CEYLON J. MED. SCI. (D) Vol. XI, Pt. 1 (May 1962)

Paragonimus infection in Wild Carnivores in Ceylon

by

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(With twelve Text Figures & five Plates)

WE recently reported the finding of lung flukes (*Paragonimus* spp.) from a civet cat (*Viverricula indica mayori*) and a fishing cat (*Felis viverrina*) shot in the Eastern Province of the Island (Dissanaike and Paramananthan, 1961 a and b). Since then we have come across *Paragonimus* infection in a rusty spotted cat (*Felis rubiginosa*) and two leopards (*Panthera pardus fusca*) from the North Central Province, and one leopard from the Northern Province.

Lung flukes of the genus *Paragonimus* are common in man in the Far Eastern countries, and several species have been found in crab-eating mammals from different parts of the world. In addition to the early descriptions of *P.westermani* from tigers which died in foreign zoological gardens, a few species of the genus have been reported on several occasions from various carnivores in India (Vevers, 1923, Gulati, 1926, Rao, 1935, and Srivastava, 1938). Although these worms had not previously been reported from Ceylon it would appear from the present findings that the infection is quite common in wild animals. It is necessary therefore, to establish the identity of the species with certainty.

In a recent review Yokogawa, Cort and Yokogawa (1960) and in a more recent paper Miyazaki (1961a), have shown that most of the species of the genus that have hitherto been described could be synonymised and they have suggested that only six or seven of them are valid species. As pointed out by them the essential features in the morphology of the flukes which help in species differentiation are, the branching of the ovaries and the arrangement of the cuticular spines. The other important characters are, the type of host, the structure of the metacercaria and the morphology of other larval stages. We describe below the characters of the specimens we have recovered from the various carnivores in this country. We have provisionally established two species here namely, *P.westermani* and *P.compactus;* but as we shall point out later, definite proof of the identity of the latter species must await the finding of the crab and snail intermediate hosts and the establishment of laboratory infections with these worms.

Distribution

From the material collected so far it is clear that the infection is common in a wide variety of carnivores in Ceylon and also from widely separated areas in the Northern, North Central and Eastern Provinces of the Island. As the map (Fig. I) indicates, *P.westermani* has been recovered from three leopards, from Vavuniya (N.P.), Habarana and Kekirawa (N.C.P.)

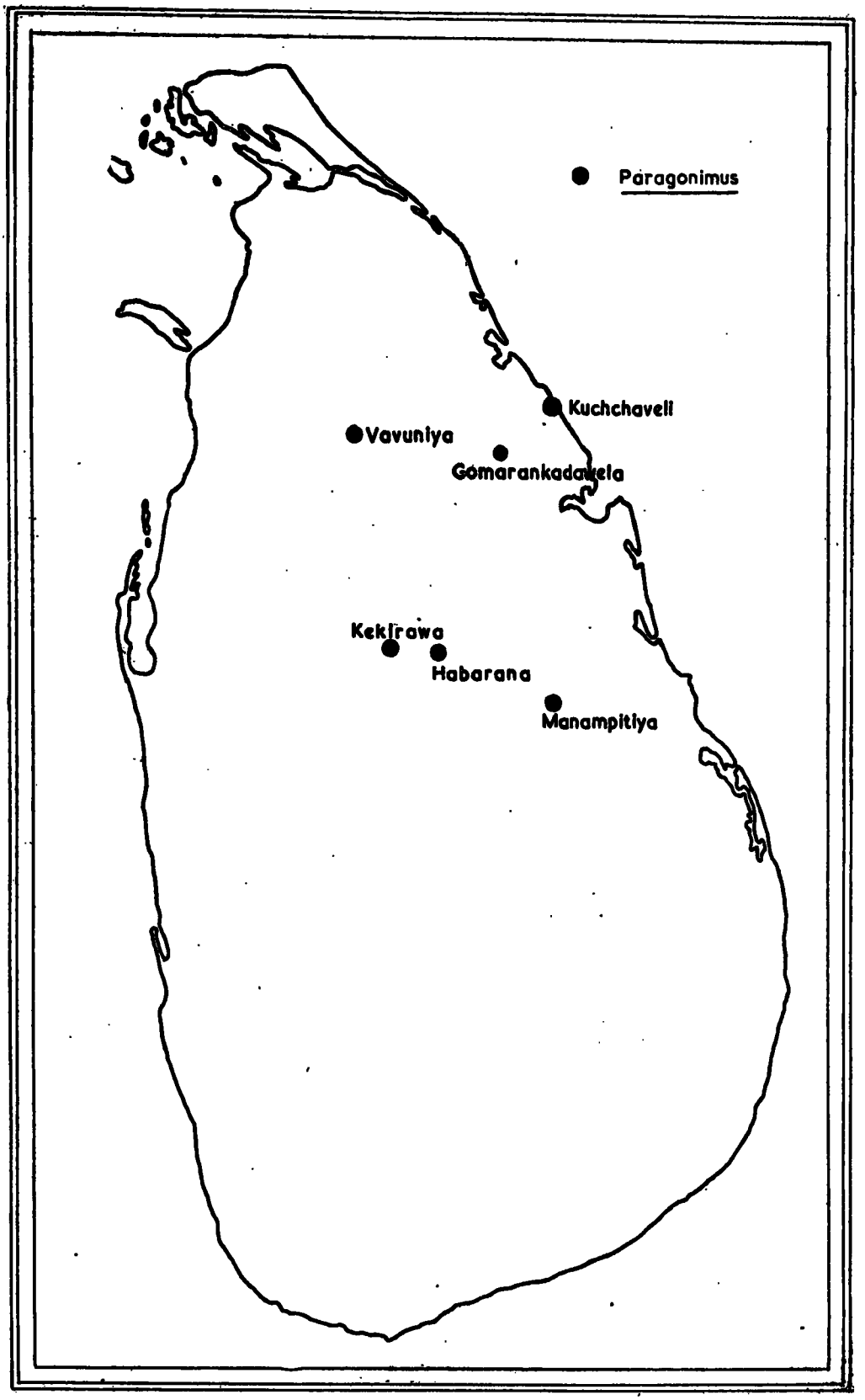

Paragonimus
Kuchchaveli
Vavuniya
Gomarankadawela
Kekirawa
Habarana
Manampitiya

FIG. I

respectively. Since we began searching for this trematode every leopard examined has been found infected. A rusty spotted cat from Manampitiya area has also been found to harbour *P.westermani*. Worms which we have provisionally identified as *P.compactus* were found in a fishing cat in Kuchchaveli and a civet cat in Gomarankadawela in the Eastern Province. No animals have so far been examined for this infection from other parts of the island, but when this is done it will be possible to have a clearer picture of the distribution of this infection throughout the country.

Description of specimens from the various hosts

(*a*) *Specimens from the civet cat*

The lungs of this animal (Fig. A) consisted of a number of cysts of small size each containing a pair of worms, and one cyst which was larger and about the size of a small glass marble (15mm by 10mm.). In all, six worms were recovered from these cysts before the lungs were fixed in 90% alcohol. The worms were first rinsed in saline, pressed between two glass slides and then fixed in 70% alcohol. They were stained in acetic acid alum carmine or Delafield's haematoxylin for further study. Two worms were removed from a cyst after fixation of the lungs, and their cuticles were peeled off and examined in glycerine for the structure and arrangement of the spines.

