

# Karyotypes of Two *Ischnochiton* Species (Ischnochitonidae: Polyplacophora)

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= 국문초록 =

연두군부 속(다관강: 연두군부과)에 속하는 두 종의 핵형

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연두군부(*Ischnochiton comptus*)와 가는줄연두군부 (*I. hakodadensis*) 두 종의 정소를 재료로 하여 핵형 분석을 실시하였다. 두 종의 염색체는 모두 중부염색체로 구성되어 있고, 염색체 수는  $n=12$ 이며, 성염색체는 관찰되지 않았다. 이들 염색체는 이들의 크기로는 별도의 염색체군으로 나누어지지 않았다.

## INTRODUCTION

Since the chromosome studies on mollusks have been mainly carried out on gastropods and bivalves, there are little data about the morphology and number of chromosomes for polyplacophorans. In the polyplacophoran group, the chromosome numbers are detected in 15 species in which only nine species were analysed for their karyotypes of chromosomes (Nakamura, 1985).

The present study was aimed for determining the number and morphology of the chromosomes of two polyplacophoran species for future comparative studies, and re-checking previously reported counts of the chromosomes in this class.

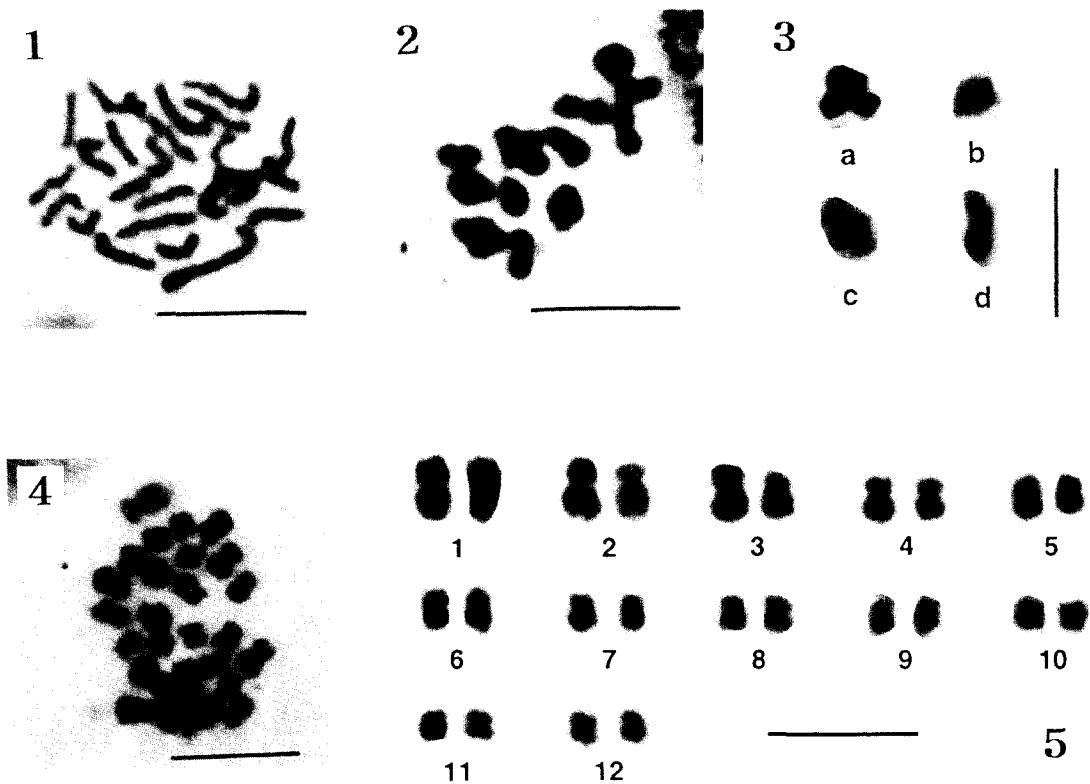
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## MATERIALS AND METHODS

Karyological examinations were made on the two polyplacophoran species, *Ischnochiton comptus* (Gould, 1859) and *I. hakodadensis* Carpenter in Pilsbry, 1893, which were collected from two localities in Korea (Table. 1).

