FISH AS THE NATURAL SECOND INTERMEDIATE HOST OF *GNATHOSTOMA SPINIGERUM*

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Abstract. Gnathostomiasis is a helminthic disease most frequently occurring in Thailand. Human infections are usually found to be caused by *Gnathostoma spinigerum*, although five species of the genus *Gnathostoma* exist in Thailand, and three of these are capable of infecting man. In Thailand, 47 species of vertebrates – fish (19), frogs (2), reptiles (11), birds (11) and mammals (4) – have been reported to serve naturally as the second intermediate (and/or paratenic) hosts of *G. spinigerum.* Of these, fish, especially swamp eels (*Monopterus albus*), were found to be the best second intermediate/paratenic hosts: they had the highest prevalence rate and the heaviest infection intensity. However, the scientific names of these fish have been revised from time to time. Therefore, for clarity and consistency, we have summarized the current scientific names of these 19 species of fish, together with their illustrations. We describe one additional fish species, *Systomus orphoides* (*Puntius orphoides*), which is first recorded as a naturally infected second intermediate host of *G. spinigerum*.

INTRODUCTION

Several helminthic zoonoses can be transmitted to humans via both marine and freshwater fish. These include capillariasis (caused primarily by *Capillaria phillipinensis*), gnathostomiasis (*Gnathostoma spinigerum*), anisakiasis (*Anisakis simplex*), dioctophymiasis (*Dioctophyme renale*), eustrongylidiasis (*Eustrongylides* spp), clonorchiasis (*Clonorchis sinensis*), opisthorchiasis (*Opisthorchis viverrini*), echinostomiasis (*Echinostoma* spp), heterophyiasis (*Heterophyes heterophyes*), metagonimiasis (*Metagonimus yokogawai*), nanophyetiasis (*Nanophyetus salmincola*) and Diphyllobothriasis (*Diphyllobothrium latum*) (Beaver *et al*, 1984; Eastburn *et al*, 1987; Eberhard *et al*, 1989; Harrell and Deardorff, 1990; Miyazaki, 1991; Anderson, 1992; Narr *et al*, 1996). In Thailand, fish-borne helminthiases are rather common; the two most prevalent infections among Thai people are opisthorchiasis and gnathostomiasis (Daengsvang, 1980, 1986; Viyanant, 1981; Jongsuksuntigul and Imsomboon, 1998).

Human gnathostomiasis is a disease primarily caused by larval and immature stages of *G. spinigerum* (Daengsvang, 1980; Miyazaki, 1991). However, four other species – *G. hispidum, G. doloresi, G. nipponicum* and *G. binucleatum* – are also known to cause disease (Araki, 1986; Ogata *et al*, 1988; Ando *et al*, 1988; Nawa *et al*, 1989; Almeyda-Artigas, 1991; Akahane *et al*, 1998; Almeyda-Artigas *et al*, 2000). There have been at least five species of *Gnathostoma* documented in Thailand: *G. spinigerum*, *G. hispidum, G. doloresi*, *G. vietnamicum* and *G. malaysiae* (Dissamarn *et al*, 1966; Daengsvang, 1973, 1980; Kamiya *et al*, 1987); however, only *G. spinigerum* is known to be responsible for human infection in the country (Daengsvang, 1980, 1986; Radomyos and Daengsvang, 1987).

In Thailand, 47 species of vertebrates – fish (19), frogs (2), reptiles (11), birds (11) and mammals (4) – are reported to serve naturally as the second intermediate (and/or paratenic) hosts of *G. spinigerum* (Daengsvang, 1980; Rojekittikhun *et al*, 1989a, 1989b). Of these animals, the fish, especially swamp eels (*Monopterus albus*), have been found to be the best second intermediate/paratenic hosts of the worm: they have the highest prevalence rate and the heaviest infection intensity; moreover, they have been found to harbor at least four species of *Gnathostoma* (Daengsvang, 1980; Rojekittikhun *et al*, 1989a, 1998a, 1998b; Setasuban *et al*, 1991; Akahane *et al*, 1995; Nuamtanong *et al*, 1998).

The Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, has conducted a considerable amount of research into *Gnathostoma* and gnathostomiasis. Advanced third-stage larvae from swamp eels and many other freshwater fish are collected regularly. However, the scientific names of these fish have been subject to periodic revision. In the interest of clarity and consistency, we have summarized the current scientific names of these 19

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species of fish with their illustrations. We also describe one additional fish species, *Systomus orphoides* (*Puntius orphoides*), which is first recorded as a naturally infected second intermediate host for *G. spinigerum*.

MATERIALS AND METHODS

Fish

Fish and eels that had grown naturally in endemic areas were the main target of this study. Fish, dead or alive, were usually purchased from local markets in Nakhon Nayok and Prachin Buri; occasionally fish were obtained from several other provinces beyond Central Thailand. The fish were transported to the laboratory in Bangkok. They were measured, weighed, and identified by species.

Press preparation (compression) method

The visceral organs of the big fish and the eels were taken out; the liver was cut into small pieces which were firmly pressed between two thick glass plates and then examined under a dissecting microscope or a big hand lens for the presence of gnathostome larvae. All muscles were cut and scraped out of bones and scales (or skins) and examined using the method used for liver tissue. For tiny fish, the whole body was pressed and examined as described above. The collected larvae were cleaned, counted and identified.

Digestion technique

The livers or muscles of the fish were chopped and put into a container containing artificial gastric juice (1% HCl and 1% pepsin). They were then incubated for 1-3 hours in a water bath at 37 ^{o}C with frequent stirring. The digested tissues were washed several times with normal saline by a simple sedimentation technique. The clear sediments were examined under a dissecting microscope.

RESULTS

The 20 species of fish in Thailand that have been found to be naturally infected with *G. spinigerum* larvae are shown in Table 1. Their previous and current scientific names, common names and Thai names together with the maximum number of larvae per fish are also tabulated. Of note is the fact that of the 20 species of 16 genera, 9 genera and 6 species have been changed. Five species, *Boesemania microlepis, Chitala ornata, Mastacembelus armatus, Ompok krattensis* and *Systomus orphoides,* harbored only one larva each. Two species, *Micronema apogon* and *Trichogaster trichopterus*, had no record of the maximum number of infected larvae. Swamp eels (*Monopterus albus*) were the most resilient carrier: 2,582 gnathostome larvae were found in the body of one eel. *Systomus orphoides*, the red-cheek barb, was for the first time, found to be a natural second intermediate host of *G. spinigerum*.

Fig 1 shows photographs of the 20 fish with their current scientific and Thai names. Eighteen pictures were taken from fresh specimens, pictures 1.2 and 1.14 were from an illustrated poster (Vidthayanon C. Fishes of Chao Phraya River), and picture 1.16 was drawn by Luang Masya (Smith, 1965). The size of each fish is indicated by a ruler in each picture.

DISCUSSION

Almost all of the 20 fish naturally infected with *G. spinigerum* larvae feed on both live or decaying animals and plants. The gourami (*Trichogaster* spp) feeds mainly on tiny living organisms and insects. Therefore, they act more likely as the second intermediate host of the nematode. Eels (*Monopterus* and *Ophisternon*), snake-head fish (*Channa* spp) and catfish (*Clarias* spp) are carnivorous creatures. Not only do they act as second intermediate hosts, they also serve as paratenic hosts. This, without doubt, increases their chance of becoming more and heavily infected.

