BODY OF REPORT

SEATO Medic Study No. 30	Studies on Paragonimus westermani in Thailand
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	Division of Medical Research Laboratories
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Introduction: Human paragonimus in Thailand was first reported by Prommas in 1928, and in 1957 Harinasuta, et. al. reported finding this parasite once again. In 1962, Vajrasthira, et. al. recognized an endemic area in the Nong Mu District of Saraburi Province in Central Thailand. Ova were found in the sputum of 38 of 140 examinees who were experiencing acute or chronic coughing.

Isshiki (1961) found ova of P. westermani (Kerbert, 1878) in the feces of a tiger imported from Thailand to a Japanese zoo. In 1964 Daengsvang, et. al. reported the recovery of P. westermani from two leopards which died in the Bang-

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kok zoo and in 1965 the junior author recognized adult P. westermani from the lungs of a leopard and a black panther which died in the Bangkok zoo. In spite of these findings, no Paragonimus sp. metacercariae were recognized until the present investigation was undertaken.

In 1963 the junior author recovered some adult Paragonimus from semi-domesticated cats in the vicinity of Udorn City, northeast Thailand. Some metacercariae possibly those of a Paragonimus species were fed to cats known to be free of natural infection. In 1964 the senior author was invited to visit Thailand by the U.S. Army for the purpose of investigating this infection. The work, carried out in the Udorn area, led to the recovery and description of the metacercariae as well as the description of the adult stage of a new species, for which the authors propose the specific name, Paragonimus siamensis, n. sp.

Materials and Methods: Thirty-five cats were collected near Udorn City, i.e., 14 in Ban Sok Down, 9 in Ban Kok Sathorn and 12 in Ban Liem. The animals were sacrificed and the lungs were immediately examined for parasites. Seven (20%) were found to harbor immature or mature Paragonimus. By village, 3 of the 14, 3 of the 9 and 1 of the 12 cats were positive. The total number of lung flukes recovered was 69, the number per animal being 1, 2, 4, 4, 9, 13 and 36. The living specimens were flattened in alcohol, stained with acid carmine and mounted in Canada balsam.

Numerous crabs were collected in the rice fields surrounding the villages from which the positive animals were recovered. The gills, livers and hearts of the crabs were pressed between two glass plates and examined for metacercariae using stereoscopic magnification. In all, 2562 crabs were examined of which 67 (2.2%) were positive for metacercariae. The maximum number per host was 45. Experimental cats fed metacercariae 3 to 5 months previously were sacrificed and 2 were found to harbor adult Paragonimus specimens. One cats was found to have two mature worms in a lung cyst and a mature (but smaller) parasite was recovered from the pleural cavity. The second cat harbored 2 fully mature worms in the lung. These specimens were fixed and stained as above. Upon examination it was found that they were morphologically indistinguishable from those specimens removed from naturally infected cats.

Description of Paragonimus siamensis n. sp. Adult (Figs. 1-4): Holotype (Fig. 1) flattened, oval in shape measuring 3.2 by 5.0 mm and covered with cuticular spines which are arranged in groups over the entire body surface. Oral sucker 0.75 by 0.56 mm; ventral sucker situated a little anteriorly to the center of body, measuring 0.72 by 0.66 mm. Short oesophagus divided into two intestines, which run posteriorly, winding 3 times. Sides of body densely covered with the vitelline gland. Ovary situated on the left side of the body and divided into 6 lobes. Coiled uterus filled with eggs located on the side opposite the ovary. Two testes simply lobed, situated posteriorly on both sides of the body, a little smaller than the ovary. Genital pore opens just behind the ventral sucker. Cirrus sac absent; small seminal receptacle present.

Forty-five paratypes which are all mature but of various degrees also flattened and oval in shape, of which 22 fully matured worms are 10.2 - 7.0 (aver. 8.4) mm in length and 5.2 - 4.0 (aver. 4.7) mm in width. Oral sucker 0.83 - 0.63 (aver. 0.73) by 0.60 - 0.32 (aver. 0.44) mm and ventral sucker 0.79 - 0.63 (aver. 0.69) by 0.71 - 0.58 (aver. 0.64) mm in fully matured worms. Ovary situated on the right side in 24 worms, on the left in 21, and divided into 6 lobes in 29 (81%) of 36 worms, into 5 in 5 worms and into 7 and 8 in 1 worm each. In the remaining 9 worms the branching of ovary was not clearly recognized. Size of testes usually a little smaller than that of the respective ovary. Every specimen without exception (Figs. 2 and 3) provided with cuticular spines which are arranged in groups.