The spines were in groups or clusters of 4—8 (Fig. II and Fig. B). It was noticed that the spines in the whole mount preparations were always smaller in size than those in the peeled cuticle preparations, (Fig. II a and b). Each spine was elongate and lanceolate, and the

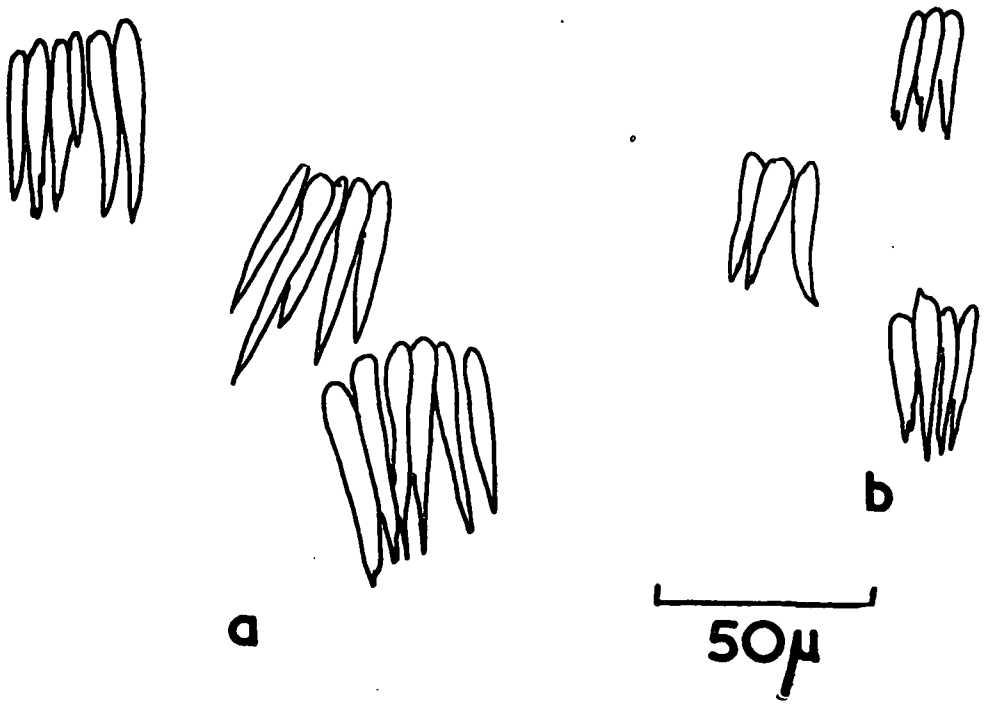


FIG. II

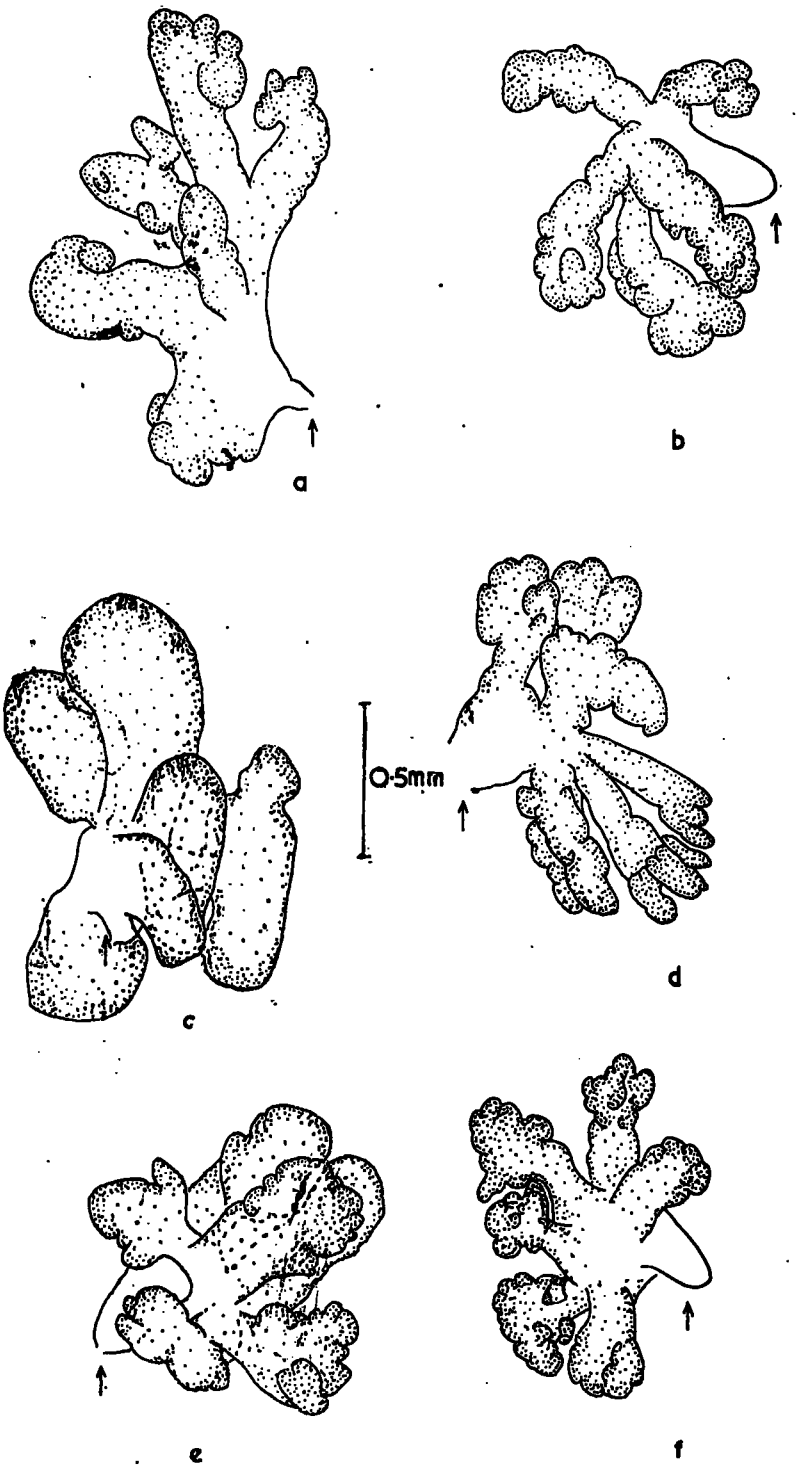


FIG. III

spines were more closely bunched together near the oral sucker region, where they were also of smaller size. The spines in peeled cuticle preparations of two worms were 33—50 μ in length (average 38.9 μ), while the spines in two mounted specimens were 15—38 μ (average 23.8 μ) in the same region. No spines were seen singly in any part of the body in either the peeled preparations or the whole mounts of the worms.

The rest of the morphology of the flukes was quite typical as seen in Figs. C and D. Seven pressed whole mounts measured 6—10mm in length by 4—5mm in width, (average 7.85mm by 4.5mm.).

The ovaries were situated beside or behind the ventral sucker on the right of the body in 5 worms and on the left side in 2 worms. They were all of the *westermani*-type and consisted of the 'handle' or stalk and 5 or 6 lobes which in more mature specimens showed lobulation at the tips. Camera lucida drawings of the ovaries of 6 specimens are shown in Fig. III.

The eggs were oval in shape, brownish in colour and measured 67.5—90 μ by 40—52 μ (average 80.8 by 46.0 μ). The wall was evenly thickened and in many specimens a knob-like thickening was seen at the pole opposite the operculum which was sometimes quite distinctly marked off from the body of the egg (Fig. IV). Eggs were also seen in nodules in the lungs apart from the cysts containing the worms (Fig. E).

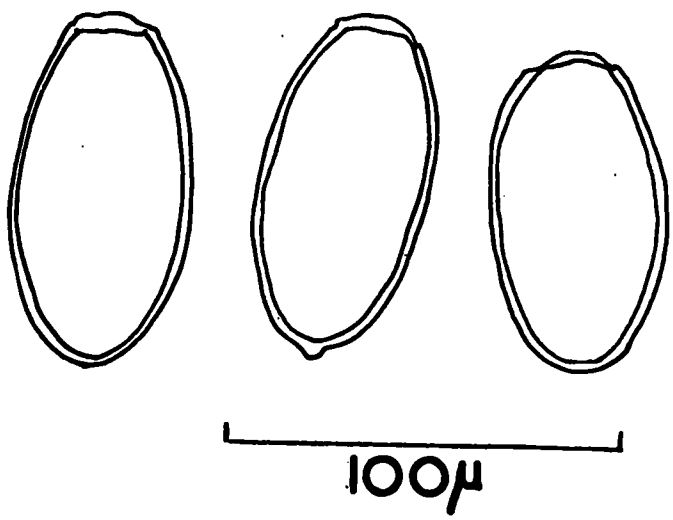


FIG. IV

(*b*) *Specimen from the fishing cat*

Only a single worm was recovered from this host and it was found creeping on the surface of the lung. There were, in addition, a number of nodules on the visceral pleura which contained numerous eggs and inflammatory cells (Fig. G).

The morphology of this worm (Fig. F) was almost the same as that of the worms from the civet cat except that it was a much younger and smaller worm. It measured 5.5mm by 3.5mm. The vitellaria were not so extensively developed and the intestinal caecae were easily seen. The spines were in groups (Fig. Vb) in most regions of the worm where they could be made out. They were 15—32.5 μ (average 26.1 μ).

