

Karyotypes on three species of Chinese mesogastropod snails, *Semisulcospira libertina*, *S. dolichostoma* and *Viviparus rivularis*

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Abstract: Three species of the families Viviparidae and Pleuroceridae, the first intermediate host of paragonimiasis, metagonimiasis and echinostomiasis were studied cytologically. The observed diploid chromosome number was as follows: *Semisulcospira libertina* 36, *S. dolichostoma* 34, and *Viviparus rivularis* 64. The mitotic chromosome complement of *S. libertina* has nine metacentric pairs and nine submetacentric pairs, and *S. dolichostoma* has three metacentric pairs and 14 submetacentric pairs of chromosomes. *Viviparus rivularis* showed two metacentric pairs and 30 submetacentric pairs of chromosomes.

Key words: chromosome numbers, karyotypes, Chinese mesogastropods, Viviparidae, Pleuroceridae

INTRODUCTION

Taxonomy of viviparid and pleurocerid snails which serve as intermediate hosts for several kinds of trematodes has been in a chaotic state for many years (Burch, 1975). The systematics of the lower taxonomic categories still remains an enigma, both from the practical viewpoint of identifying specimens

and in understanding the mechanisms of speciation and evolution (Burch, 1968). In mollusca, the literatures on karyotype analysis are not abundant due to difficulties of obtaining mitotic fields with enough quality to carry out chromosome studies. "Melaniid" (=Pleurocerid) snails are among the most common and widely distributed groups of freshwater mollusks in the world, however studies concerning their chromosomes are rare. Until now, the chromosomes of six species have been described among the poorly understood genera *Semisulcospira* and *Viviparus* occurring in China.

The greatest variation in chromosome number of any mesogastropod family occurs in the pleuroceridae. Haploid chromosome number in 22 species and one subspecies of

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Semisulcospira ranges from n=7 to n=20 (Patterson, 1969; Dillon, 1991; Nakano et al., 1994). The chromosome number of the family Viviparidae ranges from n=7 to n=14 by the literature survey (Ramamoorthy, 1958; Inaba, 1965; Park et al., 1997). In the subfamily Viviparinae, the haploid numbers of eight species range from n=7 to n=13 (Patterson, 1969; Zhou et al., 1988).

The purpose of this study was to detect the chromosome numbers and to identify the karyotypes of two pleurocerid and one viviparid Chinese snails, which serve as molluscan intermediate hosts for paragonimiasis, metagonimiasis and echinostomiasis.

MATERIALS AND METHODS

The snails in this study were collected from Houwei and Suncun Villages, Jinde County, Anhui Province, China, in October 1997. Chromosome preparations were made from gonadal tissues by the air-drying method (Park, 1994). The materials examined were gonads in active stages of gametogenesis from six specimens of *Semisulcospira libertina*, five

specimens of *S. dolichostoma* and three specimens of *Viviparus rivularis*. To observe morphological features of the chromosomes, karyotypes, relative and total lengths of the mitotic metaphase chromosomes were measured. Nomenclature of morphological types of chromosome was adopted from Levan et al. (1964).

