LARVAL STAGES OF MEDICALLY IMPORTANT FLUKES (TREMATODA) FROM VIENTIANE PROVINCE, LAOS. PART. I. METACERCARIAE

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SUMMARY

Metacercariae of five small flukes transmitted to humans, *Opisthorchis viverrini* (family Opisthorchiidae), *Centrocestus formosanus*, *Haplorchis pumilio*, *H. taichui* and *Stellantchasmus falcatus* (family Heterophyidae), were found in fish from Laos. All metacercariae, recorded from Laos for the first time, are described in detail and figured with special attention to the armature of ventrogenital com-

plex of heterophyid larvae. Fish *Hampala macrolepidota* and *Cyclocheilichthys repasson* represent new second intermediate hosts for *O. viverrini*; *Esomus longimana* for *C. formosanus*; *H. macrolepidota* and *E. longimana* for *H. pumilio*, *H. macrolepidota*, *H. dispar* and *Mystacoleucus marginatus* for *H. taichui*, and *Xenentodon cancila* for *S. falcatus*.

RÉSUMÉ : Les stades larvaires de Distomes d'importance médicale dans la province de Vientiane, Laos. Première partie : les métacercaries.

Les métacercaries de cinq espèces de Distomes (Trematoda) : *Opisthorchis viverrini* (Opisthorchiidae), *Centrocestus formosanus*, *Haplorchis pumilio*, *H. taichui* et *Stellantchasmus falcatus* (Heterophyidae) ont été trouvées pour la première fois dans des Poissons du Laos. Ces métacercaries sont décrites en détail, en particulier l'armature du complexe ventro-génital des larves d'Heterophyidae.

Les Poissons nouveaux seconds hôtes intermédiaires sont : *Hampala macrolepidota* et *Cyclocheilichthys repasson* pour *O. viverrini*; *Esomus longimana* pour *C. formosanus*; *H. macrolepidota* et *E. longimana* pour *H. pumilio*; *H. macrolepidota*, *H. dispar* et *Mystacoleucus marginatus* pour *H. taichui* et *Xenentodon cancila* pour *S. falcatus*.

INTRODUCTION

During the study on opisthorchiasis carried out in Laos in 1989 (*) snails and fish as potential intermediate hosts of trematodes were examined. Precise description of larval stages of human flukes is important because they can be misidentified with larvae of species, without medical importance, which simultaneously parasitize the same intermediate hosts. This paper presents a survey of larval stages (metacercariae) of medically important flukes found in fish

hosts during this investigation. The occurrence of cercariae in snails from Laos will be described elsewhere (Ditrich *et al.*, in prep.).

MATERIALS AND METHODS

The study was made in Vientiane province, particularly in rice fields around Vientiane city (Vientiane municipality) and at Nam Ngum water reservoir (Keo-Oudom district). Detailed description of the study area is given in papers of Ditrich *et al.* (1990) and Giboda *et al.* (1991).

A total of 782 fish of 45 species were examined for the presence of metacercariae. Methods of fish sampling and examination, as well as the list of localities and number of fish dissected, are given in the paper of Scholz (1991).

Measurements of metacercariae given in the present study in millimetres are based on examination of living or fixed (measurements in brackets) larvae.

Fish were identified by the senior author according to the books of Smith (1945) and Taki (1974). Identification was revised by M. Kottelat. Nomenclature of fish hosts is according to Kottelat (1989).

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SURVEY OF METACERCARIAE

Opisthorchiidae Braun, 1901.

1 — *Opisthorchis viverrini* (Poirier, 1886) (fig. 1).

Description: oval cyst measuring $0.19-0.25 \times 0.15-0.22$ surrounded by thick layer of host tissue (width $0.008-0.060$, overall diameter $0.20-0.31 \times 0.16-0.28$ ($0.18-0.33 \times 0.15-0.30$). Cyst containing quickly moving, C-shaped metacercaria.