Eggs (Fig. 4) removed from the end of uterus of adult worms preserved in alcohol are yellowish in color and oval in shape, measuring 88 - 78 by 49 - 39 (aver. 82 by 46) u. The eggshell is uniform in thickness. It is noticeable that the non-operculated pole is not thickened. The widest portion of the shell is usually situated near operculum.

Metacercaria (Figs. 6 and 7): The metacercaria is oval in shape and is provided with two cyst walls, the outer and inner. The outer wall is thin and fragile measuring 2.5 - 2.0 u in thickness. It adheres at one pole to the inside of the blood vessel near the heart of the crab and is sometimes eveloped by a thin fibrous membrane which seems to develop from the host tissue. Rarely, the cyst adheres to the outside of the heart. The wall of the inner cyst is more elastic and stronger, being 7.4 - 4.9 u in thickness. The diameter of the inner cyst measures 531 - 443 by 403 - 314 (average 482 by 347) u, when free of pressure. The encysted larvae is seen to contract and expand and has a conspicuous excretory bladder in the center. The intestine winds on either side of the bladder. Minute pinkish globules are usually scattered in the larval body. The oral sucker, much smaller than the ventral sucker, is provided with a stylet. The body surface is entirely covered with singly spaced cuticular spines.

Final host: Felis domestica Habitat: Lung
Locality: Vicinity of Udorn City, Udorn Province (northeastern) Thailand.
Second Intermediate Host: Paratheiphusa (Parathelphusa) germaini (Rathbun)
Holotype and 22 paratypes are deposited in the Department of Parasitology, Faculty of Medicine, Kyushu University, Fukuoka, Japan, and 23 paratypes are in the Department of Medical Zoology, SEATO Medical Research Laboratory, Bangkok, Thailand.

Discussion: The arrangement of cuticular spines and the shape of ovary are good criteria for differentiating adult Paragonimus species. In addition, the character of eggs, the comparison of relative size of ovary and testes, and the relative sizes of the oral and the ventral sucker are also useful criteria.

Including synonymes, 24 species of Paragonimus have been reported from animals, most of these being in Asia. Of these species, P. westermani (Kerbert,

1878) is most similar to P. siamensis n. sp. in the following respects: 1. Both species almost always have a 6-loped ovary, 2. the size of ovary and testes, and 3. that or the oral and ventral sucker of each individual are not remarkably different in the two species. However, these two species are easily differentiated by the arrangment of the cuticular spines. The spines of P. siamensis n. sp. are always arranged in groups, even in young worms in which no eggs are recognized in the uterus. The spines of P. westermani, by comparison, are singly spaced, although those of old worms are inclined to split into two, three, or more parts. Moreover, there is a difference in thickness of the eggshell on the non-operculated pole. In most cases the shell of P. westermani gradually thickens around the pole, but in P. siamensis n. sp. the shell is uniform in thickness.

In the larval stage there is a clear-cut difference between the two species. The metacercaria of P. westermani is spherical in shape and a little smaller than P. siamensis n. sp., but the wall of inner cyst is much thicker in the former. Moreover, the metacercaria of P. westermani is not parasitic in the blood vessels, but is to be found mainly in the gill vessels and the muscles.

The metacercaria of P. siamensis n. sp., resembles that of P. chirai (Miyazaki, 1939) in shape, but its cyst is much larger than that of the latter, which measures 384 - 253 by 280 - 192 (aver. 303 by 235) u (Miyazaki, 1947).

Another species of the genus which shows similarity to P. siamensis n. sp. in the adult stage is P. compactus (Cobbold, 1859). The cuticular spines are always arranged in groups and branching of the ovary is simple in both species. However, according to Vevers (1923), the ovary of P. compactus is divided into five lopes instead of six which is the normal form of P. siamensis n. sp. Miyazaki (1962) confirmed that one of the specimens of P. compactus which was used for the Vevers' re-description of the species had a 5-lobed ovary. Incidentally, the metacercaria of P. compactus remains unknown.