The chromosomal preparations from male gonads during spermatogenesis were made by the techniques of Nakamura (1986) with some modifications. Briefly, whole specimens were kept for 18 hours in 0.005% colchicine (Sigma) in sea water with aeration. The testes were incubated in 0.05 M KCl hypotonic solution in a clear six-well cell cul-



**Fig. 1-5.** *Ischnochiton comptus* (Gould, 1859)

**Fig. 1.** Leptotene chromosomes in male gonads.

**Fig. 2.** Diakinetic bivalents in male gonads.

**Fig. 3.** Bivalents and chiasmata positions in male gonads.

**Fig. 4.** Spermatogonial metaphase spread.

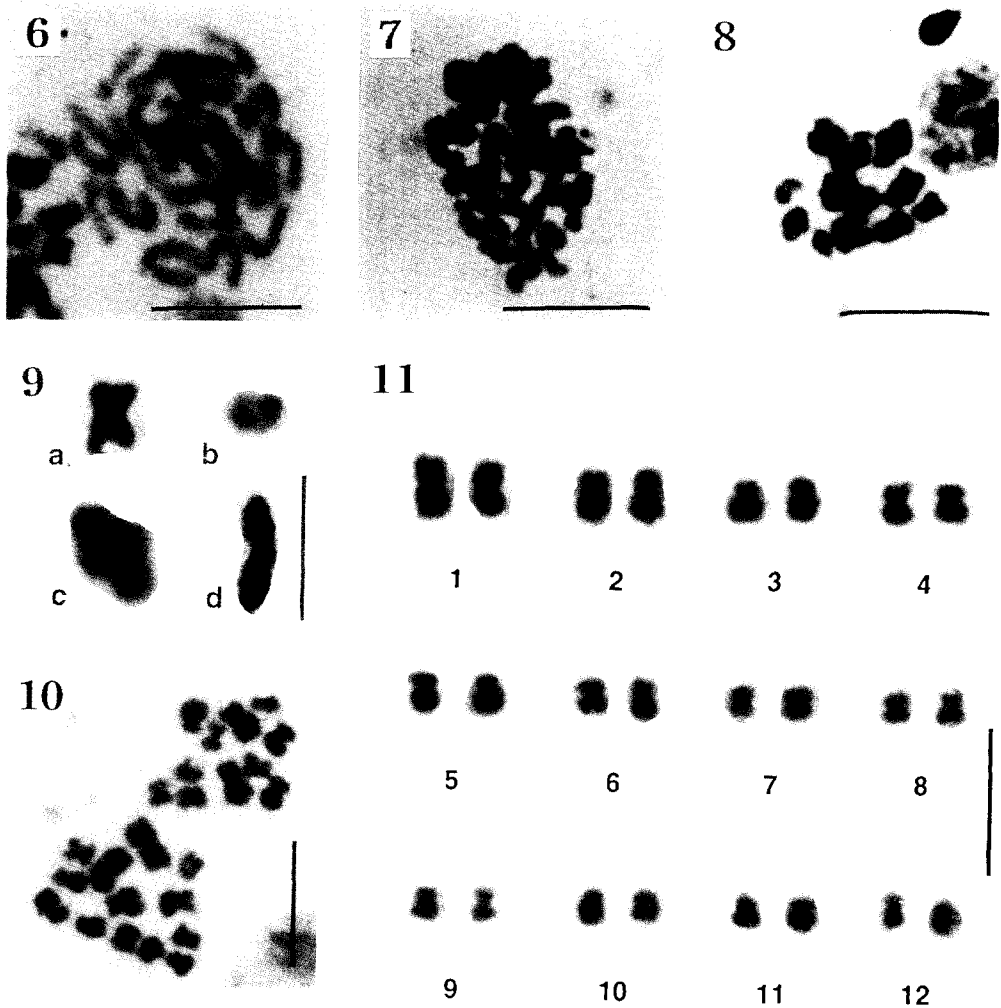
**Fig. 5.** Karyotype constructed from Fig. 4 (all scale bars indicate 10  $\mu$ m).

ture plate for 60-90 minutes. The testes were then fixed in a freshly prepared modified chilled Carnoy's fixative (3 methanol: 1 glacial acetic acid) with five times of 20 minutes each. Cell suspension was prepared by disrupting the tissue through repeated drawing in-and-out of the entire volume of the sample in 50% acetic acid.

A drop of the cell suspension was laid onto a clean slide glass which was pre-heated to 50°C. Dried slides were stained with 10% Giemsa solution (BDH) in 0.1M phosphate

buffer (pH 6.8). The stained slides were soaked into ethanol to remove excessive staining solution, and rinsed with distilled water.

Leitz Orthoplan microscope was used for observations and microphotographs. Relative length (R.L.: percentage of the total haploid length of chromosomes) and arm ratio (A.R.: length of the long arm divided by that of the short arm of the chromosomes) were calculated from the chromosomes. Chromosomes were classified by the methods of Levan *et al.* (1964).



**Fig. 6-11.** *Ischnochiton hakodadensis* Carpenter in Pilsbry, 1893

**Fig. 6.** Early leptotene chromosomes in male gonads.

**Fig. 7.** Late leptotene chromosomes in male gonads.

**Fig. 8.** Diakinetic bivalents in male gonads.

**Fig. 9.** Bivalents and chiasmata positions in male gonads.

**Fig. 10.** Spermatogonial metaphase spread.

**Fig. 11.** Karyotype constructed from Fig. 10 (all scale bars indicate 10  $\mu$ m).

## RESULTS

### 1. *Ischnochiton comptus* (Gould, 1859)

Thirty four selected chromosomal spreads from 5 individuals were karyotyped and measured for their relative lengths and arm ratios (Table 2). At meiotic diakinesis, ha-

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**Table 1.** Localities and dates of collecting materials.

