28 in size. Testes spherical or oval in posterior part of the body, anterior testis 0.09-0.19 x 0.1-0.16, posterior testis 0.06-0.17 x 0.089-0.16; cirrus sack elongate, club-shaped, 0.16-0.51 x 0.02-0.053 in size. Ovary oval or spherical, 0.071-0.13 x 0.042-0.128, median or lateral between testes. Vitellaria follicular, 23-32 in number, arranged irregularly on both side of the body or intruding into tribocytic organ, extending from ventral sucker to ovary. Uterine coils ventral to cirrus sack, genital opening at posterior extremity. Eggs few (2-3), large, measuring 0.089-0.175 x 0.048-0.083.

Host and locality: Xenochrophis piscator (Maesa stream, Doi Suthep-Pui National Park)

Site: intestine Prevalence: 100% Mean intensity: 2 Abundance: 2

## Heterophyidae Odhner, 1911

Euryhelmis sp. (adolescaria, Fig. 11)

Diagnosis. Body elongate in lateral direction, 1250; anteroposteriorly strongly pressed, 350. Oral sucker measuring  $60 \times 75$ , pharynx  $50 \times 60$  in size; prepharynx, oesophagus absent, caeca parallel with anterior edge of the body. Ventral sucker 50 in diameter. Testes situating in lateral parts of the body, lobated along posterior sides; right testis, size  $100 \times 175$ ; left testis  $100 \times 200$ . Primordium of ovary about  $50 \times 100$  in size. Vitelline follicles and eggs lacking. Excretory pore opposite to oral sucker, v-shaped, branches reaching inner end of testes.

Host and locality: Amolops afahanus (Maesa stream, Doi Suthep-Pui National Park)

Site: body surface Prevalence: 100% Mean intensity: 1 Abundance: 1

#### DISCUSSION

The species reported in this paper are common parasites of the hosts examined and are widely distributed in the adjacent countries of Thailand e.g. Burma: Bhalerao (1926, 1929, 1936), Meggitt (1927), Mehra and Negi (1928), Chatterji (1940); Laos: Scholz and Ditrich (1991, 1993); Malaysia (Fischthal and Kuntz (1965), Yeun (1962); Vietnam: Odening (1968), Moravec and Sey (1989). Considerable morphological and numerical variations have been observed on the species described in this pape; but the authors are of the opinion that these discrapencies are infraspecific variabilities of the species in question. There are, however, three species (Mesocoelium sociale, Acanthostomum (Atrophecaecum) burminis, Euryhelmis sp.) which are worthy of some remarks on the basis of our examples. Mesocoelium sociale was described for the firt time by Luhe (1901) from India and later from this (Singh, 1977) and from several other localities: Indonesia (Odhner, 1911), Burma (Meggitt, 1927); Bhalerao, 1936; Chatterji, 1940), Philippines (Tubangui, 1931; Fischthal and Kuntz, 1965) and from the overseas countries: Brazil (Travassos, 1924) and Paraguay (Odhner, 1911). Dollfus (1954) questioned its presence in South America. Freitas (1963) considered M. sociale, together with several other species of this genus to be synonymous to M. monas (Rudolphi, 1819) while Skrijabin and Morozov (1959), Yamaguti (1971) regarded it to be a valid species. Following the key recommended by Cheng (1960) for the species of the genus Mesocoelium our examples, on the basis of the position of the genital opening, well developed vitellaria and the length of caeca it is well fit for this species but the position of the testes shows considerable discrapency. Although they are at the level of the ventral sucker but they do not overlap the caeca, they are rather intercaecal. We consider this position of testes to be species specific variation and consequently it is regarded to be a valid species.

