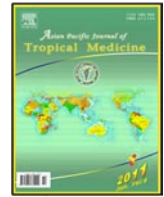




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Paragonimus worm from a New Guinea native in 1926Wenlin Wang¹, David Blair², Tian Min³, Fang Li¹, Dianhua Wang^{4*}¹Department of Parasitology, Faculty of Basic Medicine, Kunming Medical College, Kunming 650031, China²School of Tropical Biology, James Cook University, Townsville, Queensland 4811, Australia³Department of Cell Biology and Genetics, Faculty of Basic Medicine, Kunming Medical College, Kunming 650031, China⁴The Key Laboratory of Pharmaceutical Science, Kunming Medical College, Kunming 650031, China

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ABSTRACT

Objective: To reobserve and research the specimen of *Paragonimus* worm found in the left lung of a New Guinea native in 1926, which was previously identified as *Paragonimus westermani* Kerbert or *Paragonimus ringeri* Cobbold. **Methods:** Using reconstructive software and microscopy to observe some organs of the worm, and compared with other species of paragonimus. **Results:** The three dimensional (3D) views of ovary and two testes of New Guinea specimen showed that the ovary was clearly divided into six lobes. These two testes were situated oppositely in the body. One teste was divided into four branches, while another was divided into five. The cuticular spines were arranged in groups over the entire skin covered in a slide, each group was consisted of two to four single spine. **Conclusions:** Based on 3D views and measurements, we reclassified it as *Paragonimus siamensis*. This was also the first report of human case infected by *Paragonimus siamensis*.

1. Introduction

Paragonimiasis is the disease infected by lung flukes of genus *Paragonimus*. Lung flukes are found in the tropics and subtropics of East and South Asia and in Sub-Saharan Africa. It is estimated that 293 million people are at risk of paragonimiasis, and several million are actually infected [1–3]. Several species of the genus have been reported from every corner of the world, and the adequacy of species identification has begun to be scrutinized recently. We report an old case of Paragonimiasis in New Guinea.

2. Materials and methods

The sections of one *Paragonimus* worm and some skin of another worm were reserved in School Public Health & Tropical Medicine, Sydney, Australia. The number of specimen was MN.605, MN.905 (1–15).

Using microscope (OLYMPUS Ch-2) to observe and draw images of ovary and testes in the slides.

These images were scanned by scanner (HP scanjet 4570 c) for analysis. 3D views were generated and plane views were made out through linking the sideline of the 3D views.

The slide of cuticular spines on the worm skin was observed by micro-picture.

3. Results

The 3D views of ovary (Figure 1) and two testes (Figure 2) were made out through the constructure.

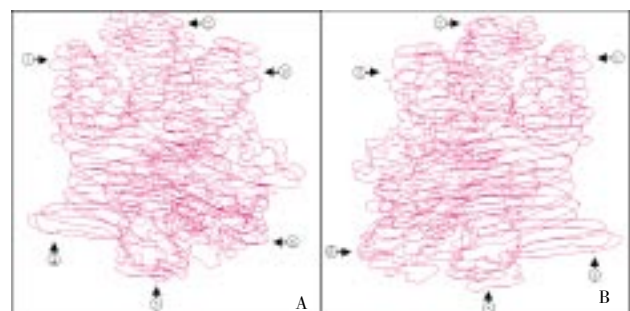


Figure 1. 3D views of ovary.

(A) Arrows show the ovary is divided into six lobes from one side view;

(B) Arrows also show the ovary has six lobes from another side view.

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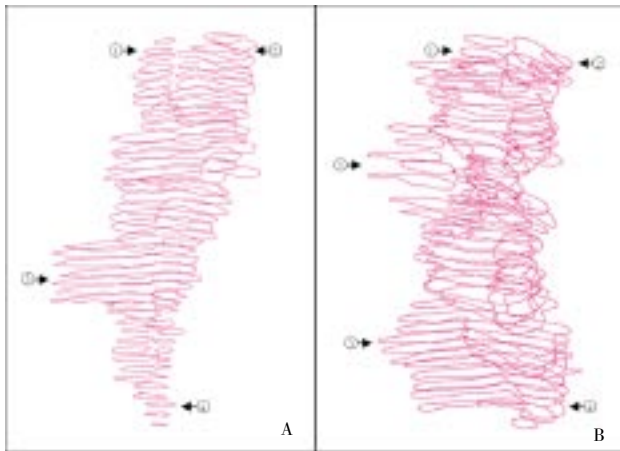


Figure 2. 3D views of two testes.
(A) Arrows show one teste is divided into four branches;
(B) Arrows show another teste is divided into five branches.

