

명주배꼽달팽이와 수정또아리물달팽이 2종의 염색체 연구¹⁾

박갑만²⁾

가톨릭관동대학교 의과대학 환경의생물학교실

요약

한국 고유의 육상달팽이인 달팽이과 명주배꼽달팽이(*Aegista tenuissima*)와 또아리물달팽이과의 수정또아리물달팽이(*Hippeutis cantori*)를 대상으로 염색체를 핵형 분석하였다. 명주배꼽달팽이의 이배체 염색체 수는 $2n=56$ 이었으며, 이는 14쌍의 metacentric 염색체, 13쌍의 submetacentric 염색체 그리고 1쌍의 subtelocentric 염색체로 구성되었다. 따라서 본 종의 기본 숫자는 $FN=56$ 으로 나타났다. 명주배꼽달팽이에서 관찰된 염색체의 전체 길이는 $1.3 \pm 0.05 \sim 5.3 \pm 0.23 \mu\text{m}$ 범위였으며, 이배체에서 염색체의 평균 전체 길이는 $61.6 \pm 2.68 \mu\text{m}$ 였다. 한편, 수정또아리물달팽이(*Hippeutis cantori*)의 염색체 수와 핵형은 $36(2n)$ 과 $13M+5SM$ 이었으며, 염색체의 평균 길이는 $59.64 \pm 1.18 \mu\text{m}$ 로 나타났다.

ABSTRACT

The karyotypes of the Korean endemic land snail, *Aegista tenuissima*, were investigated using the air-drying method. The diploid chromosome number for this species was $2n=56$, including 14 metacentric chromosome pairs, 13 submetacentric chromosome pairs and one subtelocentric chromosome pairs; therefore, the fundamental number was $FN=56$. Observed chromosomes ranged from 1.3 ± 0.05 to $5.3 \pm 0.23 \mu\text{m}$ in total length and the mean total length of the chromosomes in the diploid was $61.6 \pm 2.68 \mu\text{m}$.

1) Karyological Analysis of Korean Endemic Land Snail, *Aegista tenuissima* (Gastropoda: Bradybaenidae) and *Hippeutis cantori* (Gastropoda: Planorbidae).

2) PARK, Gab-Man, Department of Environmental Medical Biology, Catholic Kwandong University College of Medicine, E mail : gmpark@cku.ac.kr

Chromosome number and karyotypes of *Hippeutis cantori* was 36(2n) and 13M+5SM. Total length is $59.64 \pm 1.18 \mu\text{m}$.

Key words: Chromosome, Karyotype, *Aegista tenuissima*, *Hippeutis cantori*, Bradybaenidae, Planorbidae, Korea

I . INTRODUCTION

Bradybaenidae is a taxonomic family of medium-sized to small land snails, specifically terrestrial pulmonate gastropod mollusks in the superfamily Helicoidea. These snails are found mainly in Asia, with only one species occurring in Northwestern Europe: *Fruticicola fruticum*. Korean Bradybaenidae snails have been classified into 23 species by Kwon *et al.* (2001). The shell of *Aegista tenuissima* (Pilsbry & Hirase, 1908) is small to medium in size, has a low cone shape, and has a low spire. The whorl layer is 6.5 layers and is very flat. Mouth aperture is wide, the end is slightly spread, and it hangs down slightly from the body layer. It is a Korean endemic species. *Hippeutis* is a genus of minute air-breathing freshwater snails, aquatic pulmonate gastropod mollusks in the family Planorbidae. The first reported species of snails that are intermediate hosts to echinostomes are pulmonate snails in the family Planorbidae. They are hermaphroditic and is not operculated. The shell is discoidal, dextral, or sinistral. The shell of *Hippeutis cantori* is disc-shaped and small, but it is the largest in the Planorbidae. The color is yellow-white, translucent and glossy. The whorl layer is 4 layers. Body whorl edge sharply angled, shell bottom flat, top surface rounded. The bottom end is recessed and is about 1/3 of the angle diameter. Its habitat is mainly in rice fields, riverside stones and water plants, and lakesides. In Korea, it is distributed nationwide. Currently, three species in the Planorbidae have been reported in Korea (Kwon *et al.*, 2001).