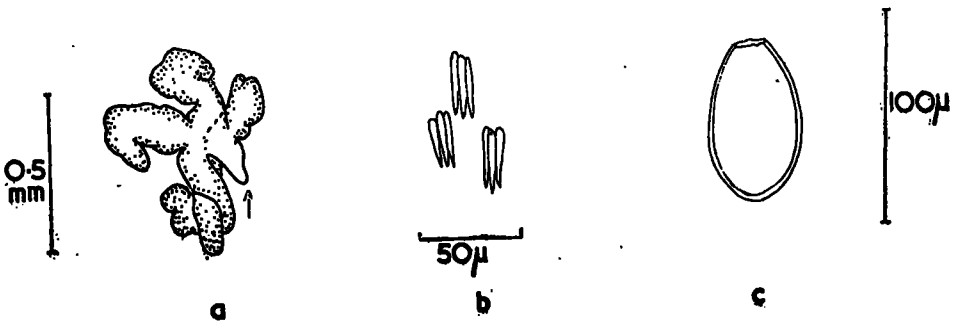


FIG. V

The ovary (Fig. Va) had 5 lobes and appeared to be somewhat smaller than the testes. The eggs (Fig. Vc) were similar to the eggs of worms from the civet cat and measured 70—90 μ by 43—52 μ (average 80.0 by 47.5 μ).

(*c*) *Specimens from the rusty spotted cat*

Both lungs of this animal (Fig. H) were literally riddled with numerous small cysts each containing a pair of worms. One cyst, however, contained 3 worms. Twenty five worms removed from the cysts were carefully pressed as before, and the lungs were then fixed. There were in addition, several worms lying free in the pleural cavity and these were smaller in size and younger.

Pressed worms from cysts were 10.5—12mm by 5—7mm (average 11.3 by 6.2mm) and those from the pleural cavity were 4—6.5mm by 2—3.5mm (average 5.5 by 3.0mm).

Preparations of peeled cuticle from the pressed worms showed clearly that the spines were arranged singly in all regions (Fig. VIa and Fig. I and J). This arrangement was seen in all whole mounts as well (Fig. VIb) and in all specimens whether from cysts or from the pleural cavity. A most striking feature of the spines was their large size. Spines from whole mounts were 42.5—65 μ (average 55.1 μ) and those from peeled cuticle preparations were 65—77.5 μ (average 70 μ).

The other structures of these flukes were typically of the *westermani*-type as seen in Figs. K, L, M. The specimens from the pleural cavity were smaller and the vitellaria and other organs were less fully developed (Figs. K and L). The ovary in the majority of specimens had 6 lobes and in the older worms these tended to be lobulated at the tips (Fig. VII). In a few, the ovary had only 5 lobes.

The eggs (Fig. VIII) were 68—86 μ by 45—53 μ (average 80.5 by 47.1 μ) the walls were not of uniform thickness in some specimens and a knob-like thickening was seen at the pole opposite the operculum in most of them.

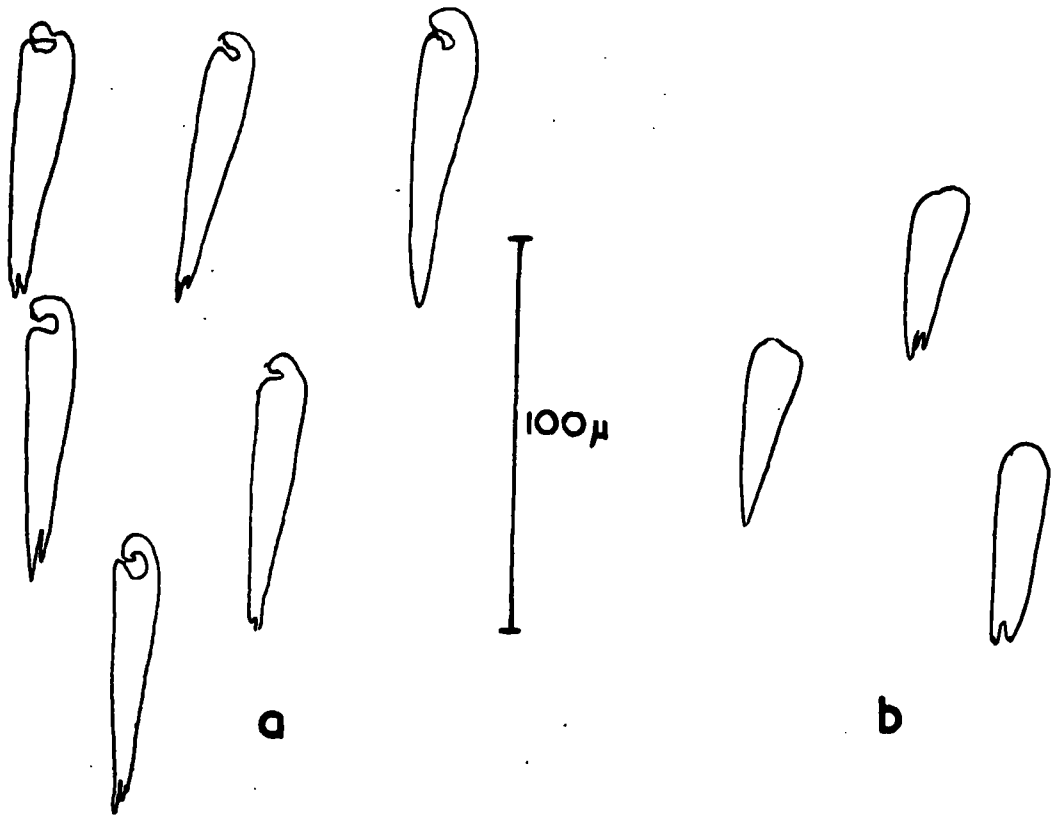


FIG. VI

(d) Specimens from leopards

The lungs of the three leopards were sent to us fixed in 10% formalin and they yielded the largest number of worms, although many were unsuitable for preparing whole mounts.

The lungs of the leopard from Vavuniya (Fig. N) yielded 21 worms, and except for one cyst that contained 3 worms the rest were found in pairs within the cysts.

Fourteen worms were recovered from the lungs of the leopard from Kekirawa, while the leopard from Habarana was so heavily infected that over a hundred worms were recovered from most of the cysts that were opened. Portions of the lungs of this animal were preserved for a museum preparation. Fig. O shows a part of the lung dissected to demonstrate the worms in pairs within a cyst.