The drum fish (*Boesemania microlepis*) is a marine fish; it can, however, live in fresh water for periods of time. Kasemsuthi *et al* (1983) have demonstrated how this kind of fish was infected: Java tilapias (*Oreochromis mossambica*, previously *Tilapia mossambica*), an amphidromous fish, was collected from the sea at Bang Saen Beach and then orally infected with *G. spinigerum* larvae after the salinity of the water was reduced to zero. The salinity of the water was then gradually increased to 30 ppt and the fish were examined at days 20 and 40 post-infection. The infection rates were 71.4% and 58.8%, respectively; the corresponding survival rates of the recovered larvae were 87.5% and 52.6%, respectively. The flesh of these infected fish, containing *G. spinigerum* larvae, was fed to a real marine fish, *Epinephelus* spp, which was examined about 1-2 months later: it was infected and the larvae were still alive (Kasemsuthi, personal communication). Another experiment done in 1974 by Nithi-Uthai showed that two species of brackish water fish, *Mugil* sp and *Chanos chanos*, could be infected with *G. spinigerum* larvae by both forced- and self-feeding. The percentages of infection were 56.0% and 80.0%, respectively, for forced feeding, and 53.3% and 30.0%, respectively, for self-feeding. However, examination of four species of brackish water fish and

Table 1
The twenty species of fish in Thailand found to be naturally infected with *Gnathostoma spinigerum* larvae.

Current scientific name	Previous name	Common name	Thai name	Length (cm)[a]	Max. no. of larvae/fish
1. *Anabas testudineus*	Same	Common climbing perch	Pla mo Thai	10-23	14
2. *Arius caelatus*	*Tachysurus caelatus*	Engraved catfish	Pla kot	20-29	5
3. *Boesemania microlepis*	*Nibea soldado*	River drumfish	Pla ma	17-60	1
4. *Channa striata*	*Ophicephalus striatus*	Striped snake-head fish	Pla chon	30-70	153
5. *Channa micropeltes*	*Ophicephalus micropeltis*	Giant snake-head fish	Pla chado	30-75	12
6. *Channa lucius*	*Ophicephalus lucius*	Blotched snake-head fish	Pla krasong	20-65	12
7. *Clarias batrachus*	Same	Batrachian walking catfish	Pla duk dan	16-40	6
8. *Clarias macrocephalus*	Same	Gunther's walking catfish	Pla duk ui	15-35	16
9. *Chitala ornata*	*Notopterus chitala*	Spotted featherback	Pla krai	35-85	1
10. *Kryptopterus cryptopterus*	Same	?	Pla kha kai	11-16	5
11. *Macrognathus siamensis*	Same	Spotted spiny eel	Pla lot chut	15-30	8
12. *Mastacembelus armatus*	Same	Armed spiny eel	Pla krathing dam	20-70	1
13. *Micronema apogon*	*Kryptopterus apogon*	Common sheatfish	Pla nam ngoen	15-77	?
14. *Monopterus albus*	*Fluta alba*	Swamp eel	Pla lai na	30-95	2,582
15. *Ompok krattensis*	*Ompok bimaculatus*	Sheatfish	Pla nua on	15-20	1
16. *Ophisternon bengalense*	*Synbranchus bengalensis*	Eel-like fish, Bengal eel	Pla lat, pla lai	25-53	40
17. *Systomus orphoides*	*Puntius orphoides*	Red-cheek barb	Pla kaem cham	8-22	1
18. *Trichogaster pectoralis*	Same	Snake skin gourami	Pla salit	15-20	5
19. *Trichogaster microlepis*	Same	Moonlight gourami	Pla kradi nang	6-15	5
20. *Trichogaster trichopterus*	*Trichopodus trichopterus*	Three-spot gourami	Pla kradi mo	5-12	?

[a] Normal length of common fish (Smith, 1965; Department of Fisheries, Ministry of Agriculture and Cooperatives, 1997).

