RESEARCH NOTES

AEDES (FINLAYA) TOGOI THEOBALD 1907, CHANTHABURI STRAIN, A LABORATORY VECTOR IN STUDIES OF FILARIASIS IN THAILAND

It has been known for a long time that the Aedine mosquito, *Aedes (Finlaya) togoi* (Taiwan strain) is a very useful laboratory vector in studies of filariasis. It has also been proven to be highly susceptible to various species of filarial parasites, i.e., subperiodic *Brugia malayi*, periodic *B. malayi*, *B. pahangi*, rural strain of *Wuchereria bancrofti*, *Dirofilaria immitis*, *Breinlia* sp. and *Setaria* sp. (Ramachandran *et al.*, 1963. *Ann. Trop. Med. Parasit.*, *57*:443). Although *Ae. togoi* has been reported in Thailand by Gould *et al.*, (1968. *Am. J. Trop. Med. Hyg.*, *17*:609), this mosquito species has not been intensively tested for the filarial susceptibility, particulary filarial parasite strains from different localities in Thailand, except a report by Choochote *et al.*, (1983. *J. Parasit. Trop. Med. Ass. Thailand*, *6*:25). The authors have demonstrated that this mosquito species from Koh Nom Sao, Chanthaburi Province, southeast Thailand, is highly susceptible to nocturnally subperiodic *B. malayi* (Narathiwat, Southern Thailand strain) and *B. pahangi* (Malaysia strain). Therefore, the present study reports the susceptibility of *Ae. togoi* (Koh Nom Sao, Chanthaburi, Southeast Thailand) to nocturnally subperiodic *W. bancrofti* (Tak, Northwest Thailand strain and Kanchanaburi, West Thailand strain) and *D. immitis* (Chiang Mai, Northern Thailand strain).

The laboratory strain of *Ae. togoi* was originally obtained from Koh Nom Sao, Chanthaburi Province, Southeast Thailand. Larvae of this mosquito species were taken from their breeding places and have been reared in the insectarium of the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand, since 1981. Subsequently, the colony has been continuously maintained for many generations in the insectary of the Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50000, Thailand, since 1983. It is easily bred and maintained in the laboratory on simple media (deionized tap-water) and larval food (ground animal chow), is a good blood feeder on golden hamster and white rat, mate readily in a 30 × 30 × 30 cm cube cage, and shows a high survival rate. It is also an autogenous mosquito.

In order to determine the filarial susceptibility rates, three-day-old adult females of *Ae. togoi* mosquitoes (fasted for 12 hours) were allowed to feed on heparinized blood of 8-year-old boy (resident of Amphur Sangklaburi, Kanchanaburi Province, West Thailand) infected with nocturnally subperiodic *W. bancrofti*, 25-year-old man (resided in Amphur Mae-Ramat, Tak Province, Northwest Thailand) infected with nocturnally subperiodic *W. bancrofti* and dog (strain from Chiang Mai Province, Northern Thailand) infected with *D. immitis*, using artificial membrane feeding technique as described by Chomcharn *et al.*, (1980. *Southeast Asian J. Trop. Med. Pub. Hlth.*, *11*: 408). Fourteen days after feeding, all mosquitoes were dissected in normal saline solution and examined under a dissecting microscope. The number of mosquitoes

Table 1

The infective rate and density load in *Ae. togoi* (Koh Nom Sao, Chanthaburi) after feeding on infected blood *(W. bancrofti, B. malayi, B. pahangi* and *D. immitis)*, all dissected 14 days after feeding.

Species and strains of filarial parasites	Mf. density per c.mm	No. dissected	No. infected (%)	Average No. larvae per infected mosquito	Total No. larvae recovered (range)
W. bancrofti, Tak strain	1.20	50	34(68.00)	4.38	149(1-10)
W. bancrofti, Kanchanaburi strain	0.85	96	42(43.75)	2.24	94(1-6)
B. malayi, Narathiwat strain*	3.45	23	12(52.17)	2.92	35
B. pahangi, Malaysia strain*	5.90	16	11(68.75)	3.91	43
D. immitis, Chiang Mai strain	3.80	52	50(96.15)	6.28	314(1-21)

* From Choochote *et al.*, 1983.

with one or more infective larvae in head, thorax and abdomen were recorded.