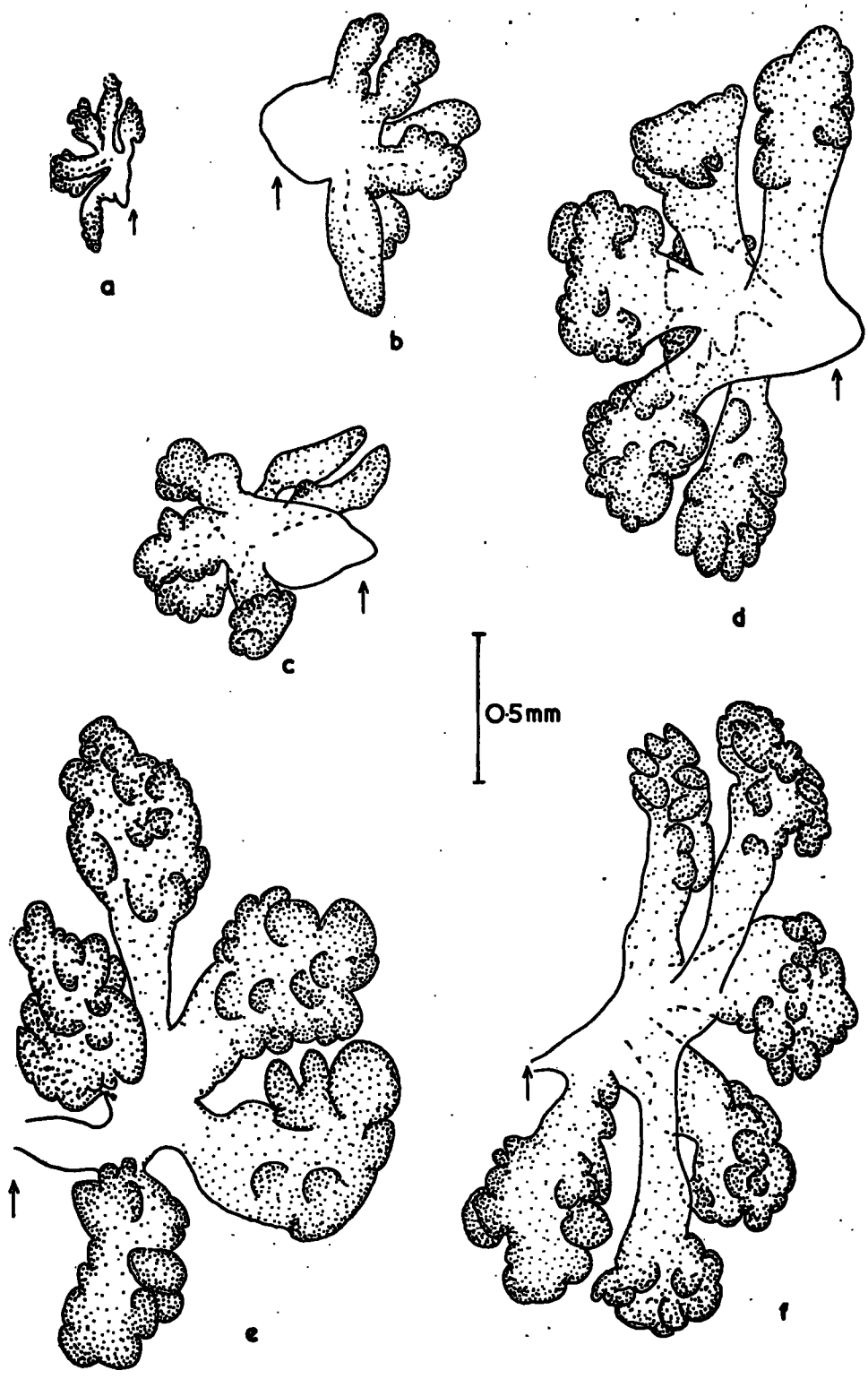


FIG. VII

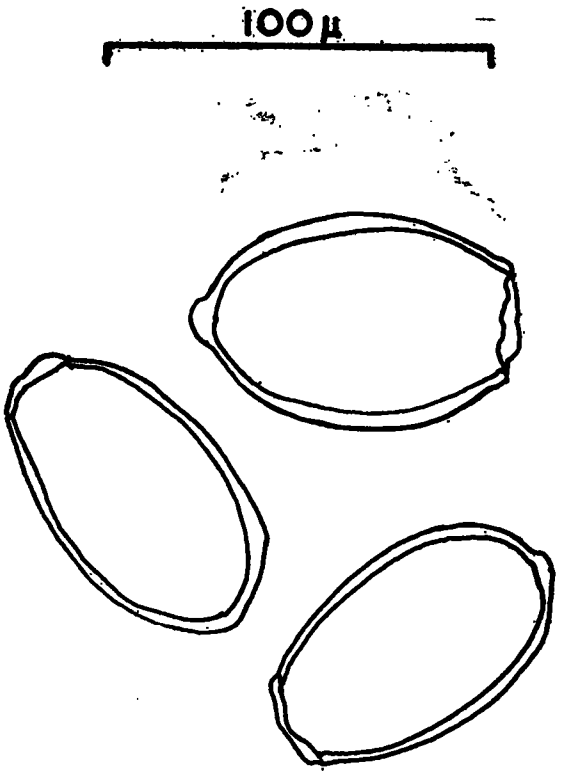


FIG. VIII

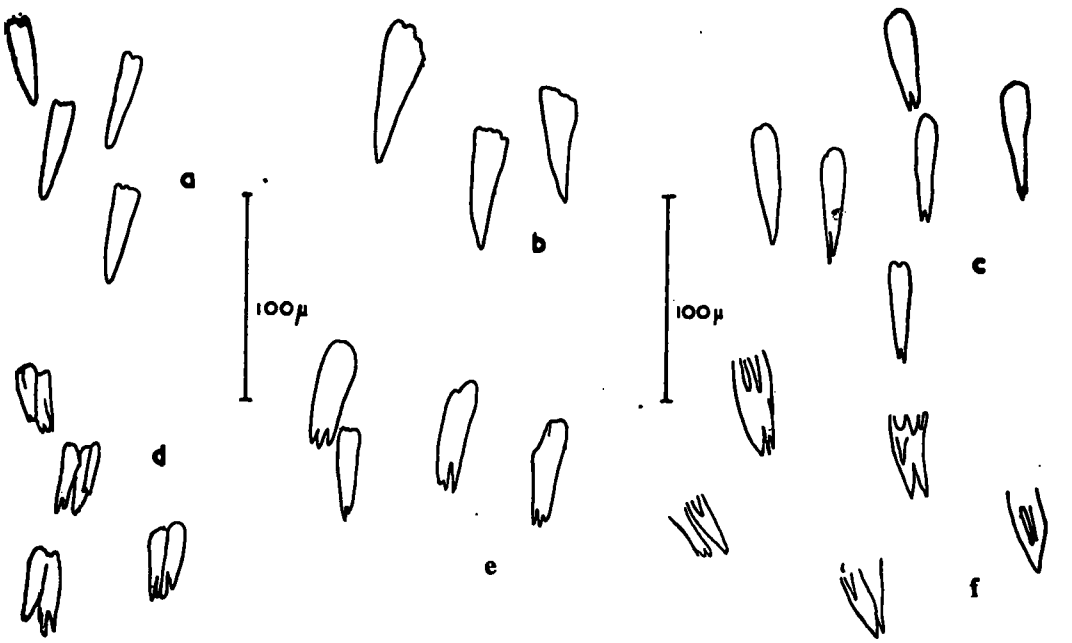


FIG. IX

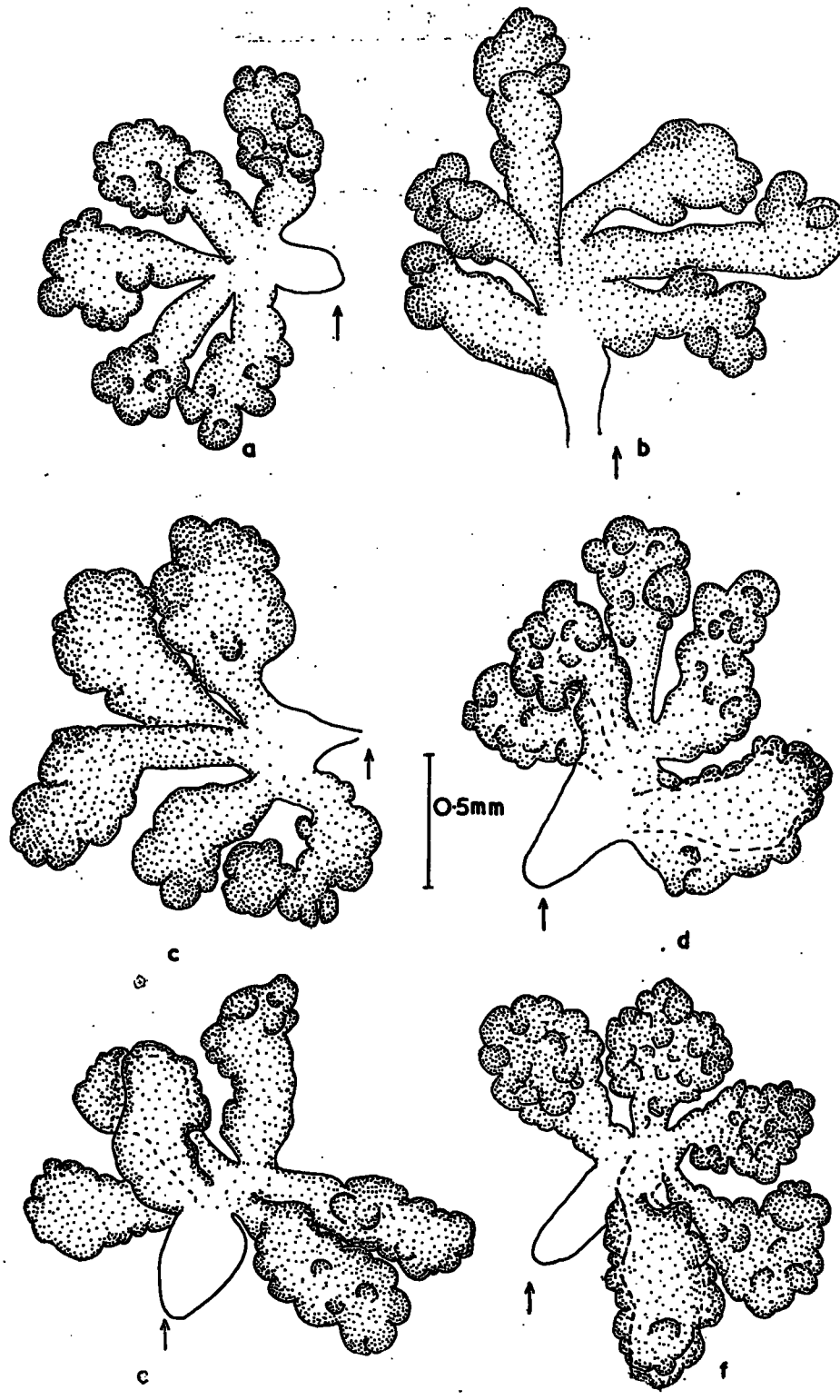


FIG. X

The measurements of the mounted worms, the spines and the eggs of worms from the three leopards are summarised in the table below (Table I).

TABLE I

Measurements of whole mounts, eggs and spines of worms from the three leopards.

Leopards	*Worms*	*Eggs*	*Spines*	
			Peeled cuticle	Whole mounts
1. Kekirawa	8—8.5mm by 5.5—6.5mm (Average 8.3 by 6mm)	75—80 μ by 40—46 μ (Average 77.8 by 42.8 μ)	30—48 μ (Average 38.7 μ)	30—45 μ (Average 39 μ)
2. Vavuniya	10—11mm by 6—7mm (Average 10.4 by 6.5mm)	75—85 μ by 45—52 μ (Average 80.3 by 49.1 μ)	55—72.5 μ (Average 65.2 μ)	43.5—60 μ (Average 51 μ)
3. Habarana	6—11.5mm by 3.5—8mm (Average 9.2 by 6.1mm)	70—78 μ by 45—50 μ (Average 75 by 47 μ)	27.5—61 μ (Average 44.9 μ)	25—41 μ (Average 35 μ)

The spines were in singles (Fig. S, T and Fig. IX). In some of the older specimens, however, the spines in certain regions showed a tendency to splitting and even separation but their appearances then were different from the grouped spines of worms from the civet cat and the fishing cat. As the table indicates, the spines in a peeled preparation of a worm from the Vavuniya leopard were quite long (average 65.2 μ).

The other structures were typically of the *westermani*-type (Fig. P, Q, R). The ovaries (Fig. X) had characteristically 6 lobes, but in a few specimens it was only possible to make out 5 lobes.

Typical eggs from worms of each of the leopards are shown in Fig. XI.

Discussion

As this is the first time lung flukes have been recorded from Ceylon we have given details of the characters that are of importance in the differentiation of the species as we felt that a correct identification was essential. All the worms recovered from the various hosts were of the *westermani*-type in their internal anatomy, particularly in regard to the ovaries, which were simple and consisted typically of six lobes. Since all the specimens from the three leopards and the specimens from the rusty spotted cat had spines that were essentially singly-spaced, we have no hesitation in identifying them as *P.westermani*. We would, however, like to stress two points with regard to these worms. First, as we have pointed out already, the spines in the worms from the rusty spotted cat were quite large and somewhat different in appearance from those of worms from the leopards except perhaps some of the worms from the Vavuniya leopard. As far as we are aware, such long spines have not hitherto been reported for *P.westermani*. Professor I. Miyazaki whom we have consulted regarding