RESULTS

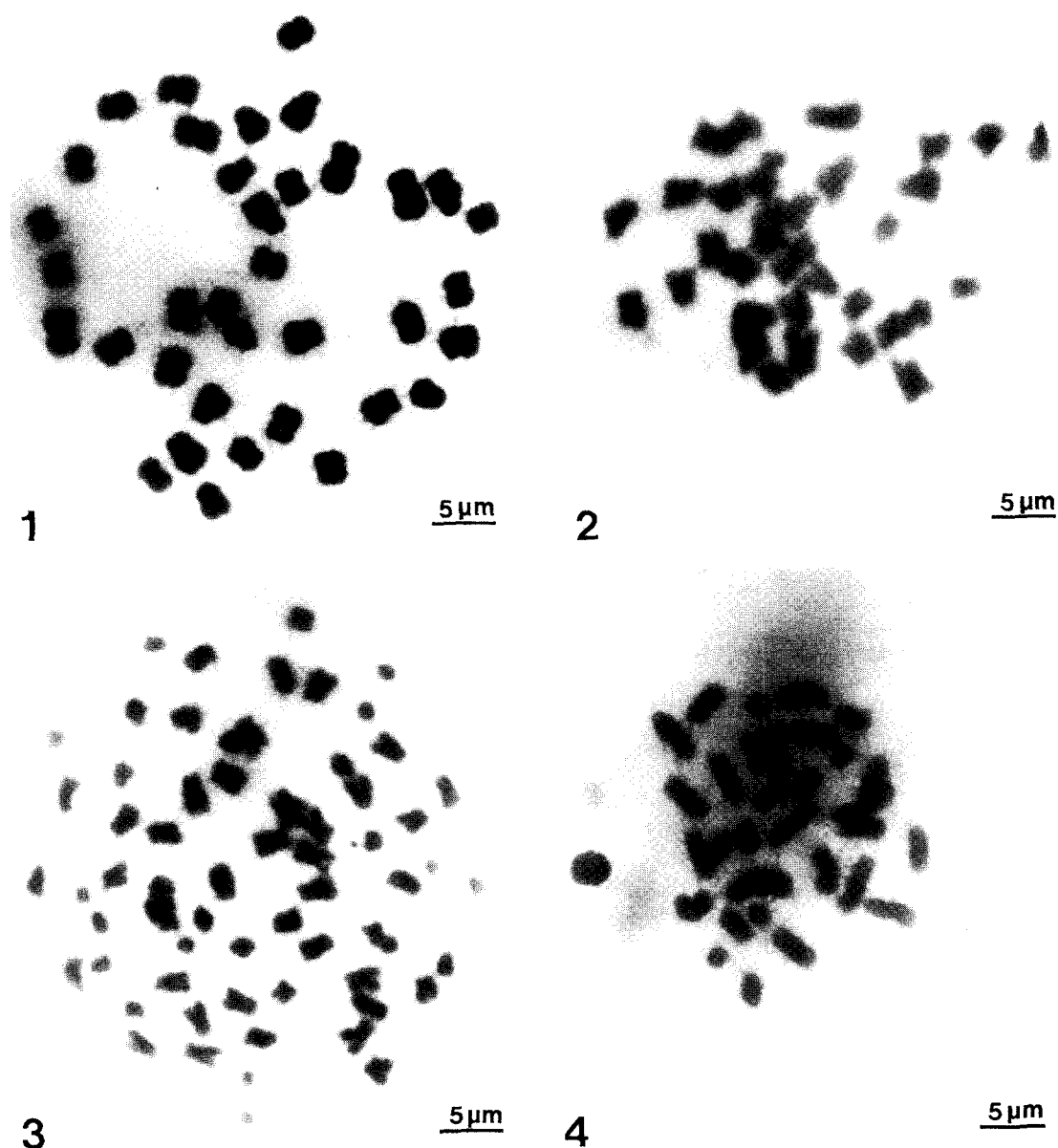
Pleuroceridae

***Semisulcospira libertina*:** Chromosomes were observed in three males and three females. The mitotic metaphase chromosomes were observed in both sexes. The diploid chromosome number was 36 (Fig. 1). The karyotypes were arranged by shape and size (Fig. 5), and Table 1 shows the results of chromosome measurement from this species. Chromosomes in males and females ranged from 2.2 to 4.2 μm in length. Mean total length of the diploid complements were $49.5 \pm 2.72 \mu\text{m}$. Karyotype consists of nine pairs of metacentrics and nine pairs of submetacentric chromosomes (Fig. 5).

Table 1. Measurements of the mitotic metaphase chromosomes of *Semisulcospira libertina*^{a)}

No. of chromosome	Length (μm)	Relative length	Type
1	4.1 ± 0.08	8.28 ± 0.22	M ^{b)}
2	2.7 ± 0.31	5.45 ± 0.44	M
3	2.5 ± 0.10	5.05 ± 0.05	M
4	2.5 ± 0.08	5.04 ± 0.17	M
5	2.3 ± 0.22	4.65 ± 0.46	M
6	2.3 ± 0.08	4.64 ± 0.23	M
7	2.2 ± 0.11	4.44 ± 0.44	M
8	2.2 ± 0.06	4.44 ± 0.26	M
9	2.2 ± 0.01	4.44 ± 0.08	M
10	4.2 ± 0.21	8.48 ± 0.40	SM ^{c)}
11	3.3 ± 0.26	6.67 ± 0.50	SM
12	3.2 ± 0.31	6.46 ± 0.47	SM
13	3.2 ± 0.17	6.46 ± 0.12	SM
14	2.7 ± 0.31	5.45 ± 0.34	SM
15	2.7 ± 0.14	5.45 ± 0.10	SM
16	2.5 ± 0.10	5.05 ± 0.05	SM
17	2.4 ± 0.05	4.85 ± 0.01	SM
18	2.3 ± 0.12	4.65 ± 0.23	SM

^{a)}Measurements were done with four sets of karyotyped mitotic cells; ^{b)}metacentric chromosome; ^{c)}submetacentric chromosome.