The infective rates of *Ae. togoi* to nocturnally subperiodic *W. bancrofti* (Tak and Kanchanaburi strain) and *D. immitis* (Chiang Mai strain) were 68.00%, 43.75% and 96.15% respectively. The details are shown in Table 1. In addition, the infective rates of this mosquito species to nocturnally subperiodic *B. malayi* (Narathiwat, Southern Thailand strain) and *B. pahangi* (Malaysia strain) reported by Choochote *et al.*, (1983. *J. Parasit. Trop. Med. Ass. Thailand, 6*:25) are also included in Table 1.

The results indicate that *Ae. togoi* (Koh Nom Sao, Chanthaburi, Southeast Thailand) serve as a good laboratory vector model for study of filariasis in Thailand. *Ae.togoi* strain from Taiwan has been extensively used research work in Thailand, now our laboratory can provide the indigenous *Ae. togoi* colony to other scientists whenever the study of filariasis is in need.

WEJ CHOOCHOTE, PRASERT KEHA, KETURAT SUKHAVAT, CHIRASAK KHAMBOONRUANG and KOM SUKONTASON. Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50000, Thailand.

SCANNING ELECTRON MICROSCOPIC STUDY OF THIRD-STAGE LARVA OF *WUCHERERIA BANCROFTI* AND *BRUGIA MALAYI* IN THAILAND

Even though extensive studies of the surface morphology of third-stage larvae of filariae from vectors by scanning electron microscope (SEM) have been documented, *i.e.*, *Wuchereria bancrofti* (Franz and Zielke, 1980. *Tropenmed. Parasit.*, *31* : 345) and *Brugia pahangi* (Aoki *et al.*, 1980. *J. Parasit.*, *66*: 449; Suguri *et al.*, 1985. *Jap. J. Parasit.*, *34* : 479), the third-stage larvae of *W. bancrofti* and *B. malayi* especially the strains from Thailand have not been studied yet. Therefore, the present studies were undertaken to examine the surface morphological structures of third-stage larvae of nocturnally subperiodic *W. bancrofti* strain from Tak Province, Northwest Thailand and nocturnally subperiodic *B. malayi* strain from Narathiwat Province, Southern Thailand. The comparative studies of other strains described by previous investigators are also discussed.

The third-stage larvae or infective stages of both nocturnally subperiodic *W. bancrofti* and nocturnally subperiodic *B. malayi* were obtained from *Aedes togoi* (Koh Nom Sao, Chanthaburi Province, Southeast Thailand) mosquitoes which were fed on infected blood using artificial membrane feeding techniques as described by Chomcharn *et al.*, (1980. *Southeast Asian J. Trop. Med. Pub. Hlth.*, *11* : 408). For nocturnally subperiodic *W. bancrofti*, infected blood were obtained from a 22-year-old man resident of Amphur Pop-Pra, Tak Province, Northwest Thailand. For nocturnally subperodic *B. malayi*, the infected blood was taken from an infected cat, the origin of the parasite was from a 20-year-old woman, a resident of Bang Paw District, Narathiwat Province, Southern Thailand. It was experimentally infected to domestic cats and are now kept in the Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50000, Thailand. The engorged mosquitoes were then reared in the insectarium (12 hours illumination, 27 ± 2°C, 50-80% RH). Fourteen days after feeding, all mosquitoes were dissected in normal saline solution and examined under a dissecting microscope. The freshly infective larvae of both filarial species recovered from infected mosquitoes were washed repeatedly in 0.1M phosphate buffer pH 7.2 and then fixed in 4% paraformaldehyde in 0.1M phosphate buffer pH 7.2. For postfixation one percent osmium tetraoxide in 0.1M phosphate buffer pH 7.2 was used. Specimens were dehydrated in a grade series of ethanol, transferred into 100% amyl acetate through the mixture of ethanol and amyl acetate, dried in a liquid CO_2, critical-point dryer, mounted on stups and coated with gold in a vacuum evaporator (EIKO IB-3). The specimens then were examined with Hitachi S-450 scanning electron microscope at an accelerating voltage of 15 KV.