these specimens from the rusty spotted cat agrees that they are *P.westermani* inspite of their long spines. Secondly, although Miyazaki (1943, and 1961 a and b) has stressed that the ovary of *P.westermani* has always six lobes, we have found a few worms from the rusty spotted cat as well as from the leopards, showing distinctly five lobes (Fig. VIIe and Fig. Xa and c).

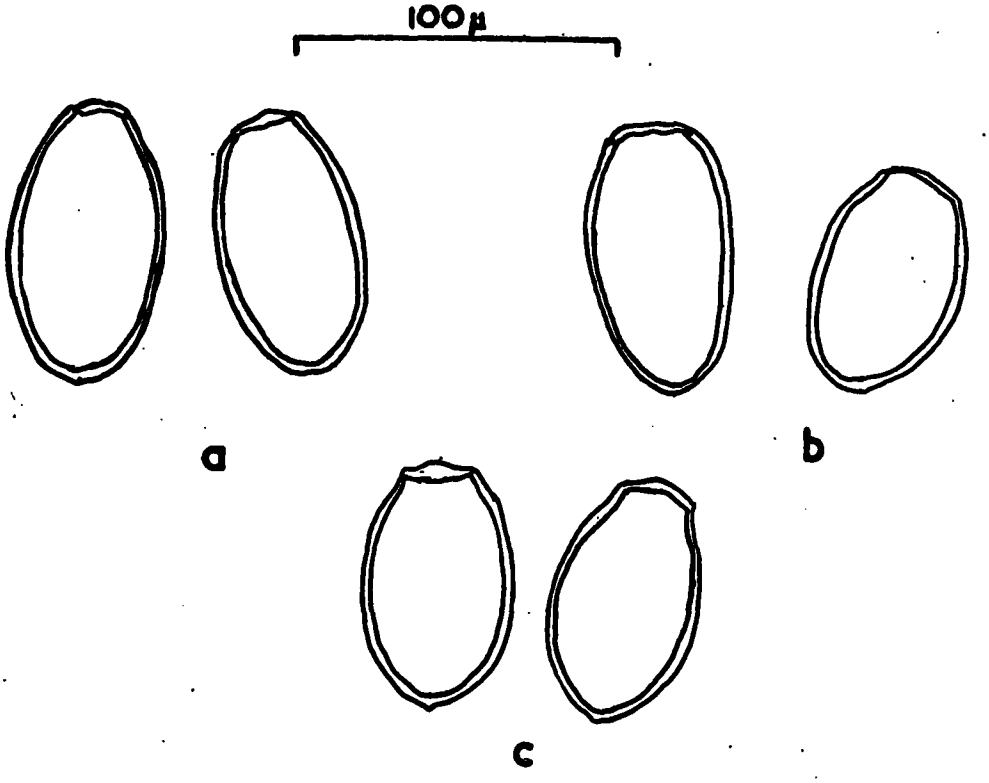


FIG. XI

With regard to the worms from the civet cat, the arrangement of the spines in groups in all regions and in all the worms studied was quite characteristic. This was also true of the single young worm that was recovered from the thoracic cavity of the fishing cat. We were therefore led to the view that these were probably *P.compactus* (Cobbold, 1859). Through the kindness of Professor J. J. C. Buckley we have had the opportunity of examining a specimen of this worm that was redescribed by Vevers (1923) from the same host as the type species. In this specimen (Fig. U) we found that the ovary had five lobes (Fig. XII) and the spines were distinctly in groups in all regions. We were able to confirm this appearance of the spines in a peeled preparation of the cuticle which was also available (Fig. V). Vevers has pointed out that the receptaculum seminis in *P.compactus* is more prominent than in other species, and Chen (1940) stating that *P.iloktsuenensis* is the only other species that has a large receptaculum seminis, suggested that *P.compactus* may be a good species. As far as this structure is concerned, we observed that Vevers' specimen which we examined was very highly compressed, with the result that all the internal structures were also markedly flattened. Furthermore, we have found that the receptaculum seminis, although prominent

in the worms from the civet cat, was variable in size and appeared smaller in older specimens. This was also the case with the worms from the rusty spotted cat. Even in the leopard worms, although the receptaculum seminis was a less prominent structure, it seemed to be smaller in the older worms. We are therefore doubtful whether this character can be made use of to distinguish *P.compactus* from *P.westermani*. A final decision on the validity of *P.compactus* as well as the confirmation of our specimens as *P.compactus* will only be possible by looking for distinguishing characters in the metacercariae of these worms. A search for the crab intermediate hosts followed by experimental infection of laboratory animals will be necessary before this.