Chromosome morphology and numbers are helpful in snail identification. In recent

years, through a considerable number of works, a large amount of information has been accumulated on the chromosomes of mollusks (Tatewaki and Kitada, 1987; Thiriot-Quiévreux, 2003; Kocot *et al.*, 2016). Cytogenetic studies of mollusks have proven important with regard to phylogenetics and the cytogenetic relationships between morphologically similar species. Karyological analyses of the Genus *Aegista* have been reported by Inaba (1959) and Lee and Kwon (1993) (Table 1). Also, chromosome numbers and karyotypes of family Planorbidae has been reported Kim and Song (1983), Szabelska *et al.* (2015) and Poonam *et al.* (2018) (Table 2). In fact, modern cytogenetic techniques have only recently been adopted for studies of Gastropoda. The family Bradybaenidae is conservative with regard to the number of chromosomes, 17 species have 28, 29 and 30 pairs of chromosomes. In this study, karyotypes of *Aegista tenuissima* and *Hippeutis cantori* were studied in order to analyze the cytogenetic relationships within the genus.

〈Table 1〉 Chromosome numbers and karyotypes of Genus *Aegista*

Species	Haploid No.	Karyotype*	Source	References
<i>Aegista chosenica</i>	28	10M+18SM	Korea	Lee & Kwon, 1993
<i>A. (Plectotropis) diversa</i>	28	14M+14SM	"	"
<i>A. (P.) quelpartensis</i>	28	15M+10SM+3T	"	"
<i>A. vatheleti</i>	29		Japan	Inaba, 1959
<i>A. tenuissima</i>	28	14M+13SM+1ST	Korea	Present study

* M, metacentric; SM, submetacentric; ST, subtelocentric; T, telocentric chromosomes

〈Table 2〉 Chromosome numbers and karyotypes of Family Planorbidae

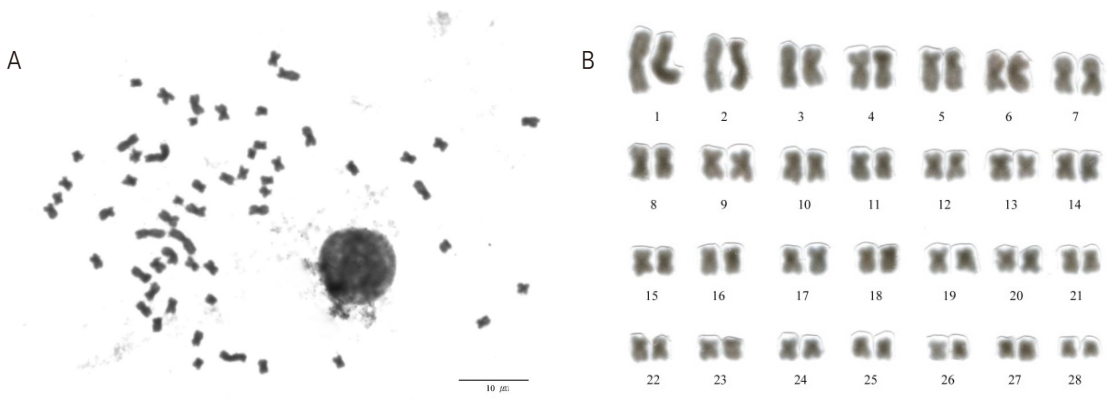
Species	Diploid No.	Karyotype	Source	References
<i>Hippeutis hemisphaerala</i>	36		Korea	Kim & Song, 1983
<i>Gyraulus convexiusculus</i>	36		"	"
<i>Planorbarius corneus</i>	36	15M+3SM	Poland	Szabelska <i>et al.</i> , 2015
<i>Gyraulus ladacensis</i>	36	14M+3SM+1ST	India	Poonam <i>et al.</i> , 2018
<i>Indoplanorbis exustus</i>	36	18M	"	"
<i>Hippeutis cantori</i>	36	14M+4SM	Korea	Present study

II. MATERIALS AND METHODS

Chromosome preparations were made on the gonads of the specimens by the usual air-drying method (Park, 2008). The prepared slides were observed under an Olympus (BX51) microscope. Chromosomal morphology was assessed from the relative length and the arm ratio was calculated based on 18 well-spread metaphase cells of *Aegista tenuissima* and 20 metaphase cells of *Hippeutis cantori*. The nomenclature proposed by Levan *et al.* (1964) was applied in this study for the karyotype analysis. Voucher specimens of the samples studied have been deposited at the Department of Environmental Medical Biology, Catholic Kwandong University College of Medicine, Korea.