Species	Localities	Data
<i>Ischnochiton comptus</i>	Hujin, Kangwon-do	14 Aug. 1993
<i>Ischnochiton hakodadensis</i>	Kotchi, Anmyon I., Chungchungnam-do	10 Jun. 1993 16 Jun. 1994

**Table 2.** Mean values and standard deviation of the relative length and the arm ratio of male chromosomes of *Ischnochiton comptus*.

Chromosome pair No.	Relative length		Arm ratio		Classification
	MEAN	SD	MEAN	SD	
1	13.59	1.250	1.21	0.131	m
2	11.49	0.823	1.20	0.127	m
3	9.78	0.381	1.23	0.198	m
4	9.12	0.424	1.24	0.156	m
5	8.56	0.362	1.22	0.179	m
6	8.03	0.435	1.27	0.244	m
7	7.60	0.371	1.22	0.129	m
8	7.19	0.357	1.27	0.165	m
9	6.77	0.353	1.29	0.139	m
10	6.33	0.377	1.31	0.219	m
11	5.99	0.351	1.26	0.190	m
12	5.60	0.380	1.26	0.135	m

**Table 3.** Mean values and standard deviation of the relative length and the arm ratio of male chromosomes of *Ischnochiton hakodadensis*.

Chromosome pair No.	Relative length		Arm ratio		Classification
	MEAN	SD	MEAN	SD	
1	13.25	1.331	1.14	0.100	m
2	11.39	0.986	1.19	0.252	m
3	10.39	0.742	1.13	0.083	m
4	9.48	0.486	1.19	0.159	m
5	8.73	0.523	1.22	0.152	m
6	7.94	0.457	1.22	0.151	m
7	7.52	0.312	1.22	0.158	m
8	7.18	0.374	1.25	0.177	m
9	6.76	0.476	1.22	0.161	m
10	6.33	0.565	1.22	0.147	m
11	5.81	0.594	1.24	0.181	m
12	5.23	0.607	1.19	0.137	m

ploid number is  $n=12$  (Fig. 2) and at spermatogonial metaphase, the diploid number of chromosomes is  $2n=24$  (Figs. 4, 5). All chromosomes were metacentric, and these chromosomes could not be divided into groups according to their lengths. At diakinesis, the separating bivalents showed several different morphologies, such as cross-shaped with one medial chiasma (Fig. 3a), ring-shaped with two terminal chiasmata (Fig. 3b), diamond-shaped containing lighter medial area with two chiasmata or one terminal and one subterminal or one terminal and one submedial chiasmata (Fig. 3c), and rod-shaped (Fig. 3d) probably with a chiasmatic bivalent. No heteromorphic chromosomes have been detected.

## 2. *Ichnochiton hakodadensis* Carpenter in Pilsbry, 1893

At the meiotic diakinesis, haploid number is  $n=12$  (Fig. 8) and at the spermatogonial metaphase, the diploid number of chromosomes is  $2n=24$  (Figs. 10, 11). The sex-determining chromosomes have not been found. Thirty one chromosomes from 7 individuals were karyotyped and measured for their relative lengths and arm ratios (Table 3). All chromosomes observed were metacentric and could not be divided into groups according to their length since their lengths decreased gradually. At diakinesis, like in *I. comptus*, the separating bivalents showed, cross-shaped (Fig. 9a), ring-shaped (Fig. 9b), diamond-shaped (Fig. 9c), and rod-shaped chromosomes (Fig. 9d).

## DISCUSSION

From the chromosomal information on the three species of the genus *Ichnochiton*, all three species were shown to have 12 chro-

sosome pairs in diploid (Table 4). However twelve pairs of chromosomes are not necessarily the characteristics of the family Ischnochitonidae. Vitturi *et al.* (1982) have been reported the same chromosome numbers from *Acanthochiton communis* (Risso) of the family Acanthochitonidae. The chromosomes of *A. communis* could be divided into two classes, 8 big and 16 small elements. In this study, chromosomes of *I. comptus* and *I. hakodadensis* could not be divided into groups by their lengths since the chromosome length of these species were gradually decreased from the 1st to the 12th chromosome pairs (Fig. 12).

All 24 chromosomes were consisted of metacentric. Even though it is certain that all of the chromosomes were metacentric, chromosomes 8th to 12th were difficult to determine the centromeric position due to their small size and degree of condensation.