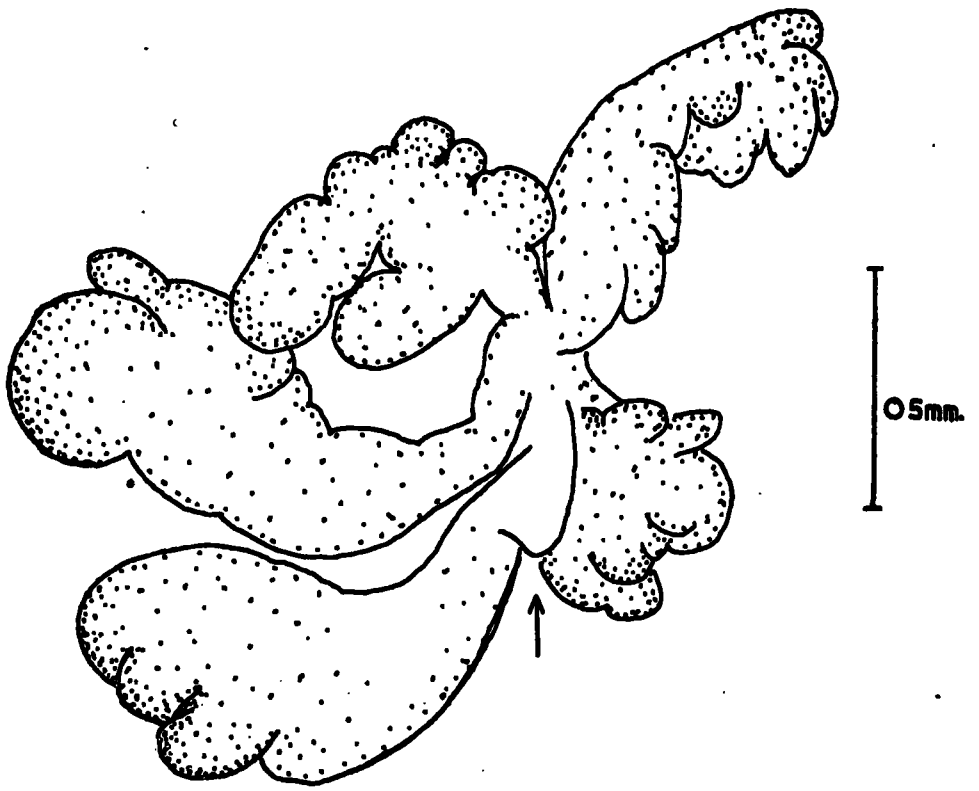


FIG. XII

It is of some significance that we have found *P.westermani* in a number of carnivores in Ceylon, for these animals must be considered as potential sources of human infection. It is true that crabs infected with the metacercaria stage of the trematode, must be ingested raw or undercooked before one can become infected. This is therefore unlikely to happen in a country like ours where the fish and flesh that is consumed is thoroughly cooked and curried. We must, nevertheless realize that fresh-water crabs of the family Potamonidae (which are the common second intermediate hosts in Far Eastern countries) are quite abundant in all parts of the Island. Fernando (1960) has recently made an excellent study of these crabs

in Ceylon and he points out that tney are eaten by poorer classes working on tea estates, and that they are also eaten on account of their supposed medicinal properties for treatment of cough. This is interesting and requires further investigation. It is possible that these crabs are used raw or undercooked in the preparation of some indigenous medicines in this country. In any event we feel that a search for human cases should be made although they are bound to be rare. A search for eggs of *Paragonimus* in the sputa or even the stools of cases of non-tuberculous haemoptysis, as well as routine intradermal tests with *Paragonimus* antigen will not be in vain, even if the findings turn out be negative.

Now that we have found *Paragonimus* infection in several different wild carnivores, a more detailed search for other vertebrate hosts, as well as for crab and snail intermediate hosts must be undertaken in all parts of the Island. We have already examined a number of crabs from Hingurakgoda, Maha Illupulama, and Paranthan. Although we have found the metacercaria of *Pleurogenoides sitapurii*, previously reported from these crabs (Dissanaike and Fernando, 1960), to be quite common in all these areas, and another unidentified metacercaria from one crab in the Maha Illupulama area, we have not so far come across the metacercariae of *Paragonimus*.

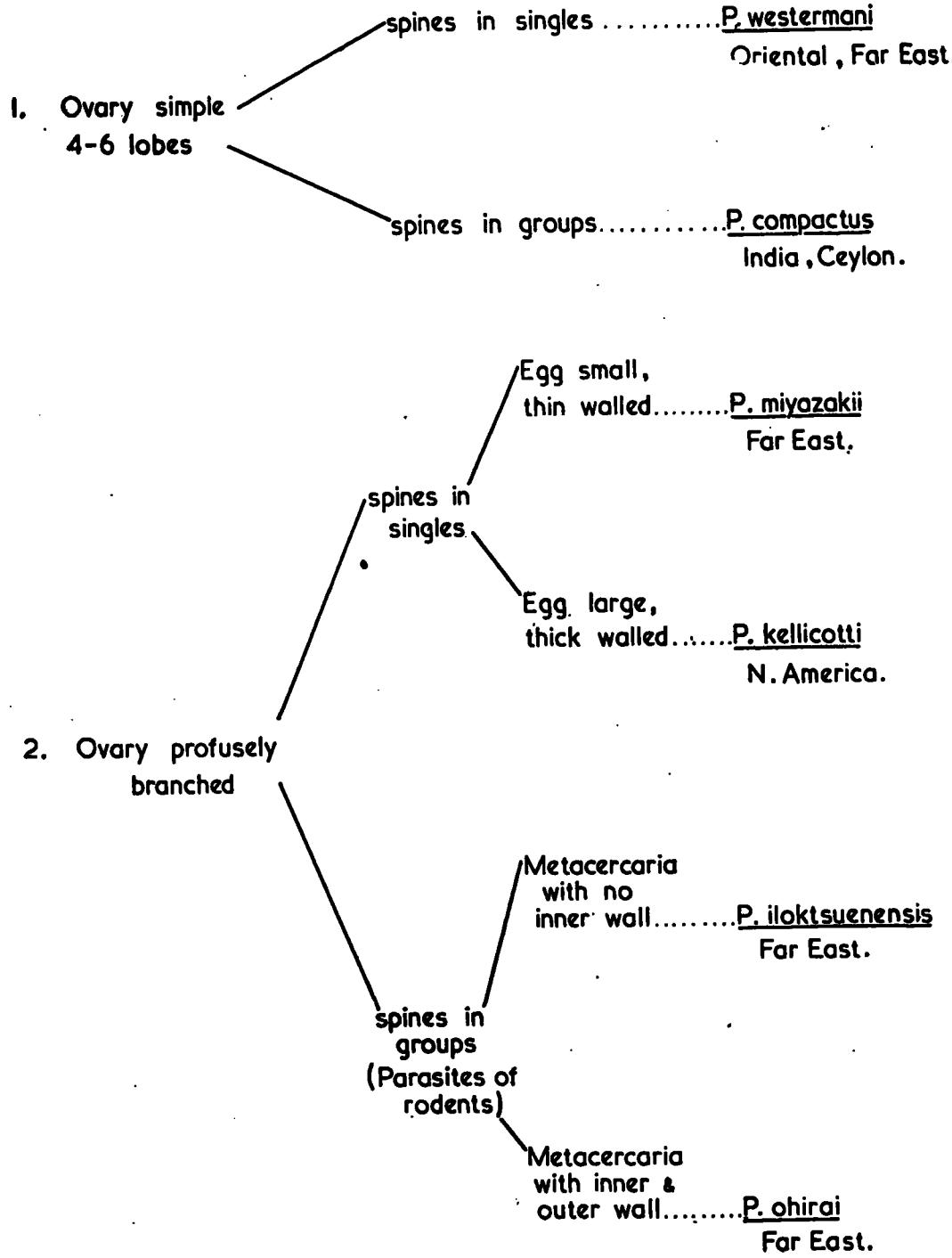
It is neither possible nor necessary in this paper to go into the interesting history of the genus or to discuss the validity of the numerous species of *Paragonimus* that have hitherto been reported. Workers like Ward and Hirsch (1915), Ameel (1934), Chen (1940) and many others have pointed out the importance of one or other structure such as the spines and ovaries in the differentiation of species. Yokogawa et al. (1960) and Miyazaki (1961a) have adequately dealt with these. We would merely like to summarise the main views of the more recent authorities in the simplified key below. Most of these workers have doubted the validity of *P.compactus* both on account of the inadequate original description of Cobbold (1859) and also the apparent inaccuracies of the accounts of spines by Vevers for the other species he described in the same paper that he redescribed *P.compactus*. As we have already pointed out earlier, however, we would like to provisionally confirm the validity of *P. compactus*. Another species, *P.rudis* from the otter in Brazil, which in fact was the first species ever to be described from this genus of worms, still awaits confirmation. And finally, a recently described species *P.yunnanensis* by Ho and others in China appears to be a valid species according to Miyazaki (1961b) but as we are unable to get any literature or adequate description of the morphology of this worm we have omitted it from the key.