In nocturnally subperiodic *W. bancrofti*, the mouth was formed as a 1.44 μm long fissure (Fig. 1). There were four pairs of oral papillae arranged in two concentric circles, each circle consisted of two subdorsal and two subventral papillae. The four smaller inner papillae measured 0.46 × 0.65 μm in diameter and appeared as nipple-like protuberance. The four outer papillae were distinct and round with a diameter of 0.72 × 1.14 μm. Apart from these oral papillae, the two amphidial opening were formed as fissure, measured 0.75 μm in length. The cuticle of larva showed transversed annulations throught the body, except at the anterior and posterior ends and measured 3.41 μm in breadth (Fig. 2). The lateral lines were observed as the interruption of the cuticular

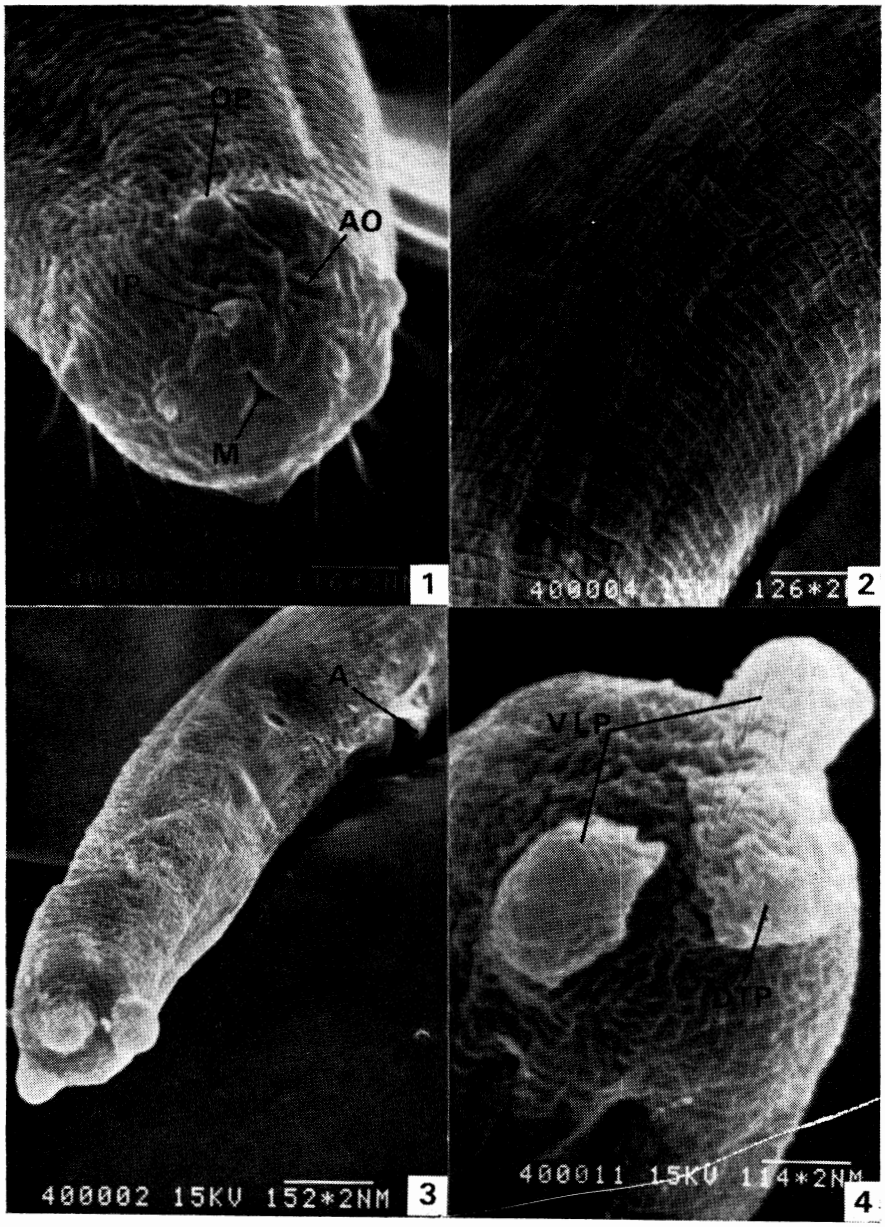

Fig. 1—Head region of third-stage larva of nocturnally subperiodic *W. bancrofti*. Note the fissure mouth (M), inner papilla (IP), outer papilla (OP), and amphidial opening (AO). × 10000.