3D views showed their morphology characters. The ovary was clearly divided into six lobes. Each lobe looked like a club and had a broad end (Figure 1). The two testes were situated oppositely in the body. One was high and another was slightly low. Their shapes were very similar to each other. One teste was divided into four or five branches and these branches were very simple. Some branches had fold or protrusion (Figure 2).

The micro-picture of cuticular spines was taken by observing their slides. The cuticular spines were arranged in groups over the entire skin covered in a slide, and each group was consisted of two to four single spine (Figure 3).



Figure 3. Micro-picture of cuticular spines.
The cuticular spines are arranged in groups (arrows).

4. Discussion

On August 13, 1926, R.W.Cilento and T.C.Backhouse^[4] took an examination on a male native from New Guinea who died after amputation for chronic ulcer of the leg. They found the left lung had a small cavity containing two

small pinkish worms. As these two worms had the main characters of the genus *Paragonimus* they were identified as *Paragonimus westermani* (*P. westermani*). The specimen was in 6 mm length, 4.5 mm breadth and 3 mm thickness. From ventral view the external organs, anterior sucker, mouth, ventral sucker could be seen. Cuticular spines were not clear, but appeared lancet-shaped and generally distributed rather than arranged in groups. The ova were expressed from the uterus of the second fluke in the cyst, varying in shape, with length from 87 to 96 μ m, breadth from 44 to 48 μ m, well marked operculum usually at broad end, vitelline cells present, yellowish brown in colour, and shell thin. In September, 1926, G.M.Heydon^[5] gave one whole and several slides which had been dissected by T.C.Backhouse. There were four *Paragonimus* species in the world in 1926, *P. westermani*, *Paragonimus ringeri* (*P. ringeri*), *Paragonimus compactus* (*P. compactus*) and *Paragonimus kellicotti* (*P. kellicotti*). H.B.Ward, E.F.Hirsch(1915)^[6], and G.M.Vevers(1923)^[7] put forward that the characters of the species were the most reliable means of differentiating the species of the genus *Paragonimus*. According to the characters of the group spines which arranged from two to four and the eggs were large with the average measurements being 0.09 by 0.055 millimetre. So the worm was identified as *P. ringeri* by G.M.Heydon. From 1926 till present time, 50 species of *Paragonimus* have been reported even though some species were synonymy. *P. ringeri* is thought to be synonymy with the triploid of *P. westermani*^[1]. So it is worthy further studying to identify which specie of *Paragonimus* the specimen of this New Guinea native is.

From above 3D views, the specimen from New Guinea is similar to these three reported species: *Paragonimus siamensis* (*P. siamensis*)^[8,9], *P. compactus*^[7], *P. westermani* ^[10]. The compare results of these species were given below.

For the sample from New Guinea, at present time the eggs covered in a slide were all ruptured, it was valueless to distinguish from other species of *Paragonimus* in egg stage. In adult worm stage, the New Guinea sample whose spines were grouped. The shape of two testes was not like ovary, so it has differences from *P. westermani*. The ovary with 5 lobes of *P. compactus* was different from New Guinea sample, too. While the shape of ovary and testes of New Guinea sample were similar to that of *P. siamensis*.

It is certainly that there are still some questions especially the synonymous to need to resolve, for example there was only different dividing ovary five or six lobes for *P. siamensis* and *P. compactus*. I.Miyaziki and D.E.Wyckoff(1965)^[8] studied the 45 paratypes of *P. siamensis*, the ovary was divided into six lobes in 29, into five lobes in 5 worms, into seven and eight in 1 worm each and in the remaining nine the branching was not clearly seen. These 5 worms with a 5 lobed ovary could not be distinguished morphologically from *P. compactus*.

It is the first report of *P. siamensis* infected human although I.Miyaziki and D.E.Wyckoff(1965)^[8] studied the

photomicrographs of *Paragonimus* eggs from the sputum of 38 cases of human paragonimiasis in Saraburi Province of central Thailand in two papers published by S.Vajrasthira et al (1959 and 1962). They were identified as those of *P.westermani*, and it is thought that those eggs were more similar in appearance to *P. siamensis ova*.