Summary

Lung flukes of the genus *Paragonimus* have been recovered from a variety of carnivores in Ceylon for the first time. An account is given of these worms from a civet cat, a fishing cat, a rusty spotted cat and three leopards.

The worms from the leopards and the rusty spotted cat have been identified as *P.westermani*, while the worms recovered from the civet cat and the fishing cat are provisionally identified as *P.compactus*. It is suggested that *P.compactus* should be regarded as a valid species and a simple key to six of the species that are recognised as valid today is given.

Key to species of Paragonimus



Acknowledgements

We are extremely grateful to Professor J. J. C. Buckley for kindly loaning us a specimen of Vevers' *P.compactus* and to Professor Ichiro Miyazaki for his invaluable opinions and suggestions. We are thankful to Dr. D. V. Arndt, Mr. E. C. Fernando and Mr. S. Nagamuthu who were responsible for supplying us with the positive animals or their organs. Our thanks are also due to Mr. E. S. Thevasagayam, Mr. K. Kumarasamy and Mr. H. Todd for sending us crabs, Mr. H. E. Bartholomeusz for technical assistance and Mr. A. U. Jayaweera for the photographs and photomicrographs.

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EXPLANATION OF PLATES

PLATE I

Figure A.—Lungs of civet cat showing cysts. Arrow indicates larger cyst. (Scale in cms.).
Figure B.—Spines from a peeled preparation of a worm from the civet cat. (× 470).
Figures C. & D.—Two whole mounts (Ventral view) of worms from the civet cat. (×9 and ×8.6 respectively).

PLATE II

Figure E.—Section of a nodule in the lung of the civet cat showing eggs. (×470).
Figure F.—Whole mount (Ventral view) of the worm from the fishing cat. (×10).
Figure G.—Section of a nodule on the surface of the lung of the fishing cat showing eggs and inflammatory cells. (×470)

PLATE III

Figure H.—Posterior view of the lungs of the rusty spotted cat showing numerous small cysts. (Scale in cms.)
Figure I.—Low power view of the spines from the ventral surface of a worm from the rusty spotted cat between oral and ventral suckers, as seen in a peeled preparation of the cuticle. (×107).
Figure J.—Higher magnification of the above. (×470).
Figures K. & L.—Ventral view of two whole mounts of worms from the pleural cavity of the rusty spotted cat. (×12).
Figure M. Whole mount (Ventral view) of a worm from a cyst of the lungs of the same host. (×7).

PLATE IV

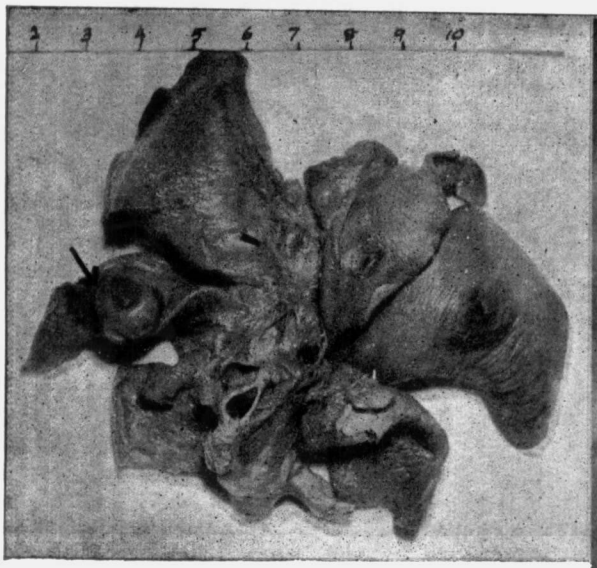
Figure N.—Lungs of leopard from Vavuniya showing cysts. (Scale in cms.).
Figure O.—Portion of the lung of the leopard from Habarana dissected to show two cysts, one empty and one showing a pair of worms 'in situ', indicated by arrow. (Scale in cms.).
Figures P. & Q.—Ventral views of two whole mounts of worms from the Kekirawa leopard. (×7).
Figure R.—Whole mount (Ventral view) of a worm from the Vavuniya leopard. (×7).

PLATE V

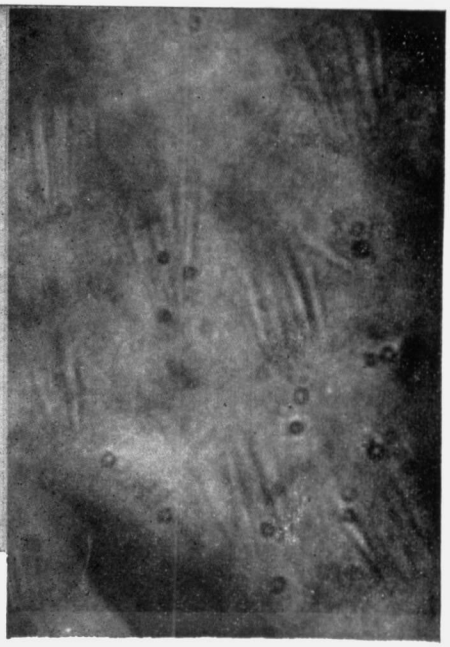
Figure S.—Spines from specimen obtained from Kekirawa leopard. (×107).
Figure T.—Spines of worm from Habarana leopard under a higher magnification. (×365).
Figure U.—Ventral view of *P.compactus* re-described by Vevers. (×8).
Figure V.—Spines from a peeled cuticle preparation of a specimen of Vevers' *P.compactus*. (×470).

EXPLANATION OF TEXT FIGURES

Figure I.—Map of Ceylon showing areas from which *Paragonimus* has been recovered from various hosts.
Figure II.—a. Spines from peeled ventral cuticle of a worm from the civet cat, between oral and ventral sucker.
b. Spines from a mounted specimen from the same host in the same region.
Figure III.—a—f. Ovaries (Ventral view) of 6 mounted specimens from the civet cat.
Figure IV.—A few eggs of a worm from the civet cat.
Figure V.—a. Ovary of the specimen from the fishing cat.
b. Spines from the same specimen between oral and ventral sucker (Ventral).
c. An egg from the same worm.
Figure VI.—a. Spines from the peeled cuticle of a specimen from the rusty spotted cat, between the oral and ventral sucker.
b. Spines from a mounted specimen from the same host, from the same region.
Figure VII.—a—f. Ovaries of 7 mounted specimens from the rusty spotted cat (Ventral view); a, b and c are of worms from pleural cavity.
Figure VIII.—A few eggs of a worm from the rusty spotted cat.
Figure IX.—Spines from various regions of ventral cuticle of leopard worms. (a. and d. from Kekirawa leopard; b. and e. from Vavuniya leopard; and c. and f. from Habarana leopard. Various degrees of splitting are shown).
Figure X.—a—f. Ovaries of 6 mounted specimens from the three leopards (Ventral view).
Figure XI.—a—c. Eggs taken from worms from the three leopards.
Figure XII.—Ovary (Ventral view) of the specimen of Vevers' *P.compactus*.



