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RESEARCH NOTE

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Chromosomes and Chromosome Breakage in the Lung Fluke, *Paragonimus heterotremus*

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Abstract

The chromosomes of the lung fluke, *Paragonimus heterotremus*, were studied by air-drying technique. The results showed the chromosome numbers were 2n = 22 and n = 11, which consisted of one pair of large metacentric, four pairs of medium subtelocentric, three small metacentric or submetacentric, and three small submetacentric or subtelocentric, whereas the relative lengths of the chromosomes were 20.5 ± 0.4, 12.6 ± 0.2, 11.2 ± 0.2, 9.3 ± 0.2, 8.9 ± 0.2, 7.8 ± 0.2, 7.4 ± 0.1, 6.0 ± 0.1, 6.0 ± 0.1, 6.0 ± 0.1 and 4.2 ± 0.0%, respectively. The results also revealed chromosome breakage on the small chromosome (No. 8).

Keyword: lung fluke, *Paragonimus heterotremus*, chromosomes, chromosome breakage

The lung fluke, *Paragonimus*, is a harmful parasite causing paragonimiasis in humans and animals, mainly in Asia, West Africa, and South and Central America. Over forty species have been described, and more than ten species have been reported to infect humans. In Thailand, six species of *Paragonimus* have been reported in a variety of mammalian hosts: *P. siamensis, P. bangkokensis, P. harinasutai, P. macrochis, P. westermani* and *P. heterotremus* [1]. Among these, *P westermani* and *P. heterotremus* are infective to humans. Only *P. heterotremus* has been proven to be the main cause of human paragonimiasis in this country [2-5]. Cytological studies of lung flukes have been conducted and reported extensively for various species of parasites, but only a few papers have dealt with Thai *Paragonimus* chromosomes, which have been described by Terasaki in 1989 for *P. heterotremus*, and Komalamisra in 2000 for *P. siamensis* [6-7]. Although the chromosome of *P heterotremus* has been preliminarily explored, there is still a need for more information. This report aimed to investigate and clarify the chromosomes of Thai *P. heterotremus*.

Metacercariae of *P. heterotremus* were obtained from waterfall crabs, *Larnaudia beasekomae* Bott, 1970, collected from Pakphli District, Nakhon Nayok Province. After oral inoculation of metacercariae into cats, 60 days later, adult flukes were recovered from the lungs of the cats. The chromosomes were prepared from the gonadal tissues of the flukes according to the air-drying method of Terasaki and stained in 5% Giemsa solution for 10 minutes [8]. Finally, all the prepared slides were used for cytological examination.

Mainly, the chromosome numbers found in the genus *Paragonimus* were 2n = 22, n = 11, except those of *P. pulmonalis* (3n = 33) and some strains of *P. westermani* (3n = 33, 4n = 44), which were found in China and Japan [2, 8-14]. This report revealed the chromosome number of *P. heterotremus* to be 2n = 22, n = 11, and could be divided into 3 groups (large, medium, and small) according to their sizes, which resembled other reported species. Based on the chromosome

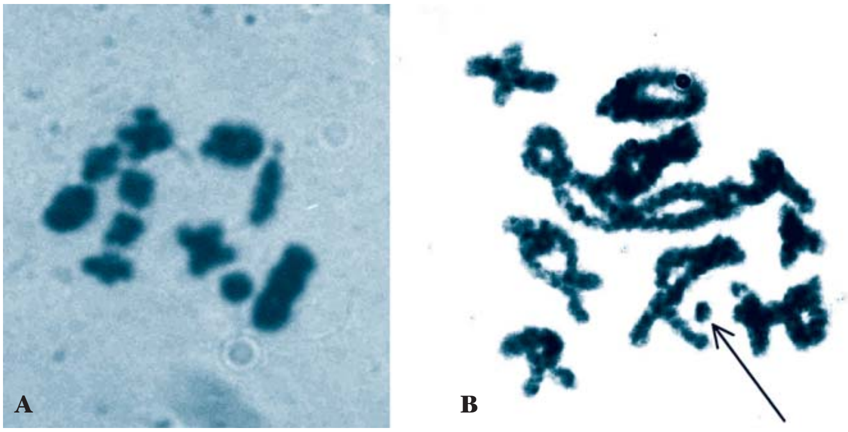

Fig 1 A. Chromosomes of *Paragonimus heterotremus* (n = 11, 2n = 22).
B. Small-chromosome fragment in the diplotene stage (arrow).