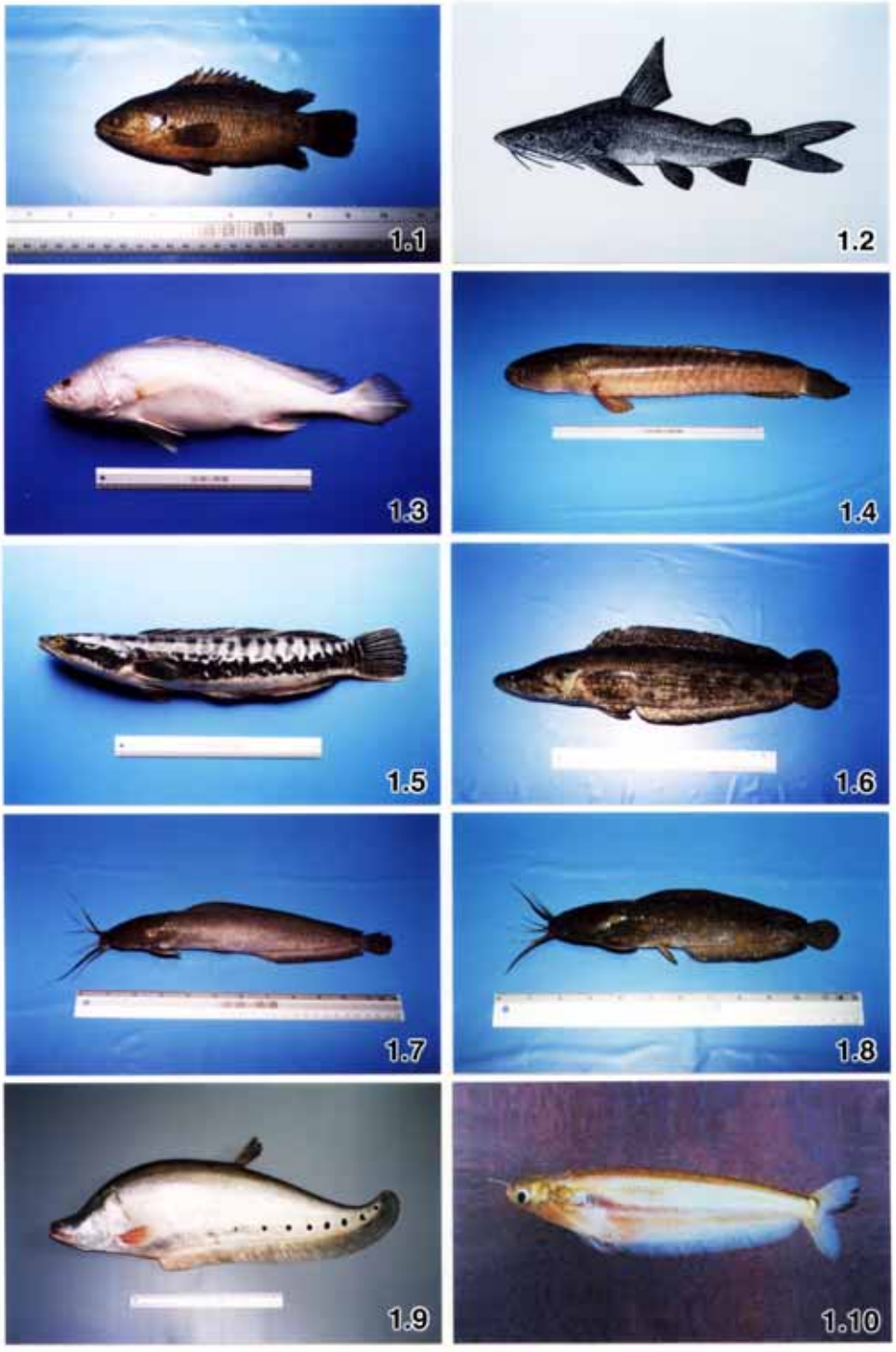