Fig. 2—Showing the transversed annulations of body cuticle. Note the lateral lines as interruption of the cuticular annulations and the longitudinal folds cleary seen. × 5700.

Fig. 3—The tail end of third-stage larva. Note the crescent-shaped anus (A) and three caudal papillae. × 2900.

Fig. 4—A higher magnification of tail end. Note the two ventrolateral papillae (VLP) and dorsoterminal papilla (DTP). × 9600.

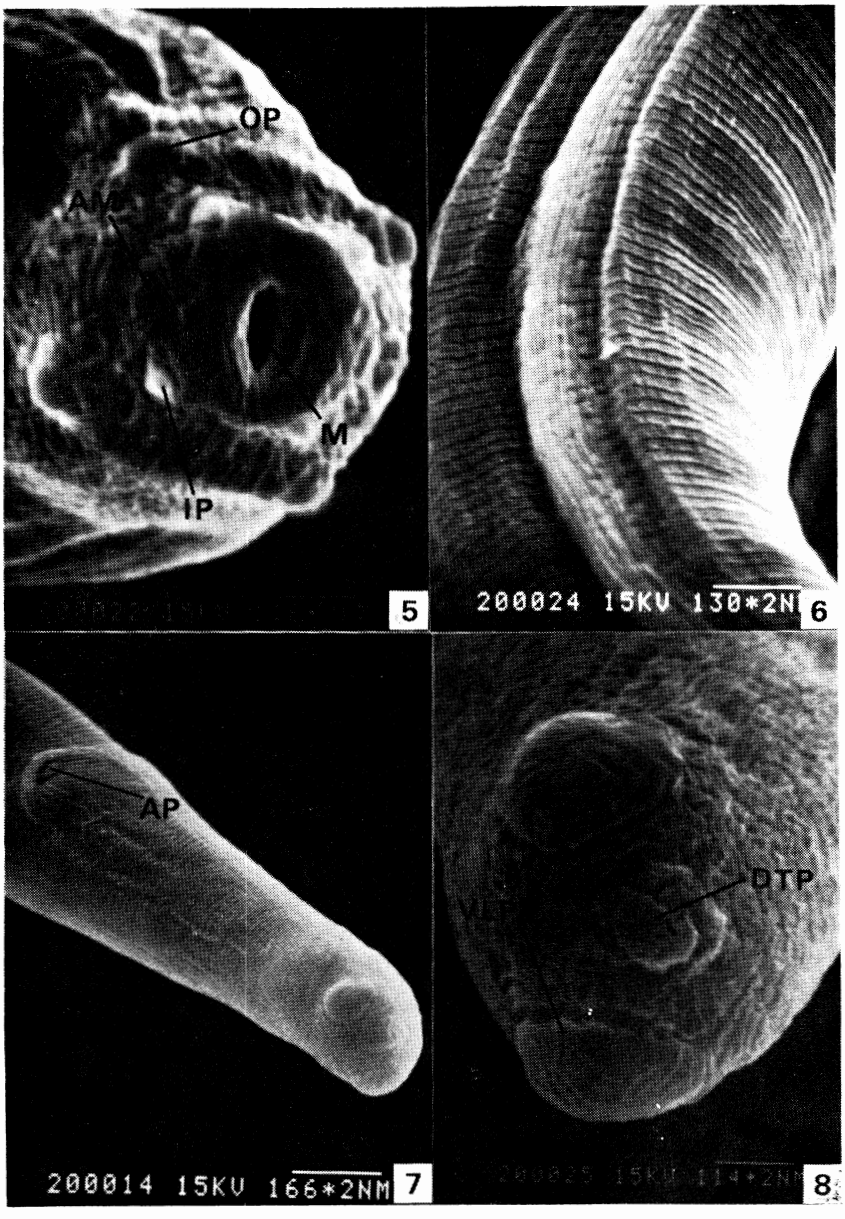

Fig. 5—Head region of third-stage larva of nocturnally subperiodic *B. malayi*. Note the circular mouth (M), inner papilla (IP), outer papilla (OP) and amphid (AM). × 8700.

Fig. 6—Showing the transversed annulations of body cuticle. Note the lateral lines as interruption of the cuticular annulations and the longitudinal folds clearly seen. × 8700.