Bhalerao's species, now is nominated Acanthostomum (Atrophecaecum) burminis (Bhalerao, 1926) (Yamaguti, 1971) was originally described as Acanthochasmus burminis Bhalerao, 1926 from Burma. The genus Acanthochasmus was erected by Looss (1899) but as this name was pro-

occupied, Looss (1900) changed it Acanthochasmus. This view was accepted by e.g. Dollfus (1950) but others (Dawes, 1956, Yamaguti, 1971) preferred the genus name Acanthostomum. Bhalerao (1926) in the description of A. (A.) burminis, the crusial structural elements of the alimentary system (atrophied caeca and the presence of ani, which were later (Bhalerao, 1940) the basis of the creation of his new genus Atrophecaecum) were described as they are simple and without ani. In his recent paper, (Bhalerao, 1940) he cited that the structure of the alimentary system of this species has been inadequately described in his former (1926) paper and he presented an amended and quite different structure of the alimentary system. Khalil (1963) proposed to reduce the four closely related genera: Acanthostomum, Atrophecaecum, Gymnatrema and Haplocaecum to subgenera of Acanthostomum. Yamaguti (1971) has accepted the subgeneric splitting of Acanthostomum (Acanthostomum, Atrophecaecum) but the other two subgenera, Gymnotrema and Haplocaecum) were regarded to be synonyms with Acanthostomum and Atrophecaecum respectively. Acanthostomum (A.) burminis was reported subsequently by Thapar and Ali (1929). Agarwal (1966) and Srivastava (1982) from India. The latter author on the ground of the examination of a series of specimens came to the conclusion that this species performes a range of variations in almost all characters, even in the atrophy to the caecum therefore he regarded the related species: Acanthostomum hindusthanensis Baugh, 1956, A. simhai Khalil, 1963; A. (A.) alii Karykarte, 1967 and Haplocaecum proctoporum Dwivedi, 1966 to be synonymous to Acanthostomum (A.) burminis (Bhalerao, 1926). Nasir, (1974) in his survey of the genus Acanthostomum (A.), out of the nominated species (approximately more than 30 species) had recognized only two species A. (A.) scyphocephalum Braun, 1899 and A. (A.) imbutiforme Molin, 1850. We think that Nasir (1974) went too far because symple on the theoratical basis we can suppose that a helminth parasite which has Old and New World distribution and a broad variety of definitive hosts (marine and freshwater fishes, reptiles) should performed higher diversity than that of a single species. Secondly, it also shows that how much work should be done to clarify the scope of the individual variations on the one hand and the discontinuity of the specific characters on the other. Species of the genus Euryhelmis, using the analogy of the life cycle of the species Euryhelmis squamula (Rudolphi, 1819) have two intermediate hosts (gastropods, amphibians) and definitive hosts which are different carnivors, mainly species of mustelids (In Thailand there are 10 species of mustelids and a few in the study area [Cabitt and Stewart-Cox, 1995]). In the ecosystem of the Doi Suthep-Pui National Park all of the prerequistes are present which are necessary for the circulation of the life cycle of this species. The body shape and form of this adolescaria, the position of the caeca as well as the structure of the testes suggest a new species. Its designnation, however, needs to study the adult specimens as well.

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### บทคัดย่อ

สัตว์ครึ่งบกครึ่งน้ำ 5 ชนิต (Amolops afahanus, Ichthyophis supachaii, Kaloula pulchra, Mycrohyla sp., Rana sp.) และ สัตว์เลี้ยยคลาน 6 ชนิต (Amphiesma deschauensis, Cosymbotus platyurus, Liolepis belliana, Cuora amboiensis, Hemidactylus frenatus, Xenochrophis piscator) ที่จับได้จากลำน้ำแม่สา อุทยานแห่งชาติดอยสุเทพ-ปุย จังหวัดเชียงใหม่ และจากบางท้องที่ของจังหวัดเชียงใหม่ นำมาตรวจสอบหาพยาธิใบไม้ พบทั้งสิ้น 11 ชนิตคือ Diplodiscus amphichrus, Encyclometra bungara, Glyphthelmins staffordi, Pleurogenoides sphaericus, Ganeo tigrinus, Postochigenes majeedi, Mesocoelium sociale, Acanthostomum (Atrophecaecum) burminis, Paradistomum geckonum, Gogatea serpentium และ Euryhelmis sp. ทุกชนิตที่พบเป็นรายงานการพบจากสถานที่ใหม่