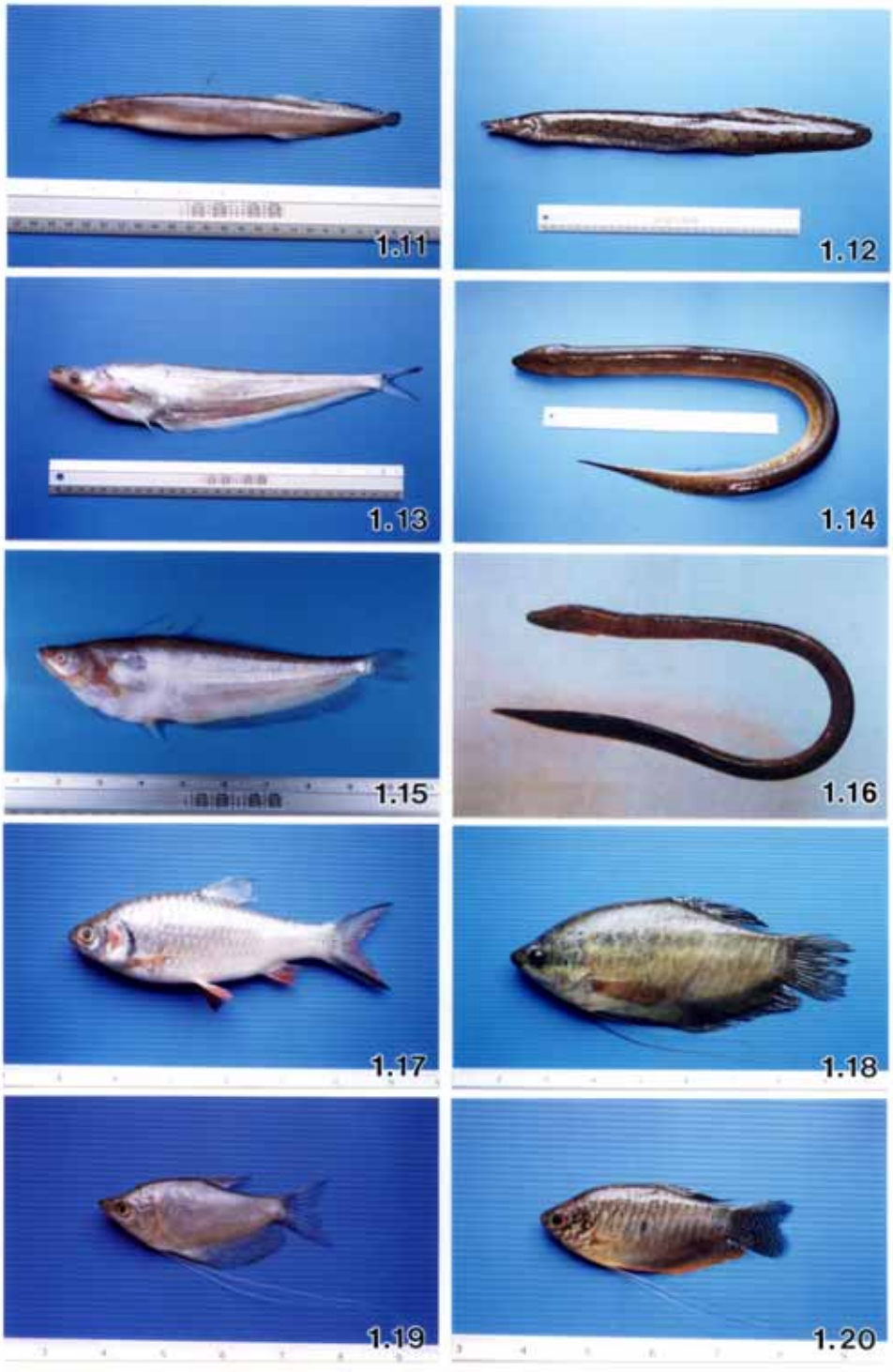