The disease was found in a native of the village of Awursingi of New Guinea in 1926, the location was near the southeast coast of the island of New Britain. Although the time was very long till present, it is a necessary and challenging work to further investigate and study local *Paragonimus* and paragonimiasis. Molecular data is now being used extensively for lung flukes. Molecular phylogenies produced to date indicate that *Paragonimus* species infecting humans were scattered on the tree and were not a monophyletic group^[11–19].

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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References

- [1] Blair D, Agatsuma T, Wenlin W. Paragonimiasis .In: Murrell KD, Fried B. World class parasites. *Food-borne parasitic zoonoses fish and plant-borne parasites*. New York: Springer Science & Business Media, LLC; 2007, p. 117–50.
- [2] Keiser J, Utzinger J. Emerging foodborne trematodiasis. *Emerg Infect Dis* 2005; **11**(10): 1507–14.
- [3] Procop GW. North American Paragonimiasis (Caused by *Paragonimus kellicotti*) in the context of global Paragonimiasis. *Clin Microbiol Rev* 2009; **22**(3): 415–46.
- [4] Cilento RW, Backhouse TC. Paragonimiasis: its first recorded occurrence in the territory of New Guinea. *Med J Australia* 1927; **15**: 79–81.
- [5] Heydon GM. *Paragonimus ringeri* in a New Guinea Native. *Med J Australia* 1927; **24**: 204–5.
- [6] Ward HB, Hirsch EF. The species of *Paragonimus* and their differentiation. *Ann Trop Med Parasitol* 1915; **9**:109–63.
- [7] Vevers GM. Observations on the genus *Paragonimus* braun with a re-description of *P. compactus* (Cobbold,1859) 1899. *J Helminthol* 1923; **1**: 9–20.
- [8] Miyazaki I, Wykoff DE. On a new lung fluke *Paragonimus siamensis* n.sp. found in Thailand(Trematoda: Troglotrematidae). *Jap J Parasit* 1965; **14**(3): 251–7.
- [9] Kannangara DWW, Karunaratne GMS. *Paragonimus siamensis*–the fourth lung fluke reported from Ceylon. *Ceylon J Med Sci* 1969; **18**(2): 61–5.
- [10]Burton JB, Clint EC, Thomas NO. *Human parasitology*. 3 rd ed. New York: Elsevier Academic Press; 2005, p. 217–21.
- [11]Nolan MJ, Cribb TH. The use and implications of ribosomal DNA sequencing for the discrimination of digenean species. *Adv Parasitol* 2005; **60**: 101–63.
- [12]Iwagami M, Rajapakse RP, Paranagama W, Agatsuma T. Identities of two *Paragonimus* species from Sri Lanka inferred from molecular sequences. *J Helminthol* 2003; **77**(3): 239–45.
- [13]Blair D, Chang ZS, Chen MG, Cui AL, Wu B, Agatsuma T, et al. *Paragonimus skrjabini* Chen, 1959 (Digenea:Paragonimidae) and related species in eastern Asia: a combined molecular and morphological approach to identification and taxonomy. *Syst Parasitol* 2005; **60**(1): 1–21.
- [14]Jones MK. Recent advances in the diagnosis of human Schistosomiasis. *Asian Pac J Trop Med* 2008; **1**(1): 63–68.
- [15]Cristobal S,Berry-Caba.Schistosomiasis and the Philippine campaign. *Asian Pac J Trop Med* 2008; **1**(2): 41–60.
- [16]Nmorsi OPG,Isaac C, Ukwandu NCD,Ekundayo AO,Ekozien MI.Schistosoma haematobium and Plasmodium falciparum co-infection with protection against Plasmodium falciparum malaria in Nigerian children. *Asian Pac J Trop Med* 2009; **2**(2): 16–20.
- [17]Agatsuma T,Hirai H. Genetic variation of *Paragonimus* species. In:Arizono N,Chai JY, Nawa Y, Takahashi Y, eds. *Asian Parasitology, Vol.1:Food-Borne Helminthiasis in Asia. Japan: FAP Journal*. 2005:P.155–165.
- [18]Choi JH, Lee JH, Yu HS, Jeong HJ, Kim J, Hong YC, Kong HH, Chung DI.Molecular and biochemical characterization of hemoglobinase, a cysteine proteinase, in *Paragonimus westermani*. *Korean J Parasitol* 2006; **44**: 187–196.
- [19]Kumar S, Tamura K Nei M. MEGA3:integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Briefings Bioinformatics* 2004; **5**: 150–163.