Free metacercaria measuring $0.32-0.82 \times 0.10-0.14$ ($0.31-0.82 \times 0.08-0.21$), containing brownish pigment scattered throughout body. Body surface covered with small spines. On each body side, 12-13 sensillae $0.009-0.012$ long present. Subterminal, oval or rounded oral sucker measuring $0.048-0.080 \times 0.045-0.080$ ($0.044-0.074 \times 0.044-0.076$). Large ventral sucker, $0.046-0.085 \times 0.048-0.082$ ($0.040-0.097 \times 0.046-0.095$) in size, situated in posterior half of body. Prepharynx very short ($0.003-0.004$), pharynx measuring $0.020-0.031 \times 0.013-0.025$ ($0.017-0.028 \times 0.015-0.025$). Oesophagus long ($0.07-0.15$); intestinal caeca reaching posteriorly up to posterior half of excretory bladder. Cephalic, drop-shaped glands, 5-6 in number, situated on each side of oesophagus and opening near posterior margin of oral sucker. Small glands (« skin glands » after Vajrasthira *et al.*, 1961) scattered between oral sucker and cephalic glands. Nerve ring (commissure) located behind pharynx. Excretory bladder large, $0.06-0.16 \times 0.06-0.14$ ($0.05-0.18 \times 0.07-0.12$) in size, containing oval or rounded granules measuring $0.002-0.004$. Flame-cell formula (pattern) of metacercariae $2 [(3 + 3) + (3 + 3 + 3)] = 30$ (fig. 1).

Hosts: *Hampala dispar* — infected 16 fish from 111 examined, mean intensity of infection 6 (range 1-47); *H. macrolepidota* — 3/34, 6 (1-16); *Barbodes gonionotus* — 1/61, 7; *Puntius brevis* (syn. *P. leiacanthus*) — 3/22, 11 (1-12); *Puntius* sp. 1 — 2/13, 4 (1-6); *Puntius* sp. 2 — 1/39, 2; *Cyclocheilichthys repasson* — 17/27, 15 (1-66).

Localization: muscles.

Localities: Nam Ngum water reservoir — 9 localities (see Ditrich *et al.*, 1991), drain near Ban Phonmi (Phonhong surroundings), irrigation systems of rice fields in Vientiane suburb (Nong Sang Thoo settlement, canal Bung Salakham).

Comments: metacercariae under study correspond morphologically with larvae of *O. viverrini* described by Vajrasthira *et al.* (1961). Only small differences in arrangement and location of individual groups of flame cells of *O. viverrini* metacercariae from Laos, and larvae described and figured by the above authors, were recorded (fig. 1).

Fish species *Hampala macrolepidota* and *Cyclocheilichthys repasson* are new intermediate hosts for *O. viverrini*.

FIG. 1. — *Opisthorchis viverrini* (Poirier, 1886).

A: metacercaria partly released out of cyst, B: encysted metacercaria, C: free metacercaria.

The precise localization of *O. viverrini* metacercariae were observed only in a limited number of infected fish. In *Cyclocheilichthys repasson* specimens from a canal near Phonmi village, more than one third of larvae (36 %) were localized in body muscles, while only 13 % of larvae were found in muscles of pectoral fins. Similarly, Vichasri *et al.* (1982) recorded most *O. viverrini* metacercariae in body muscles of fish from northeastern Thailand. The above data, as well as results of Tesana *et al.* (1985), differ from Harinasuta and Vajrasthira (1960), who concluded that muscles of pectoral fins are the preferred site of *O. viverrini* metacercariae.

Heterophyidae Odhner, 1914.

2 — *Centrocestus formosanus* (Nishigori, 1924) (fig. 2).

Description: cyst oval, thick-walled, measuring $0.18-0.24 \times 0.14-0.20$. Thickness of outer cyst wall $0.009-0.019$; inner wall very thin, transparent. Metacercariae mostly C-shaped, measuring $0.46-0.49 \times 0.12-0.15$.

FIG. 2. — *Centrocestus formosanus* (Nishigori, 1924).

A: free metacercaria, B: head end, C: circumoral spines, D: encysted metacercaria (after Scholz, 1991).

Body surface densely covered with short spines. Oral sucker oval or nearly rounded, $0.041-0.057 \times 0.046-0.049$ in diameter. Circumoral spines present, arranged in two alternating rows of 32 triangular spines measuring $0.012-0.013 \times 0.003-0.004$ (16 larger spines), and $0.010-0.011 \times 0.003$ (smaller spines). Ventral sucker $0.036-0.045 \times 0.039-0.043$ in size. Prepharynx relatively long ($0.055-0.057$), longer than oesophagus ($0.032-0.039$). Pharynx measuring $0.026-0.034 \times 0.027-0.034$. Intestine containing numerous discoidal bodies, intestinal caeca short, not reaching to level of anterior margin of excretory bladder. Small, weakly visible anlage of ovary localized in front of X-shaped excretory bladder, anlagen of two testes situated laterally to it.

Host: *Esomus longimana* — 10/58, 11 (1-37).

Localization: gills.

Localities: Vientiane — Nong Sang Thoo settlement, Kham-savath village.

Comments: the species *C. formosanus* is a widely distributed parasite of fish-eating birds and mammals including man (Velasquez, 1982). It has been recorded in China, Taiwan, Japan, Philippines, and India (Premvati and Pande, 1974, Yanohara, 1985).