Fig 1- Illustrations of the 20 fish species in Thailand found to be naturally infected with *G. spinigerum* larvae. Their current scientific names, with Thai names in parentheses, are as follows: 1.1. *Anabas testudineus* (pla mo Thai), 1.2. *Arius caelatus* (pla kot), 1.3. *Boesemania microlepis* (pla ma), 1.4. *Channa striata* (pla chon), 1.5. *Channa micropeltes* (pla chado), 1.6. *Channa lucius* (pla krasong), 1.7. *Clarias batrachus* (pla duk dan), 1.8. *Clarias macrocephalus* (pla duk ui), 1.9. *Chitala ornata* (pla krai), 1.10. *Kryptopterus cryptopterus* (pla kha kai), 1.11. *Macrognathus siamensis* (pla lot chut), 1.12.



Mastacembelus armatus (pla krathing dam), 1.13. *Micronema apogon* (pla nam ngoen), 1.14. *Monopterus albus* (pla lai na), 1.15. *Ompok krattensis* (pla nua on, pla cha-on), 1.16. *Ophisternon bengalense* (pla lat, pla lai), 1.17. *Systomus orphoides* (pla kaem cham), 1.18. *Trichogaster pectoralis* (pla salit), 1.19. *Trichogaster microlepis* (pla kradi nang), 1.20. *Trichogaster trichopterus* (pla kradi mo).

34 species of marine fish from the Gulf of Thailand and adjacent areas for natural infection of gnathostome larvae did not demonstrate any infection (Nithi-Uthai, 1974).

In Thailand, there are about 145 species of cyprinoid fish (Smith, 1965). Until this study, none had been found to serve as the host of *Gnathostoma*. In this study, the red-cheek barb (*Systomus orphoides*) was shown for the first time to be infected. If a great number of fish of any species were to be subjected to careful examination, it would be more than likely that they too would find their way onto the list of naturally infected second intermediate hosts of *Gnathostoma.*

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Gnathostoma nematodes require two intermediate hosts and one definitive host to complete their life cycles (Fig. 1). In general, the adult worms live and spawn in a tumor-like mass in the stomach of the definitive host (e.g., cat, tiger, leopard or dog in the case of G. spinigerum).Â Humans are not definitive hosts of Gnathostoma spp., and L3 cannot mature into adults in them [31].Â Recent studies have demonstrated that G. spinigerum ES antigens modulate monocyte function via inhibition of Fc gamma receptor I expression, and trigger apoptosis of the peripheral blood mononuclear cells mainly via the extrinsic pathway [37, 38]. A second intermediate host, generally a fish but may also be an amphibian or other vertebrate, consumes the infected crustacean and provides the muscle tissue in which the larvae molt and become advanced third-stage larvae. At this point, the G. spinigerum larvae are very infective to their definitive hosts, but they may simply roam throughout the tissues of a wrong host without reaching sexual maturity. With 36 different species as paratenic hosts the worms are able to have a widespread distribution.Â 1966. Further Investigations On Natural And Experimental Hosts Of Larvae Of Gnathostoma Spinigerum In Thailand. American Journal of Tropical Medicine and Hygiene, 15: 727-729. Department of Parasitology, Faculty of Medicine, Chiang Mai University, THAILAND, 2004. Gnathostoma spinigerum infects vertebrate animals. In the natural definitive host (cats, dogs, wild animals) the adult worms reside in a tumor which they induce in the gastric wall. They deposit eggs that are immature when passed in the feces. After maturation in water, the egg releases a first stage larva (L1). After ingestion by a small crustacean (Cyclops) (first intermediate host), the L1 develops into a L2.Â Alternatively, the second intermediate host may be ingested by another animal (paratenic host) in which the L3 does not develop further, but remains infective to the next predator. Humans become infected by eating undercooked fish or poultry containing L3s, or reportedly by drinking water containing L2-infected Cyclops. Fish as the natural second intermediate host of Gnathostoma spinigerum. W. Rojekittikhun, J. Waikagul, Tossapon Chaiyasith. Biology, Medicine. The Southeast Asian journal of tropical medicineâ€¦ 2002. Gnathostomiasis is a helminthic disease most frequently occurring in Thailand. Human infections are usually found to be caused by Gnathostoma spinigerum, although five species of the genusâ€¦ Expand. 30. PDF. View on PubMed. Save. Alert. Human infections are usually found to be caused by Gnathostoma spinigerum, although five species of the genus Gnathostoma exist in Thailand, and three of these are capable of infecting man. In Thailand, 47 species of vertebrates--fish (19), frogs (2), reptiles (11), birds (11) and mammals (4)--have been reported to serve naturally as the second intermediate (and/or paratenic) hosts of G. spinigerum.Â We describe one additional fish species, Systomus orphoides (Puntius orphoides), which is first recorded as a naturally infected second intermediate host of G. spinigerum. Download full-text PDF. Source.