Figs. 1-4. Meiotic and mitotic chromosomes of three Chinese snail species. **Fig. 1.** Diploid chromosomes in metaphase I of *Semisulcospira libertina*. **Fig. 2.** Diploid chromosomes in metaphase I of *Semisulcospira dolichostoma*. **Fig. 3.** Diploid chromosomes in metaphase I of *Viviparus rivularis*. **Fig. 4.** Haploid chromosomes of *V. rivularis*.

***Semisulcospira dolichostoma*:** Thirty-four chromosomes were observed at spermatogonial metaphase in 24 mitotic cells studied. The mean total chromosome length, based on measurements from three sets of karyotyped

cells, was $50.3 \pm 2.29 \mu\text{m}$. The length of chromosomes ranged from 2.1 to 4.8 μm (Table 2). Fig. 6 shows the karyotype constructed from the chromosomes shown in Fig. 2, which was one of the most elongated complements. This species has 34 chromo-

Table 2. Measurements of the mitotic metaphase chromosomes of *Semisulcospira dolichostoma*^{a)}

No. of chromosome	Length (μm)	Relative length	Type
1	4.8 \pm 0.10	9.54 \pm 0.27	M
2	3.4 \pm 0.06	6.76 \pm 0.27	M
3	2.5 \pm 0.44	4.97 \pm 0.17	M
4	3.8 \pm 0.14	7.55 \pm 0.46	SM
5	3.6 \pm 0.13	7.16 \pm 0.05	SM
6	3.5 \pm 0.05	6.96 \pm 0.25	SM
7	3.4 \pm 0.02	6.76 \pm 0.44	SM
8	3.1 \pm 0.12	6.16 \pm 0.30	SM
9	2.9 \pm 0.08	5.76 \pm 0.54	SM
10	2.7 \pm 0.36	5.37 \pm 0.19	SM
11	2.6 \pm 0.22	5.17 \pm 0.20	SM
12	2.5 \pm 0.16	4.97 \pm 0.17	SM
13	2.4 \pm 0.18	4.77 \pm 0.13	SM
14	2.4 \pm 0.01	4.77 \pm 0.06	SM
15	2.4 \pm 0.12	4.77 \pm 0.03	SM
16	2.2 \pm 0.06	4.37 \pm 0.15	SM
17	2.1 \pm 0.04	4.17 \pm 0.14	SM

^{a)}Measurements were done with three sets of karyotyped mitotic cells.

somes: three pairs of metacentrics and 14 pairs of submetacentrics.

Viviparidae

Viviparus rivularis: From three male specimens, spermatogonial mitotic divisions were observed in 18 cells. In these cells, 64 chromosomes (2n) were also counted (Fig. 3). Meiotic chromosomes in male were observed (Fig. 4). The lengths of the chromosomes in male ranged from 1.6 to 4.6 μm . Mean total length of the diploid complements in male were 96.4 \pm 7.86 μm (Table 3). Karyotypes consist of two pairs of metacentrics and 30 pairs of submetacentric chromosomes (Fig. 7).

DISCUSSION

One of the most commonly encountered genera of freshwater snails in the Far East Asia is *Semisulcospira*. More than 18 species and subspecies of this genus have been described from China (Yen, 1939). Identification of these species is difficult due to the great amount of variation in shell shape, structure and coloration. In the Pleuroceridae, the family to which *Semisulcospira* is presently referred about chromosomes, 12 species have been studied from Japan and eight species

from Korea (Patterson, 1967; Burch, 1968; Park, 1994). *Semisulcospira libertina* (= *bensoni*) is regarded as the most important intermediate host of *Paragonimus westermani* (Yokogawa et al., 1960a, 1960b). Several nominal subspecies of *S. libertina* have also been implicated as intermediate hosts for paragonimiasis and metagonimiasis in humans and other mammals in the Far East. However, a little is known concerning the biological relationships of the nominal species of *Semisulcospira* over its wide range of geographic distribution (China, Formosa, Japan and Korea), and the relationship of *S. libertina* to other melaniid snails. *Semisulcospira dolichostoma* is a pleuracerid species in mainland China, and no information on the susceptibility of human-infecting trematodes is available. Although some viviparid snails such as *Cipangopaludina* spp. act as the second intermediate host of echinostome trematods, the parasitological role of *V. rivularis* has not been reported yet. Therefore, further studies on the susceptibility tests of these snails to trematodes are needed. Patterson (1967) found 18 bivalents in each of the specimens of *S. libertina* that were studied from four localities in Japan. Inaba and Tanaka (1953) reported *S. libertina* to have eight pairs of chromosomes