III. RESULTS

A microphotograph of somatic metaphase chromosomes and the karyogram organized in order of decreasing size are shown Fig. 1, and measurements of the chromosomes are listed Table 3. 18 mitotic metaphase stage of *Aegista tenuissima* were analyzed. This species showed a diploid chromosome number of $2n=56$, with 14 metacentric, 13 submetacentric and one sub-telocentric chromosome pairs. Observed chromosomes ranged from $1.3\pm$ to $5.3\mu\text{m}$ in length (Table 3). This species showed no inter-specimen variability in chromosome counts. Also, sexual dimorphism



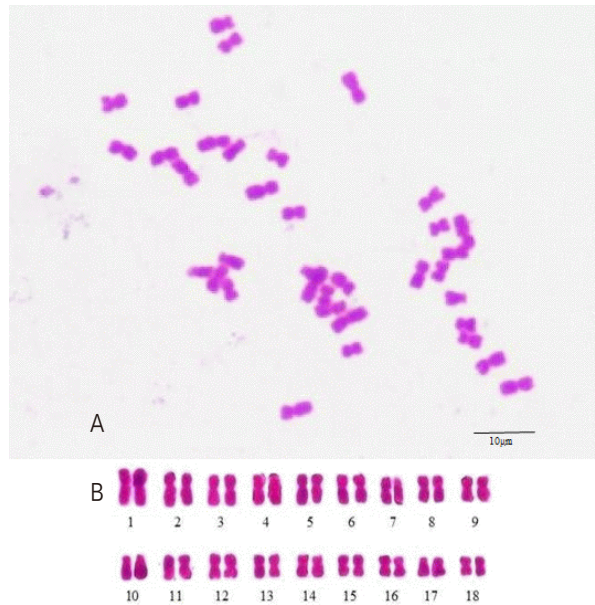
〈Fig. 1〉 Metaphase chromosomes of *Aegista tenuissima* (A) and karyotype constructed from A (B).

(Table 3) Relative lengths and total lengths (μm) of chromosomes of *Aegista tenuissima*

Chromosome	RL \pm SE	TL \pm SE	Type
1	8.6 \pm 0.08	5.3 \pm 0.23	M
2	6.5 \pm 0.13	4.0 \pm 0.35	M
3	5.4 \pm 0.12	3.3 \pm 0.23	M
4	5.1 \pm 0.06	3.1 \pm 0.16	M
5	4.9 \pm 0.11	3.0 \pm 0.15	ST
6	4.6 \pm 0.16	2.8 \pm 0.43	M
7	4.2 \pm 0.07	2.6 \pm 0.19	SM
8	3.9 \pm 0.06	2.4 \pm 0.15	M
9	3.8 \pm 0.10	2.3 \pm 0.28	M
10	3.6 \pm 0.04	2.2 \pm 0.10	SM
11	3.4 \pm 0.04	2.1 \pm 0.11	SM
12	3.3 \pm 0.05	2.0 \pm 0.13	M
13	3.3 \pm 0.04	2.0 \pm 0.11	M
14	3.3 \pm 0.14	2.0 \pm 0.10	M
15	3.1 \pm 0.06	1.9 \pm 0.11	M
16	2.9 \pm 0.13	1.8 \pm 0.12	SM
17	2.8 \pm 0.10	1.7 \pm 0.09	SM
18	2.8 \pm 0.09	1.7 \pm 0.07	SM
19	2.8 \pm 0.08	1.7 \pm 0.06	SM
20	2.6 \pm 0.18	1.6 \pm 0.13	M
21	2.6 \pm 0.14	1.6 \pm 0.11	SM
22	2.6 \pm 0.12	1.6 \pm 0.08	SM
23	2.4 \pm 0.13	1.5 \pm 0.08	M
24	2.4 \pm 0.11	1.5 \pm 0.06	SM
25	2.4 \pm 0.09	1.5 \pm 0.05	SM
26	2.3 \pm 0.14	1.4 \pm 0.04	M
27	2.3 \pm 0.08	1.4 \pm 0.03	SM
28	2.1 \pm 0.36	1.3 \pm 0.05	SM