From the medical point of view, it is very significant that inhabitants of the above-mentioned villages near Udorn City have a custom of eating raw carbs. An exact survey of human paragonimiasis in these villages is very necessary, though no human cases have yet been reported.

Summary: In the vicinity of Udorn City, situated in northeast Thailand, the authors found 69 adult lung flukes from 7 (20%) of the 35 cats examined. In addition, they found the metacercariae of the same lung fluke from 67 (2.2%) of the 2,562 crabs (Parathelphusa germaini) collected in the same area. After careful study and detailed comparisons with known species of lung flukes, the authors proposed the name Paragonimus siamensis n. sp. for this new lung fluke.

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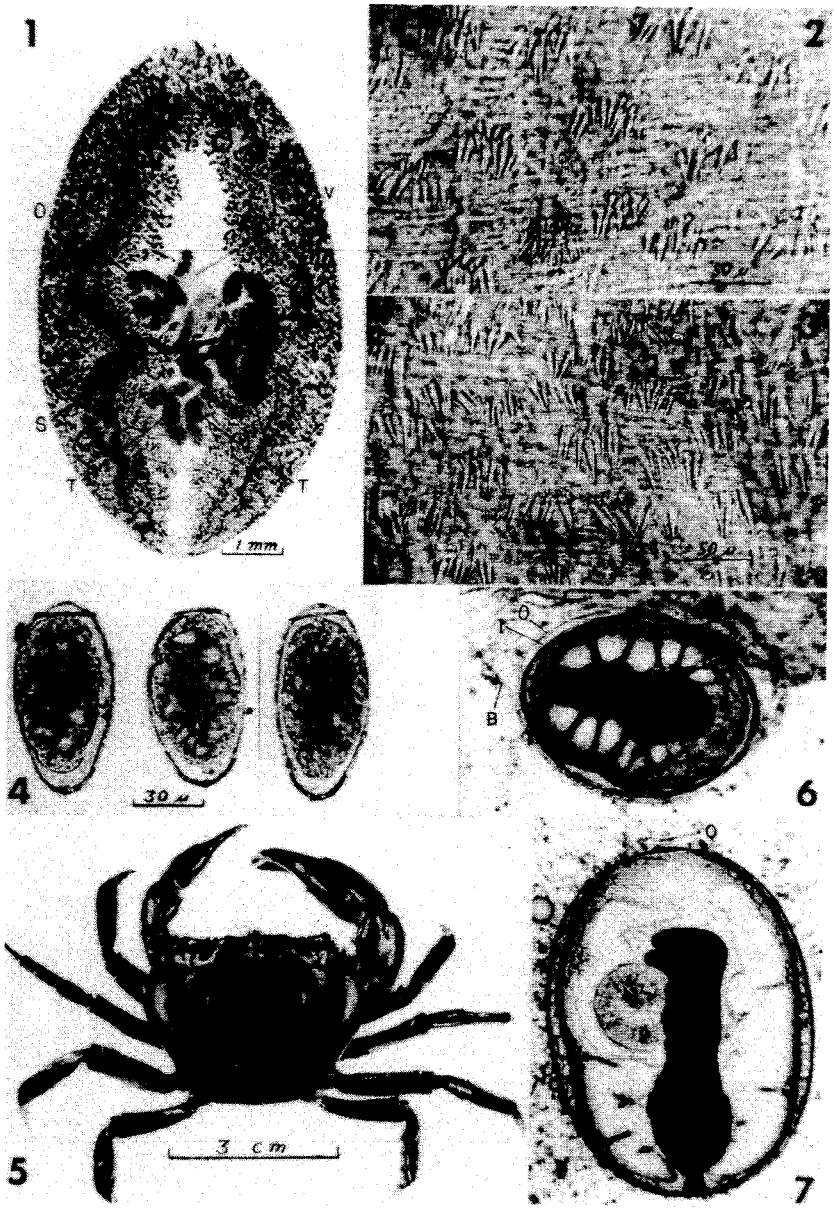
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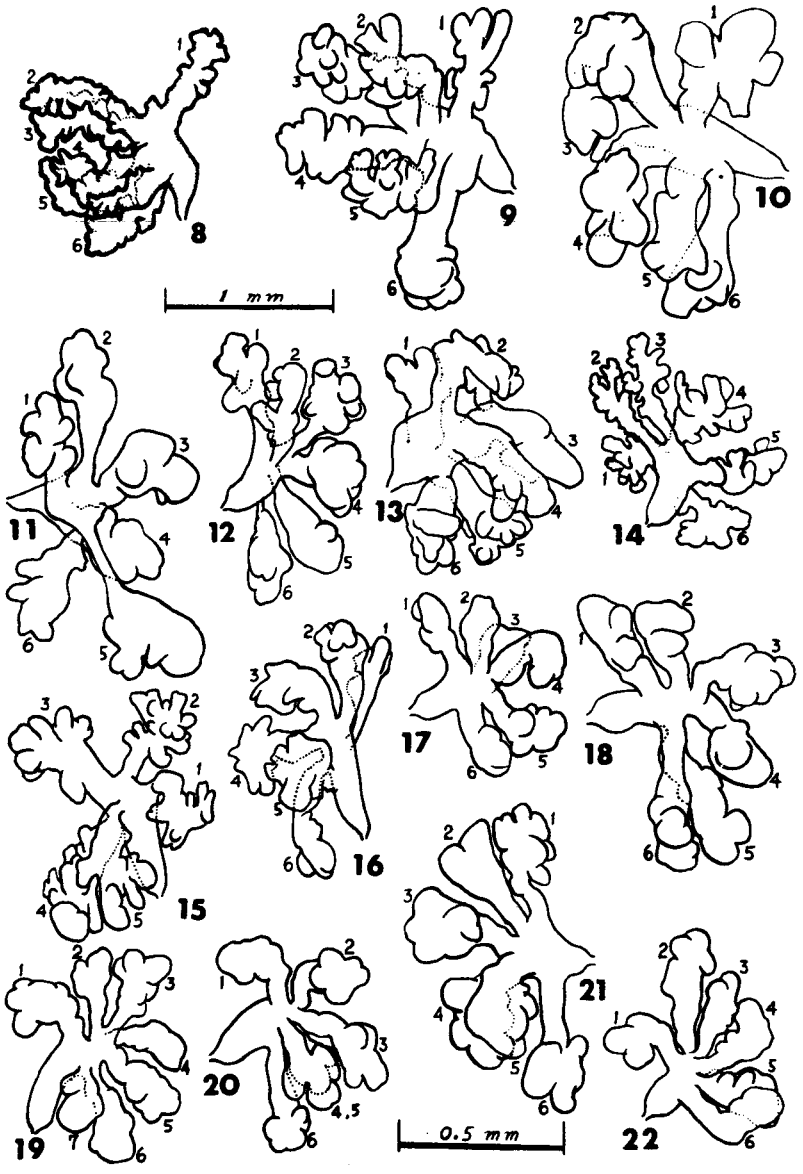
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Fig. 1. Doral view of the holotype of Paragonimus siamensis n. sp.

O: Ovary S: Seminal receptacle
T: Testis V: Ventral sucker

Fig. 2. Cuticular spines between the oral and ventral suckers.

Fig. 3. Spines laterally from the vental sucker.

Fig. 4. Uterine eggs preserved in alcohol.

Fig. 5. Male of Parathelphusa (Parathelphusa) germaini (Rathbun), the second intermediate host of P. siamensis n. sp. (photographed by Mr. Minei).

Fig. 6. Metacercaria of P. siamensis n. sp. parasitic in the blood vessel, free from pressure.
B: Blood vessel I: Inner cyst O: Outer cyst

Fig. 7. Removed metacercaria of the new species, under cover glass pressure.
O: Outer cyst

Figs. 8 to 22: Ovaries of the holotype (8) and paratypes (9 to 22) of P. siamensis n.sp. of which 8 to 17 and 18 to 22 are drawn under the same magnification, respectively.