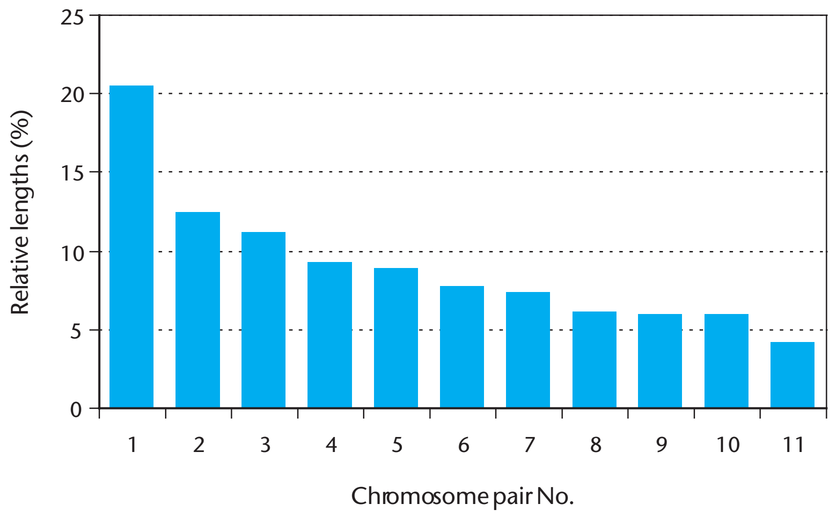

Fig 2 Bar chart showing relative length of *Paragonimus heterotremus* chromosomes.

nomenclature recommended by Levan [15], it consisted of one pair of large metacentric, four pairs of medium subtelocentric, three small metacentric or submetacentric, and three small submetacentric or subtelocentric [Fig 1]. On the basis of 35 metaphase figures, the length of each chromosome was measured by ImageJ 1.34s (http://rsb.info.nih.gov/ij/), and then the percentage relative length of each chromosome was calculated [relative length (%) = (length of each chromosome/total length of all chromosomes) x 100], resulting in 20.5 ± 0.4, 12.6 ± 0.2, 11.2 ± 0.2, 9.3 ± 0.2, 8.9 ± 0.2, 7.8 ± 0.2, 7.4 ± 0.1, 6.0 ± 0.1, 6.0 ± 0.1, 6.0 ± 0.1 and 4.2 ± 0.0%, respectively; a bar chart was constructed and is shown in Fig 2. This report also revealed chromosome breakage on the small chromosome (No. 8), of which a small chromosome fragment was seen and recognized in the diplotene stage of some cell plates [Fig 1]. A chromosome deletion may occur when the chromosome breaks and a piece is lost. This, of course, involves loss of genetic information and results in what could be considered "partial monosomy" for that chromosome. Chromosomes are the information manuals of cells. Genes are arranged on chromosomes in a linear fashion, like beads arranged on a string. Genes monitor cells how to make proteins, which perform many vital functions in the body. When chromosomes break, genes are disrupted and do not function correctly. This leads to produce abnormal proteins, resulting in various health problems. For instance, in parasitic diseases, Corcoran *et al* showed that the structural genes for *Plasmodium falciparum* antigen could be lost due to chromosome deletion [16]. Alano *et al* revealed that *Plasmodium falciparum* carrying a subtelomerically deleted chromosome 9 could not form even the earliest form of gametocyte and be detectable with antibodies against gametocyte-specific antigen [17]. Additional information concerning chromosome aberrations of parasites has been reported [18-21]. Although chromosome healing may occur spontaneously when a chromosome is accidentally broken, some problems related to paragonimiasis (*P. heterotremus*) may involve chromosome breakage or partial chromosome loss by the parasite.

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