

Karyological Studies of *Biomphalaria tenagophila* (d'Orbigny, 1835) (Gastropoda: Planorbidae) from Rio de Janeiro, Brazil

Gab Man Park^{1,*} and Tai-Soon Yong²

¹Department of Environmental Medical Biology, Kwandong University College of Medicine, Gangneung 210-701, Korea; ²Department of Environmental Medical Biology and Institute of Tropical Medicine, Yonsei University College of Medicine, Seoul 120-752, Korea

Abstract: The karyotypes of *Biomphalaria tenagophila* collected from Rio de Janeiro, Brazil were studied using the air-drying method. Somatic cells of this species had $2n=36$. The 18 chromosome pairs were identified and classified into 3 groups. The diploid cell has 7 pairs of metacentric, 8 pairs of submetacentric, and 3 pairs of subtelocentric chromosomes. Observed chromosomes ranged from 2.4 to 6.4 μm , and the total length was 122.3 μm . This is the first report on the chromosome of *B. tenagophila*.

Key words: *Biomphalaria tenagophila*, karyotype, freshwater snail, Brazil

Biomphalaria tenagophila is a species of air-breathing freshwater snail, an aquatic pulmonate gastropod mollusk in the family Planorbidae. *B. tenagophila* is a neotropical species, and its native distribution include Peru, Uruguay, Argentina, and southern Brazil [1-4]. Santos et al. [5] was the first to record *B. tenagophila* on Ilha Grande, Rio de Janeiro, in Brazil. This species is a medically important pest because of transferring the disease, intestinal schistosomiasis [6]. Intestinal schistosomiasis is the most widespread of all types of schistosomiasis [7,8]. *B. tenagophila* is an intermediate host and a vector of *Schistosoma mansoni* [9]. *S. mansoni* infects about 83.31 million people worldwide [10].

The study of an organism genome at the chromosomal level can be used in differentiating one species from another, i.e., the analysis of chromosome numbers, size, and centromere positions [11-13]. In fact, modern cytogenetic techniques have since mid-1990s been adopted for studies of Gastropoda. The subfamily Biomphalarinae has been known to be a conservative group in regard to haploid chromosome numbers 18 (Table 1). In this study, the karyotype of *B. tenagophila* was studied

in order to analyze their genetic relationships.

The 9 specimens used in this study were collected in Museu de Arte Moderna (22°54'48.12"S, 43°10'19.17"W), Rio de Janeiro, Brazil, September 2012, and examined shortly after collection (Fig. 1). The chromosome preparations were made on gonad of the specimens by the usual air-drying method [14]. The prepared slides were observed under an Olympus (BX51) microscope. The identical specimens used for this study have been deposited at the Department of Environmental Medical Biology, Kwandong University College of Medicine, Korea.

A microphotograph of somatic metaphase chromosomes and the karyogram are shown in Fig. 2, and measurements of the chromosomes are presented in Table 2. Fig. 2B shows the karyotype of this species arranged by size. The present cytological preparations showed 10 well-spread mitotic cells on a slide. This species showed a diploid chromosome number of $2n=36$, with 7 metacentric, 8 submetacentric, and 3 subtelocentric chromosome pairs. Observed chromosomes ranged from 2.4 to 6.4 μm in length. This species was no inter-specimen variability in chromosome counts. Also, sexual dimorphism of chromosomes was not found in this study.

Most species of the *Biomphalaria* have been investigated and at present, *B. glabrata*, *B. pfeifferi*, *B. madagascariensis*, *B. sudanica*, *B. tanganyicensis*, *B. alexandrina*, and *B. truncatus* have a haploid chromosome complement of 18 (Table 1). The chromosomes are relatively small and monomorphic. Conservation of

•Received 12 May 2014, revised 24 June 2014, accepted 24 June 2014.

*Corresponding author (gmpark@kd.ac.kr)

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Table 1. Chromosome numbers and karyotypes of subfamily Biomphalarinae

Species	Haploid no.	Karyotype ^a	Source	References
<i>Biomphalaria alexandria</i>	18		Egypt	Natarajan and Gismann [20]
	18	10M+4SM+2A+2T	Egypt	Abdel-Haleem [19]
	18	8M+8SM+2ST	Egypt	Bakry and Garhy [18]
<i>B. choanomphala</i>	18		Tanzania	Burch [16]
<i>B. glabrata</i>	18		Puerto Rico	Burch [21]
	18	10M+4SM+2A+2T	USA	Raghunathan [17]
	18	10M+4SM+2A+2T	Egypt	Abdel-Haleem [19]
<i>B. sudanica</i>	18		Sudan	Burch [21]
<i>B. tanganyicensis</i>	18		Tanzania	Natarajan and Gismann [20]
<i>B. pfeifferi</i>	18		Tanzania South Africa	Natarajan and Gismann [20]
<i>B. p. gaudi</i>	18		Liberia, Ghana	Natarajan and Gismann [20]
<i>B. p. madagascariensis</i>	18		Madagascar	Natarajan and Gismann [20]
<i>B. tenagophila</i>	18	7M+8SM+3ST	Brazil	This study
<i>B. truncatus</i>	18	10M+4SM+2ST+2T	Egypt	Bakry and Garhy [18]

^aA, acrocentric; M, metacentric; SM, submetacentric; ST, subtelocentric; T, telocentric chromosomes.