Table 3. Measurements of the mitotic metaphase chromosomes of *Viviparus rivularis*^{a)}

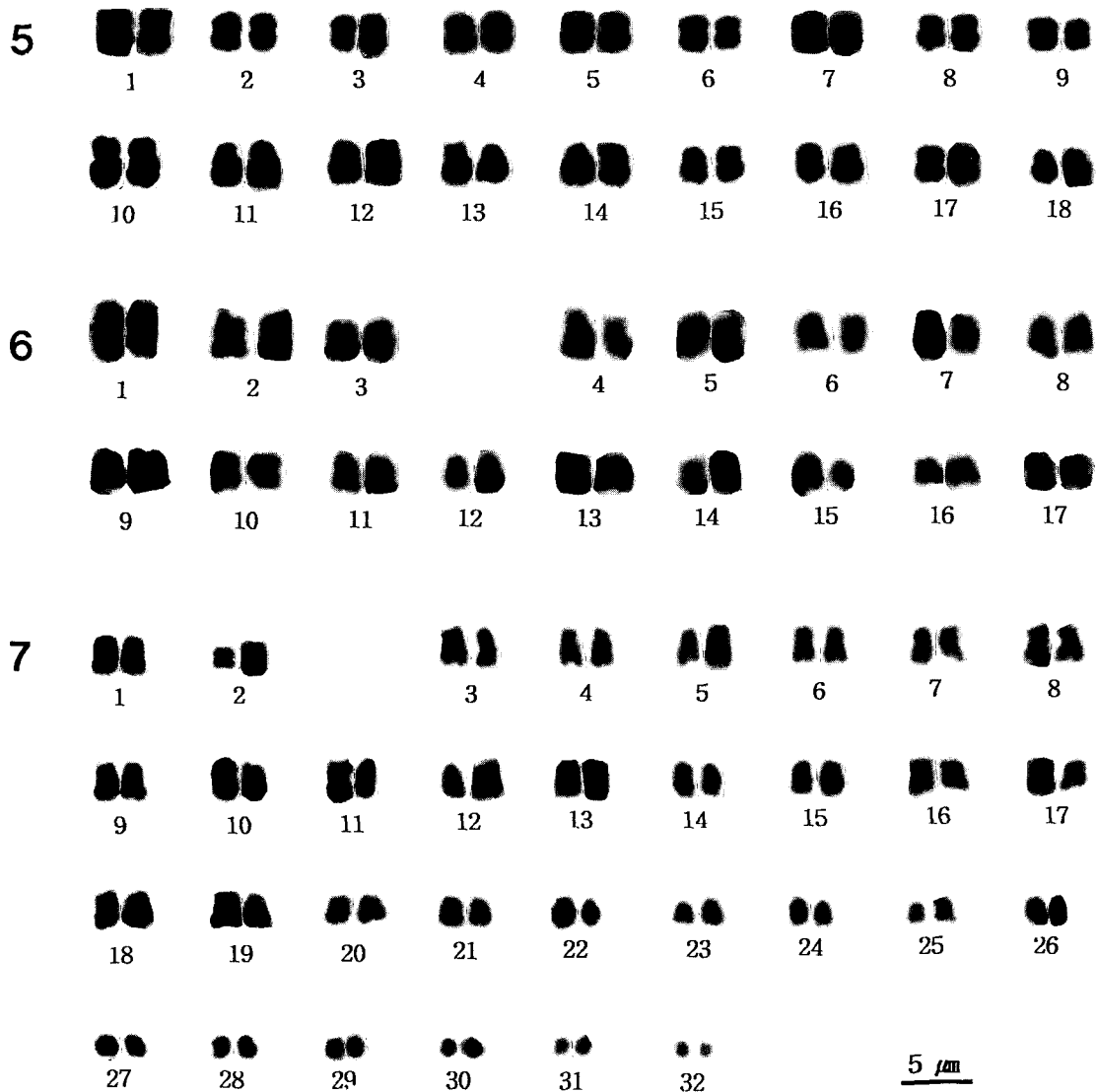
No. of chromosome	Length (μm)	Relative length	Type
1	3.6 \pm 0.14	3.73 \pm 0.44	M
2	2.1 \pm 0.02	2.18 \pm 0.42	M
3	4.6 \pm 0.26	4.77 \pm 0.18	SM
4	4.0 \pm 0.42	4.15 \pm 0.37	SM
5	3.9 \pm 0.13	4.05 \pm 0.04	SM
6	3.8 \pm 0.11	3.94 \pm 0.19	SM
7	3.8 \pm 0.08	3.94 \pm 0.15	SM
8	3.7 \pm 0.15	3.84 \pm 0.17	SM
9	3.6 \pm 0.14	3.73 \pm 0.44	SM
10	3.5 \pm 0.21	3.63 \pm 0.07	SM
11	3.4 \pm 0.11	3.53 \pm 0.27	SM
12	3.4 \pm 0.14	3.52 \pm 0.25	SM
13	3.3 \pm 0.12	3.42 \pm 0.23	SM
14	3.3 \pm 0.07	3.42 \pm 0.13	SM
15	3.3 \pm 0.10	3.42 \pm 0.11	SM
16	3.2 \pm 0.22	3.32 \pm 0.15	SM
17	3.2 \pm 0.14	3.32 \pm 0.12	SM
18	3.1 \pm 0.08	3.22 \pm 0.16	SM
19	3.1 \pm 0.04	3.22 \pm 0.13	SM
20	3.0 \pm 0.22	3.11 \pm 0.11	SM
21	2.9 \pm 0.25	3.00 \pm 0.08	SM
22	2.8 \pm 0.06	2.90 \pm 0.04	SM
23	2.7 \pm 0.17	2.80 \pm 0.03	SM
24	2.6 \pm 0.28	2.70 \pm 0.09	SM
25	2.6 \pm 0.11	2.70 \pm 0.01	SM
26	2.4 \pm 0.31	2.49 \pm 0.24	SM
27	2.3 \pm 0.04	2.39 \pm 0.10	SM
28	2.1 \pm 0.02	2.18 \pm 0.21	SM
29	2.0 \pm 0.15	2.07 \pm 0.04	SM
30	1.8 \pm 0.04	1.87 \pm 0.21	SM
31	1.7 \pm 0.28	1.76 \pm 0.34	SM
32	1.6 \pm 0.14	1.66 \pm 0.15	SM