A



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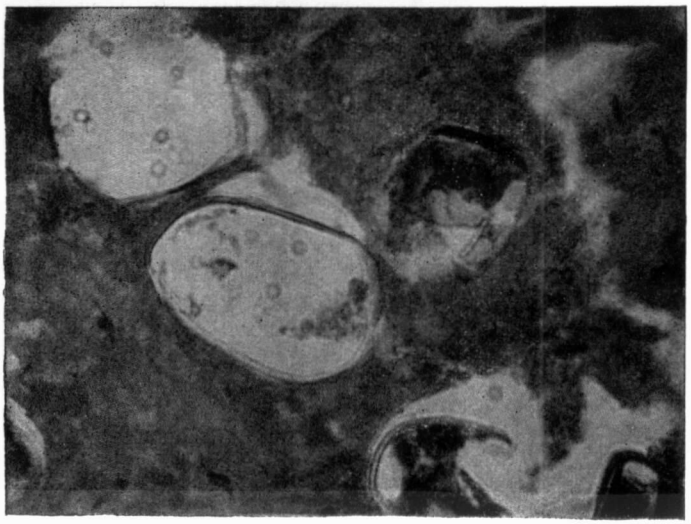

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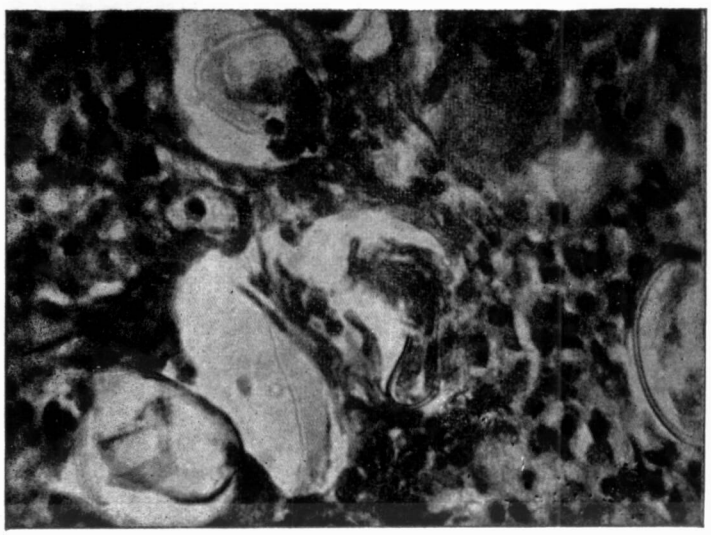

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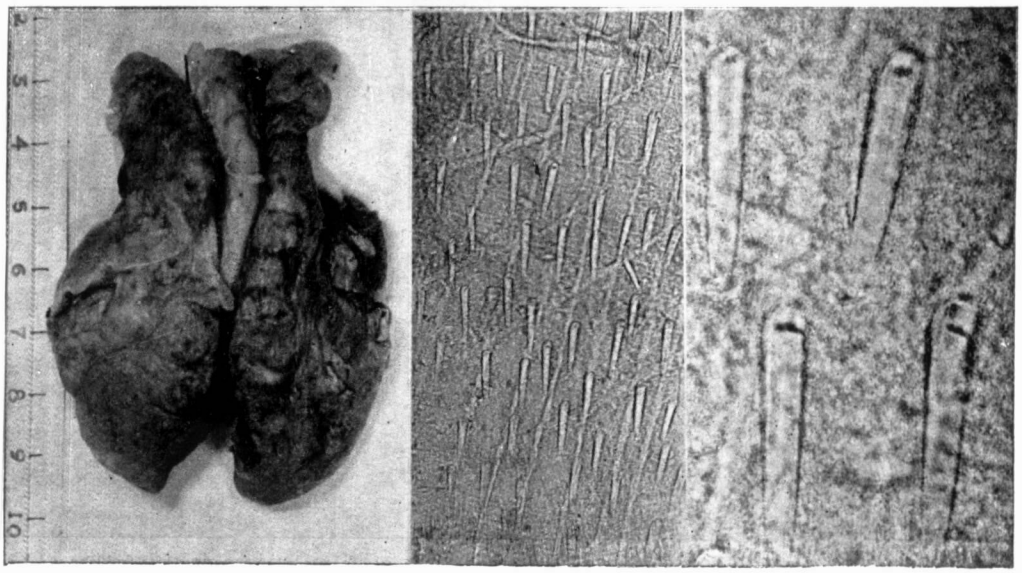
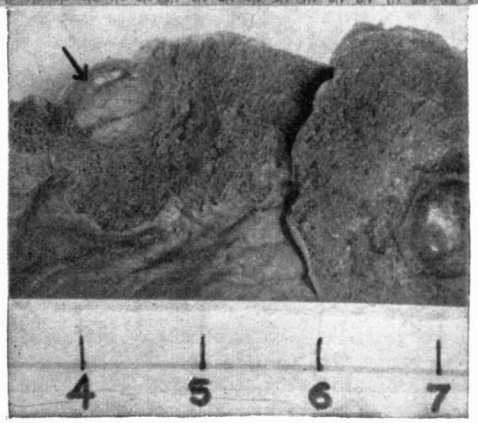


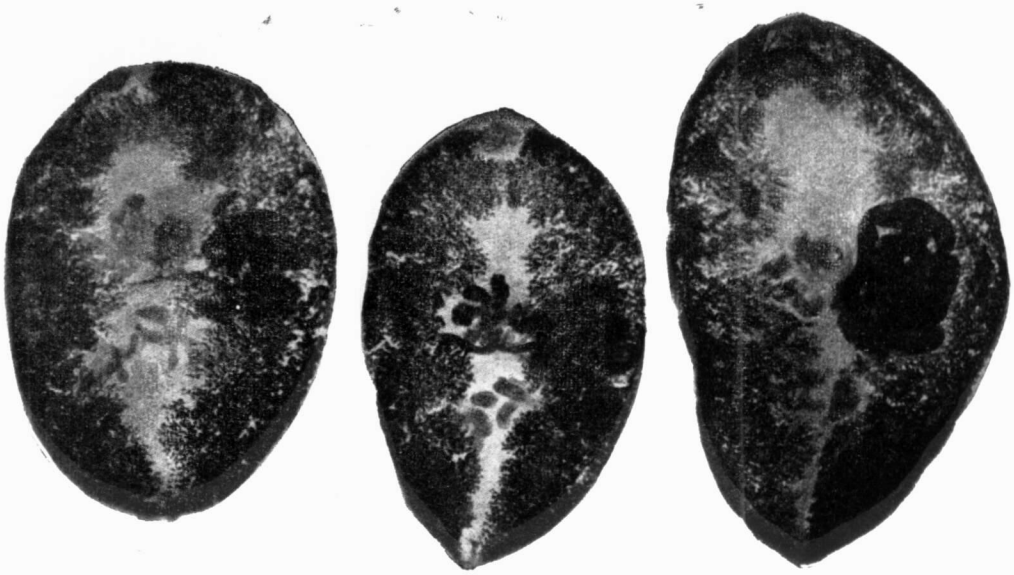
H I J

K L M

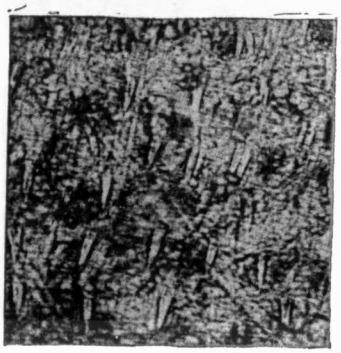
N

O





P Q R



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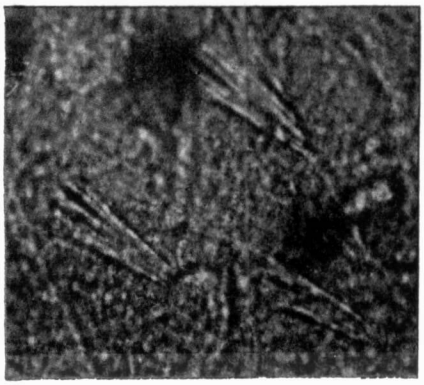

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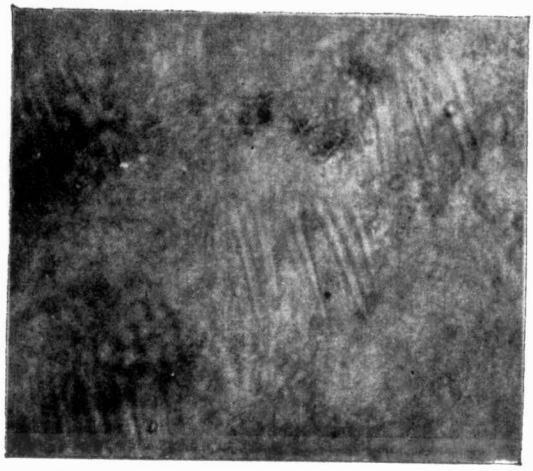

V