Fig. 1. Shell of *Biomphalaria tenagophila* from Rio de Janeiro, Brazil.

chromosome number has been pointed out for many gastropoda groups [15]. Burch [16], in the 1960s, utilized preparations from the ovo-testis and reported a haploid complement of 18; however, these results were deduced from the meiotic stage, where it was often difficult to identify each and every chromosome. However, with the development of cytological methods such as hypotonic treatment of tissue samples and pre-treatment with colchicines (air drying technique), clearer deductions were possible on the chromosome number and morphology [11]. The morphology of mitotic metaphase chromosomes has been reported in 10 species of the subfamily Biomphalarinae with $n = 18$ and $2n = 36$ (Table 1).

The present results revealed that the diploid chromosome number of *B. tenagophila* is 36. With regard to karyotype analysis in the genus *Biomphalaria*, only 4 papers about *B. glabrata*, *B. truncatus*, and *B. alexandria* has been published [17-19]. Though the chromosome number is the same between *B. glabrata*, *B. alexandria*, *B. truncatus*, and *B. tenagophila*, the karyotype is dif-

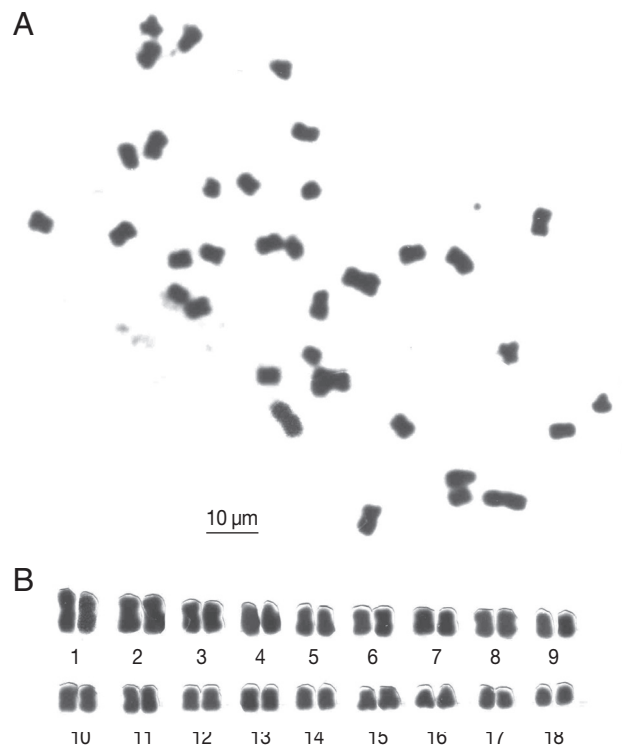


Fig. 2. Metaphase chromosomes of *Biomphalaria tenagophila* (A) and karyotype constructed from A (B).

ferent. *B. glabrata* and *B. alexandria* have 10 pairs of metacentric, 4 pairs of submetacentric, 2 pairs of acrocentric, and 2 pairs of telocentric chromosomes, and *B. truncatus* has 10 metacentric, 4 submetacentric, 2 subtelocentric, and 2 telocentric chromosomes, whereas *B. tenagophila* has 7 metacentric, 8 submetacentric, and 3 subtelocentric chromosomes. The results of this study on the mitotic metaphase chromosomes provided a

Table 2. Total lengths (μm) and relative lengths of chromosomes of *Biomphalaria tenagophila*

Chromosome	Total length \pm SE	Relative length \pm SE	Type
1	6.4 \pm 0.21	9.3 \pm 0.42	M
2	6.2 \pm 0.80	9.0 \pm 0.35	M
3	5.5 \pm 0.45	8.0 \pm 0.63	M
4	5.3 \pm 0.11	7.7 \pm 0.16	ST
5	4.8 \pm 0.43	7.0 \pm 0.65	SM
6	4.6 \pm 0.19	6.7 \pm 0.32	M
7	4.4 \pm 0.10	6.4 \pm 0.19	SM
8	4.2 \pm 0.14	6.1 \pm 0.27	M
9	4.0 \pm 0.10	5.8 \pm 0.54	SM
10	3.9 \pm 0.08	5.7 \pm 0.35	M
11	3.7 \pm 0.23	5.4 \pm 0.15	SM
12	3.6 \pm 0.07	5.2 \pm 0.23	SM
13	3.4 \pm 0.13	4.9 \pm 0.13	M
14	3.2 \pm 0.11	4.7 \pm 0.16	SM
15	3.0 \pm 0.15	4.4 \pm 0.33	SM
16	2.8 \pm 0.12	4.1 \pm 0.12	ST
17	2.6 \pm 0.09	3.8 \pm 0.08	SM
18	2.4 \pm 0.26	3.4 \pm 0.21	ST

Based on measurements from 4 sets of karyotyped cells. SE, standard error.

much more elucidated observation of the chromosomes of this species. Further studies are needed with various analyses for the investigation of the karyo-systematic evolution, and accumulation of karyological information is very much required in the subfamily Biomphalarinae.

CONFLICT OF INTEREST

We have no conflict of interest related to this study.

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