^{a)}Measurements were done with five sets of karyotyped mitotic cells.

($n=8$, $2n=16$). In this study, however, all specimens of this species had 18 bivalents. Burch (1968) also found *S. libertina* to have a haploid chromosome number of $n=18$. We therefore mention that Inaba and Tanaka's report (1953) of only eight pairs of chromosomes for this species is in error. Burch (1968) extended our cytological knowledge of *Semisulcospira* by studying eight additional species in which the haploid chromosome numbers ranged from $n=8$ to $n=20$. With such a wide variation in chromosome numbers, the pleuroceridae has been proven to be a group of considerable cytogenetic interest. Park (1994) has reported

on the chromosomes of eight species of pleuroceridae in Korea. The six species of *Semisulcospira* in his study had chromosome number of $n=18$ and $2n=36$. It is obvious that chromosome number is more conserved in species of *Semisulcospira* in Korea than those in Japan or China. Park (1994) reported that the karyotyping is especially helpful for analyzing *Semisulcospira* in Korea, since all species studied so far from Korea have the same chromosome number ($n=18$, $2n=36$). *Semisulcospira forticosta* and *S. tegulata* can be distinguished by their karyotypes.

In the Viviparidae, the chromosome number of all species studied so far is $n=7$ to $n=14$



Figs. 5-7. Karyotypes of three Chinese snail species.

Fig. 5. Karyotype of the chromosomes in Fig. 1 (*Semisulcospira libertina*). **Fig. 6.** Karyotype of the chromosomes in Fig. 2 (*Semisulcospira dolichostoma*). **Fig. 7.** Karyotype of the chromosomes in Fig. 3 (*Viviparus rivularis*).

(Patterson, 1969; Zhou et al., 1988; Park et al., 1997). In the most primitive viviparid subfamily, Bellamyinae, the haploid chromosome numbers of 11 species studied range from 8 to 11 (Ramamoorthy, 1958; Inaba, 1965; Zhou et al., 1988). In the subfamily Viviparinae, the haploid number of eight species ranges from n=7 to n=13 (Rainer, 1963). Recently, Park et al. (1997) reported the chromosome numbers in two species of echinostome vector snails of

the family Viviparidae in Korea. In the present study, however, the chromosome number of *V. rivularis* is different from those of the former reports.

According to the above comparison, it is suggested that *S. libertina* is more conserved than *S. dolichostoma* or *V. rivularis*. DNA sequence analysis of specific genes could be valuable for further classification of the relationship of vector snails in different

localities.

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