Fig. 7—The tail end of third-stage larva. Note the crescent-shaped anus with anal prolapse (AP) and three caudal papillae. × 2300.

Fig. 8—A higher magnification of tail end. Note the two ventrolateral papillae (VLP) and rudimentary dorsoterminal papilla (DTP). × 8500.

annulations and longitudinal folds were cleary observed on the surface, all over the body, except at the anterior and posterior ends. The posterior end was curved slightly ventrally (Fig.3). The anus was located approximately 98 μm in front of the posterior end, measured 6.50 μm in length and was prominent and crescent shaped with a raised anterior border. The caudal extremity was round and carried three papillae which were rather similar in size (Fig. 4). There were two bubble-like ventrolateral papillae with a diameter of 1.74 × 2.19 μm and 1.69 × 1.96 μm for the left and right sides and one dorsoterminal papilla diameter measured 2.31 × 2.30 μm.

In nocturnally subperiodic *B. malayi*, the orifice of the mouth was circular shaped and measured 6.10 × 12.43 μm (Fig. 5). There were eight cephalic papillae arranged in two concentric circles, each circle consist of two subdorsal and two subventral papillae. Those of the inner row were smaller, appeared as nipple-like protuberance and in diameter measured 3.81 × 5.87 μm. The four outer papillae were distinct and spherical with a diameter of 7.51 × 9.35 μm. Apart from these oral papillae, the two amphids measured 5.88 × 7.50 μm in diameter could be cleary seen. There were numerous transversed annulations on the cuticular surface, except at the body extremities, these measured 3.11 μm in width. The lateral lines were observed as interruption of the cuticular annulations and longitudinal folds were found throughout the body, except at the body extremities (Fig. 6). The posterior end was curved slightly ventrally (Fig. 7). The anus was located approximately 75.15 μm in front of the posterior end, measured 10.41 × 33.80 μm in length and was prominent and crescent shaped with a raised anterior border. Some of the specimens showed anal prolapse 38.89% (7/18). There were three caudal papillae, two ventrolateral and one dorsoterminal (Fig. 7). The two ventrolateral caudal papillae were cleary seen rather similar in size and appeared spherical shaped, measured approximately 13.03 × 16.29 μm, whereas the dorsoterminal papillae was round and rudimentary in appearance and measured 6.23 × 9.68 μm (Fig. 8).

Observations on the surface topography of third-stage larvae of nocturnally subperiodic *W. bancrofti* strain from Tak Province, Northwest Thailand and nocturnally subperiodic *B. malayi* strain from Narathiwat Province, Southern Thailand in the present studies revealed that the morphological features of both filarial species are generally in agreement with the descriptions of previous investigatiors on *W. bancrofti* (Franz and Zielke, 1980. *Tropenmed. Parasit., 31* : 345) and *B. pahangi* (Aoki *et al.*, 1980. *J. Parasit., 66* : 449; Suguri *et al.*, 1985. *Jap. J. Parasit., 34* : 479), i.e., the structure of head, body cuticle, anus and terminal caudal papillae. The difference in the current studies are the presence of anal prolapse (38.89%; 7/18) in nocturnally subperiodic *B. malayi*, while it was not evident in *W. bancrofti* and *B. pahangi*. This might be a common characteristics of third-stage larva of nocturnally subperiodic *B. malayi* strain from Narathiwat Province, Southern Thailand or for other strains. Another is the small papilliform processes on the left side, between the anus and tail end of *B. pahangi*, whereas it was absent in nocturnally subperiodic *B. malayi*.

WANCHAI MALEEWONG, WEJ CHOOCHOTE*, KETURAT SUKHAVAT*, CHIRASAK KHAMBOONRUANG* and CHANNARONG ARUNYANART. Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, *Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50000, Thailand.

THE NATURAL FIRST INTERMEDIATE HOST OF *PARAGONIMUS* IN THAILAND.

In Thailand paragonimiasis is recognized as an important helminthic infection. So far, six species of *Paragonimus* have been found existing in the country namely, *Paragonimus westermani*, *P. heterotremus*, *P. bangkokensis*, *P. harinasutai*, *P. siamensis* and *P. macrorchis*. For all species, the natural second intermediate hosts, the natural and experimental definitive hosts are known except the natural snail hosts have not been found (Vajrasthira, 1986, Publication of The 25th Aniversary of the Faculty of Tropical Medicine, p. 98).