A comparison between two species revealed that there were no morphological difference, the relative length and the size of each chromosome pairs. The spermatocyte bivalent showed ring-, cross-, diamond- and rod-shaped chromosomes, as similarly occurred in *L. corrugata* and *A. communis* (Vitturi *et al.*, 1982), and *C. olivaceus* (Vitturi, 1982).

The chromosome banding techniques, such as C- and NOR-banding, should be introduced to the chitons to compare the banding pattern and to estimate the karyological evolution of the class Polyplacophora for further studies.

## SUMMARY

Chromosomes prepared from the male gonads of the two polyplacophoran species, *Ichnochiton comptus* and *I. hakodadensis* were analysed. The numbers of chromosomes of the two species were 24 in diploid and 12 in ha-

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**Table 4.** Chromosome numbers of polyplacophorans listed in previous literatures (modified from Nakamura, 1985).

Species	Chromosome number		Collection area	References
	2n	n		
Ischnochitonidae				
<i>Lepidochitona corrugata</i> ( <i>Middendorffia caprearum</i> )	24	12	Palermo, Italy	Vitturi <i>et al.</i> , 1982
* <i>Lepidozona coreanica</i>	24	12	Shimonoseki, Japan	Nishikawa and Ishida, 1969
* <i>Ischnochiton comptus</i> ( <i>I. comptus</i> f. <i>comptus</i> )	24	12	Shimonoseki, Japan	Nishikawa and Ishida, 1969
* <i>I. boninens.</i> s ( <i>I. comptus</i> f. <i>isaoi</i> )	24	12	Shimonoseki, Japan	Nishikawa and Ishida, 1969
Mopaliidae				
<i>Katharina tunicata</i> ( <i>Katherina tunicata</i> )	12		Oreg., USA	Dolph and Humphrey, 1970
* <i>Placiphoralla stimpsoni</i> ( <i>P. japonica</i> )	24		Shimonoseki, Japan	Nishikawa and Ishida, 1969
Chitonidae				
<i>Chiton olivaceus</i>	26	13	Palermo, Italy	Vitturi, 1982
			Shimonoseki, Japan	Nishikawa and Ishida, 1969
* <i>Liolophura japonica</i>	24	12	Miura Peninsula, Kanagawa, Japan	Kawai, 1976
* <i>Onithochiton hirasei</i>	24	12	Shimonoseki, Japan	Nishikawa and Ishida, 1969
Acanthochitonidae				
<i>Acanthochitona communis</i>	24	12	Venice, Italy	Vitturi <i>et al.</i> , 1982
<i>A. crinitus</i>	18	9	Roscoff, France	Colombera and Tagliaferri, 1983
* <i>A. defilippii</i>	16	8	Shimonoseki, Japan	Nishikawa and Ishida, 1969
	16	8	Hayama, Kanagawa, Japan	Kawai, 1976
<i>A. discrepans</i>	18	9	England	Certain, 1951(cited from Nishikawa and Ishida, 1969)
* <i>A. rubrolineata</i>	16	8	Shimonoseki, Japan	Nishikawa and Ishida, 1969

Remarks: \*Species also distributed in Korea; ( ) scientific names in original papers.

ploid. In the case of *I. comptus*, this chromosome count coincides with the report of Nishikawa and Ishida (1969). The sex-determining chromosomes have not been detected. All of the chromosomes were classified of metacentric and could not be grouped into different classes according to their lengths.

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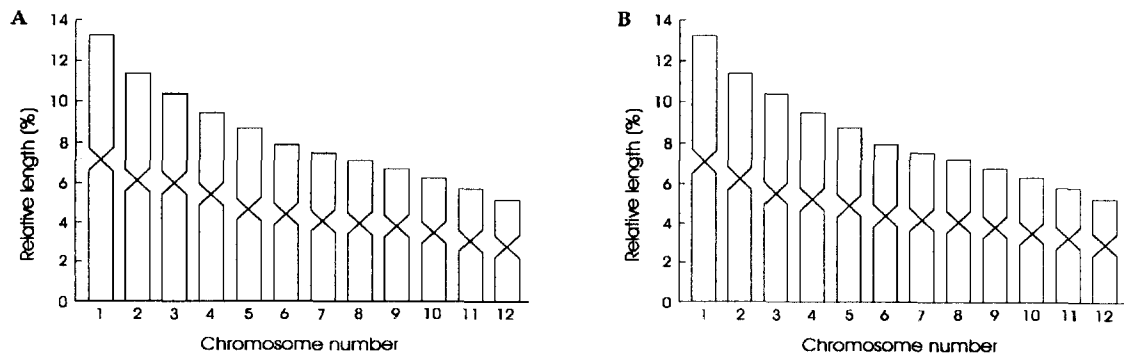


Fig. 12. Idiograms of the *I. comptus* (A) and *I. hakodadensis* (B) drawn from the data in the Table 3 and Table 4, respectively.

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