3 — *Haplorchis pumilio* (Looss, 1896) (fig. 3).

Description (two fixed specimens measured): oval, thin-walled cyst, $0.19-0.24 \times 0.18-0.20$ in diameter, containing quickly moving, C-shaped larva. Thickness of wall about $0.003-0.005$. Excysted metacercaria measuring $0.58-0.61 \times 0.14-0.19$. Body surface covered with numerous spines, posteriorly less densely arranged and smaller. Oral sucker widely oval, measuring $0.035-0.054 \times 0.058-0.062$. Ventral

FIG. 3. — *Haplorchis pumilio* (Looss, 1896).

A: encysted metacercaria, B, C: sclerites of ventrogenital complex, D: free metacercaria.

sucker measuring $0.031-0.054 \times 0.021-0.038$ modified into ventrogenital complex *. Sucker armed with crown of 32-36 sclerites $0.005-0.006$ long, interrupted dorsally. Sclerites with shape of two concavesided triangles joint by their apices. Length of teeth of triangles $0.001-0.002$. Pair of antero-lateral lobes with a few simple, $0.002-0.004$ long spines, often with fused bases. Mid-dorsal lobe armed with 7-9 long, simple spines $0.003-0.005$ in length (fig. 3). Prepharynx relatively long (0.07). Pharynx 0.040×0.038 in size. Length of oesophagus 0.15 . Intestinal caeca reaching up to anterior margin of excretory bladder. Anlage of testis measuring $0.024-0.067 \times 0.034-0.075$, situated in front of excretory bladder.

Hosts: *Hampala macrolepidota* — 1/34, 10; *Esomus longimana* — 1/153, 1.

Localization: muscles.

Localities: Nam Ngum water reservoir (1 locality), Vientiane — canal Bung Salakham.

Comments: in species identification of members of the genus *Haplorchis* Looss, 1899, the armature of ventrogenital sac represents the most reliable morphological feature (Pearson, 1964, Pearson and Ow-Yang, 1982). The morphology of this armature revealed that metacercariae from Laos are conspecific with *H. pumilio*.

(*) Armature of ventrogenital complex described according to terminology of Pearson (1964) and Pearson and Ow-Yang (1982).

FIG. 4. — *Haplorchis taichui* (Nishigori, 1924).

A: encysted metacercaria, B, D: sclerites of ventrogenital complex, E: free metacercaria.

H. pumilio metacercariae has hitherto been recorded in fish from Africa (Kenya, Egypt) and several Asian countries, e. g. Israel, India, China, Taiwan, Philippines (Hsü, 1950; Pearson, 1964; Pande and Shukla, 1972; Velasquez, 1973; Khalifa *et al.*, 1977; Sommerville, 1982 *a, b*). However, we did not succeed in finding any data on the occurrence of *H. pumilio* metacercariae from countries of the past Indochina (Vietnam, Cambodia, Laos). Moreover, both fish species, in which metacercariae were found, have not been reported as second intermediate hosts of *H. pumilio*.

4 — *Haplorchis taichui* (Nishigori, 1924) (fig. 4).

Description: thin-walled oval cysts measuring $0.025\text{--}0.025 \times 0.016\text{--}0.023$ ($0.019\text{--}0.031 \times 0.018\text{--}0.029$). Thickness of wall about $0.004\text{--}0.005$. Metacercaria measuring $0.32\text{--}0.44 \times 0.14\text{--}0.21$ ($0.32\text{--}0.61 \times 0.14\text{--}0.24$). Body surface covered with numerous scale-like spines up to 0.005 long, posteriorly becoming finer and less densely arranged. Oral sucker $0.042\text{--}0.057 \times 0.030\text{--}0.053$ ($0.032\text{--}0.057 \times 0.035\text{--}0.065$) in size. Ventral sucker measuring 0.042×0.049 ($0.027\text{--}0.046 \times 0.024\text{--}0.050$) armed with ventral, crescentic (semi-lunar) group of 12-14 long hollow spines ($0.006\text{--}0.022 \times 0.002 \times 0.003$ in size) and 3-4 smaller, simple sclerites. Band of minute spines present dorsally to

FIG. 5. — *Stellantchasmus falcatus* (Onji et Nishio, 1916). A: encysted metacercaria, B, C: spination of ventral sucker.

inner ends of central sclerites of crescentic group and on medial face of ventral lips. Prepharynx relatively short (up to 0.015). Pharynx measuring $0.027\text{--}0.038 \times 0.026\text{--}0.034$ ($0.024\text{--}0.048 \times 0.022\text{--}0.036$). Oesophagus $0.04\text{--}0.11$ long ($0.05\text{--}0.16$). Intestinal caeca filled with refractile discoid bodies, $0.009\text{--}0.010$ in diameter. Testis anlage ($0.019\text{--}0.052 \times 0.026\text{--}0.057$ in size) situated in posterior part of body.