M, metacentric; RL, relative length; SE, standard error; SM, submetacentric; TL, total length

of the chromosomes was not found in this study. Considering these results, cytologically, it was found that *Aegista* is different from other species although belonging to the same genus (Table 1). Proliferation divisions of spermatogonia of *Hippeutis cantori* were observed in 20 cells, and 36 (2n) chromosomes were observed (Fig. 2). Total length of the diploid complements was $59.64 \pm 1.18 \mu\text{m}$ (Table 4). The karyotype consisted of 36 (2n) and 13M+5SM.



〈Fig. 2〉 Metaphase chromosomes of *Hippeutis cantori* (A) and karyotype constructed from A (B).

〈Table 4〉 Relative lengths and total lengths (μm) of chromosomes of *Hippeutis cantori*

Chromosome	RL \pm SE	TL \pm SE	Type
1	7.8 \pm 0.61	4.6 \pm 0.11	M
2	7.1 \pm 0.25	4.2 \pm 0.23	SM
3	6.5 \pm 0.31	3.9 \pm 0.21	M
4	6.4 \pm 0.23	3.8 \pm 0.16	M
5	6.4 \pm 0.05	3.8 \pm 0.15	SM
6	6.4 \pm 0.02	3.8 \pm 0.10	M
7	6.2 \pm 0.23	3.7 \pm 0.08	M
8	5.9 \pm 0.20	3.5 \pm 0.03	M
9	5.5 \pm 0.28	3.3 \pm 0.16	M
10	5.2 \pm 0.43	3.1 \pm 0.09	SM
11	5.1 \pm 0.07	3.1 \pm 0.04	M
12	5.1 \pm 0.02	3.0 \pm 0.01	M
13	4.9 \pm 0.06	2.9 \pm 0.02	M
14	4.6 \pm 0.09	2.8 \pm 0.12	SM
15	4.5 \pm 0.10	2.7 \pm 0.06	M
16	4.4 \pm 0.04	2.6 \pm 0.02	M
17	4.2 \pm 0.10	2.5 \pm 0.11	SM
18	4.0 \pm 0.07	2.4 \pm 0.04	M

IV. DISCUSSION

Out of 291 gastropod species reported, 207 were karyotyped and 27 included banding techniques, reflecting the increasing knowledge of chromosome morphology over the last three decades (Thiriot-Quévieux, 2003). Chromosome variations, in terms of both the number and karyotype pattern, have been implicated as a primary isolating mechanism for speciation (Kongim *et al.*, 2013). The number of chromosomes in the genus *Aegista* studied to date by some researchers and including the present study were $n=28$ and $n=29$ (Table 1). Through this study, this species was found to have the same number of chromosomes ($2n=28$ from *A. chosonica*, *A. diversa* and *A. quelpartensis*) (Lee & Kwon, 1993). In some cases, different karyotypes were observed within the same species ($2n=29$ from *A. vatheleti*) (Inaba, 1959). This may be due to differences in the techniques used to obtain the chromosome preparations. Evidence from chromosome morphology is still rarely used to study the relationships and evolution of gastropod taxa. Information on karyotypes is usually available for only a few isolated species. Therefore, cytotaxonomic relationships based on karyotypes remain scarce because of this irregular sampling (Thiriot-Quévieux, 2003). When comparing several species in the same family, some authors have attempted to infer the evolutionary relationships (Chambers, 1982; Aparicio, 1983; Thiriot-Quévieux, 1988; Dillon, 1991). The karyotypes of six species from Planorbidae studied were reported (Table 2). The chromosome numbers of five species in Planorbidae studied by Kim & Song (1983), Szabelska *et al.* (2015) and Poonam *et al.* (2018) and including the present study were $n=18$. The facts indicated that the chromosomes of Planorbidae seem to be numerically more stable than those of other molluscan families (Ramesh & Pandit, 2014; Poonam *et al.*, 2018). Karyotypic evolution may have no bearing on phylogenetic schemes in the Planorbidae with same chromosome number and similar karyotype.

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