In April this year, a survey team from the Department of Helminthology collected three species of snails, *Bithynia siamensis siamensis*, *Indoplanorbis exustus* and *Filopaludina martensi martensi*, from a well in the rice filed in Nakhon Nayok, Central Thailand, a known endemic area of paragonimiasis. By the crushing technique, it was found that one out of 140 *Filopaludina martensi martensi* harboured rediae and cercariae of *Paragonimus*.

The redia was cylindrical (Fig. 1A), filled with 1-8 (av. 4) cercariae, the average size of 20 rediae was 558 microns in length and 190 microns in width. The cercaria (Fig. 1B), when alive, moved actively at the bottom of the container, body was elongated ovoidal, the average size of 20 cercariae was 308 microns in length and 88 microns in width, oral sucker armed with a 10 microns long stylet, tail was short 33 microns in length and 33 microns in width.

These rediae and cercariae mophologically resemble the same stages of *P. peruvianus* reported by Malek *et al.*, (1985, *J. Parasit.*, *71*:253) and *P. ohirai* reported by Kawashima *et al.*, (1984. *Southeast Asian J. Trop. Med. Pub. Hlth.*, *15* : 148). Based on the gross morphology, it was difficult to say at this

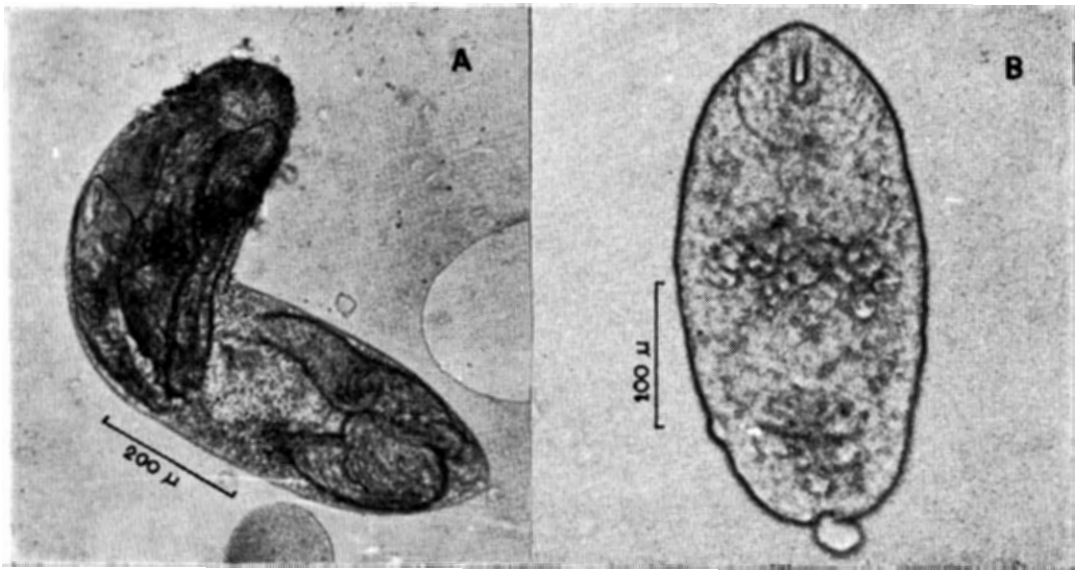

Fig. 1—(A). Redia and (B). Cercaria of *Paragonimus* sp. from *Filopaludina martensi martensi*. Living specimens.

period which species these larvae belong to. The experiments to obtain the adult worm for identification is in progress.

Filopaludina (Siamopaludina) martensi martensi (Frauenfeld) is a common local freshwater snail, the local name is "Hoi Kum" and it is generally accepted as edible by the Thais. "Hoi Kum" was previously reported as the intermediate host of *Angiostrongylus cantonensis* by Harinasuta *et al.*, (1965. *J. Med. Ass. Thailand, 48* : 3). Closer observations should now be taken on the potential role of this natural snail host in the transmission of paragonimiasis in Thailand. Further studies on the ecology and its life history are in progress.

This study is supported by Mahidol University Research Grant No. A/S1-1987.

SANAN YAEMPUT and JITRA WAIKAGUL. Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Bangkok.