Hosts: *Hampala macrolepidota* — 2/34, 2 (1-2); *H. dispar* — 4/111, 7 (1-19); *Mystacoleucus marginatus*? juv. — 8/16, 6 (1-22); *Barbodes gonionotus* — 4/61, 3 (2-5).

Localisation: muscles.

Localities: Nam Ngum water reservoir (2 localities), river Mekong in Vientiane.

Comments: *H. taichui* metacercariae have been recorded in fish from any Asian countries, e. g., Israel, India, China, Taiwan, Philippines (Hsü, 1950; Pearson, 1964; Nath and Pande, 1970; Velasquez, 1973). In Thailand, *H. taichui* larvae were found in *Puntius* (= *Barbodes*) *gonionotus*, *Puntius leiacanthus* (= *brevis*) and *P. orphoides* (Kliks and Tantachamrun, 1974). However, the fish of the genus *Hampala* have not been referred to as a host of *H. taichui* metacercariae.

5 — *Stellantchasmus falcatus* Onji et Nishio, 1916 (fig. 5).

Description: metacercaria surrounded by oval or nearly rounded cyst measuring $0.020\text{--}0.021 \times 0.016\text{--}0.017$ ($0.020\text{--}0.032 \times 0.018\text{--}0.026$). Thickness of cyst wall $0.004\text{--}0.005$. Size of metacercaria $0.27\text{--}0.35 \times 0.12\text{--}0.25$ ($0.20\text{--}0.24 \times 0.13\text{--}0.16$).

Body surface covered with numerous spines up to long 0.003-0.004, posteriorly finer and less densely arranged. Oral sucker measuring 0.038×0.049 ($0.026-0.043 \times 0.026-0.045$). Oval ventral sucker measuring 0.025×0.022 ($0.023-0.027 \times 0.025-0.027$), armed with minute spines encircling mouth and forming two dense, elevated latero-ventrally situated masses. Prepharynx relatively short ($0.02-0.03$). Pharynx 0.030×0.030 ($0.023-0.027 \times 0.020-0.025$). Intestinal caeca reaching only to a level of testis anlagen. Measurements of anlagen $0.024-0.035 \times 0.027-0.039$.

Expulsor elongated ($0.045-0.062 \times 0.023-0.026$ in size), situated behind ventral sucker.

Host: *Xenentodon cancila* — 1/2, 15.

Localization: fins.

Locality: Nam Ngum water reservoir.

Comment: *S. falcatus* metacercariae were found in *Mugil cephalus*, *M. dussumieri*, *Anabas testudineus*, *Liza menada*, *Acanthogobius flavimanus* from Egypt, Israel, Taiwan, Hong Kong, Korea, Japan, Philippines, Hawaii (Chen, 1950; Noda, 1959; Pearson, 1964; Komiya and Suzuki, 1966; Lee and Cheng, 1970; Velasquez, 1982; Chai and Sohn, 1988; Radomyos *et al.*, 1990). In northeast Thailand, metacercariae of *S. falcatus* were recorded in fins of *Dermogenys pusillus* (Kliks and Tantachamrun, 1974).

DISCUSSION

The examination of fish from Vientiane province confirmed the presence of metacercariae of some medically important flukes. From trematode species, metacercariae of which were found in fish, the small liver fluke, *Opisthorchis viverrini*, is the most important parasite from a medical point of view (see Harinasuta and Vajrasthira, 1960; Wykoff *et al.*, 1965, etc.).

All four heterophyid flukes found in fish from Laos can parasitize humans eating raw fish. Cases of human infection with heterophyid trematodes are numerous mainly in Southeast Asia and Japan where dishes prepared from raw or poorly cooked animals are popular (Kagei *et al.*, 1964; Manning *et al.*; Kliks and Tantachamrun, 1974; Tantachamrun and Kliks, 1978; Soe *et al.*, 1984; Radomyos *et al.*, 1990). In some cases, atypically located worms can cause serious complications (see Manning *et al.*, 1971; Vasquez, 1982).

All larval stages of medically important flukes have been reported from Laos for the first time. Moreover, no data are available on the occurrence of all developmental stages including adults of heterophyid trematodes in that country. This fact is caused, above all, by the absence of detailed helminthological investigations of this problem.

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