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The Chromosomes of Four Chiton Species (Polyplacophora)

Seungshic Yum[1] and Byung Lae Choe

Department of Biology, Sung Kyun Kwan University, Suwon 440-746, Korea

= 국문요약 =

다판류 4 종의 염색체

염 승 식 · 최 병 래

성균관대학교 생물학과

한국산 다판류 4종의 정소를 재료로 warm drying method를 이용하여 핵형 분석을 실시하였다. 연두군부과(Ischnochitonidae)에 속하는 북방줄군부(*Lepidozona albrechtii*)의 염색체 수는 2n=24, n=12, 형태적으로는 10쌍의 중부염색체, 한쌍의 중부 또는 차중부염색체, 그리고 한쌍의 차중부염색체로 구성되어 있으며, 줄군부(*Lepidozona coreanica*)의 염색체는 2n=24, 중부염색체 8쌍, 중부 또는 차중부염색체 한쌍, 그리고 세쌍의 차중부염색체로 나타났다. 두 종 모두 각 염색체쌍의 형태는 중부 및 차중부염색체로 구성되어 있었으며, 크기로는 별도의 염색체군으로 나누어지지 않았다. 따가리과(Mopaliidae)에 속하는 따가리(*Placiphorella stimpsoni*)의 염색체는 2n=24, n=12, 핵형분석결과 6쌍의 중부염색체, 한쌍의 차단부, 그리고 5쌍의 말단부염색체였으며, 1번과 2번 염색체쌍은 다른 염색체쌍들 보다 월등히 큰 것으로 나타났다. 군부과(Chitonidae)에 속하는 꼬마군부(*Chiton kurodai*)의 염색체는 2n=24, n=12, 중부염색체 7쌍, 차중부 4쌍, 그리고 한쌍의 차단부염색체였고, 1번 염색체쌍이 크기에 의해 다른 염색체쌍들과 구분되었다.

본 연구의 결과와 과거의 연구 결과를 종합하면, 연두군부 과는 2n=24; 따가리과는 2n=12 및 24; 군부과는 2n=24 및 26의 염색체를 갖고 있는 것으로 요약되며, 특히 군부속(Genus *Chiton*)에서는 속내 종간 숫적 변이가 나타남이 확인되었다

INTRODUCTION

Chromosomal studies on Polyplacophora have been noticeably useful in systematics (Nishikawa and Ishida, 1969; Dolph and Humphrey, 1970; Kawai, 1976; Vitturi, 1982; Vitturi *et al.*, 1982; Colombera and Tagliaferri, 1983). However, concerning the cytotaxonomic studies of chitons, there are little data about the morphology and the number of chromosomes for this taxonomic group. Fifteen species in the Polyplacophora have been studied cytologically (Nakamura, 1985; Choe *et al.*, 1995). Nakamura (1985) reviewed chromosome numbers and karyological data for 14 species in 4 of recent Polyplacophora families. Choe *et al.* (1995) reported

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[1]Present address: Department of Developmental Genetics, National Institute of genetics, Mishima, Shizuoka 411, Japan

2n=24, n=12 of *Ischnochiton comptus* and *I. hakodadensis* in Korea. In order to estimate the karyological evolution among the class Polyacophora, it is necessary not only the extend the range of studies to cytologically unexplored species but also to re-check previously reported numbers and morphology of chromosomes.

In the present paper, karyological studies are presented for four chiton species, *Lepidozona albrechtii* (von Schrenck, 1862) and *L. coreanica* (Reeve, 1847) in the family Ischnochitonidae; *Placiphorella stimpsoni* (Gould, 1859) in Mopaliidae; and *Chiton kurodai* Taki & Taki, 1929 in Chitonidae. Karyological data of *L. albrechtii* and *C. kurodai* are given for the first time and chromosome counts of the remainder two species are compared to the previous report of Nishikawa & Ishida (1969).

MATERIALS AND METHODS

Samples employed in this study were collected from three localities in Korea (Table 1). The chromosomal preparations from male gonads during spermatogenesis were made by the warm drying method of Nakamura (1986) with somewhat modifications (Choe *et al.*, 1995). Voucher specimens of the species used in this study have been placed in the Dept. of Biology, Sung Kyun Kwan University.

Table 1. Localities and collection data for chromosome analysis.

Species	Localities	Collection date
Family Ischnochitonidae		
Lepidozona albrechtii	Hujin, Kangwon-do, East Coast of Korea	Sept. 4, 1994
Lepidozona coreanica	Kotchi, Anmyon Island, Chungchungnam-do, West Coast of Korea	Jun. 16, 1994
Family Mopaliidae		
Placiphorella stimpsoni	Chonjin, Kangwon-do East Coast of Korea	Sept. 4, 1994
Family Chitonidae		
Chiton kurodai	Hujin, Kangwon do East Coast of Korea	Aug. 14, 1993

RESULTS

1. *Lepidozona albrechtii* (von Schrenck, 1862)

Karyological data were obtained from ten metaphases for chromosome analysis. karyotypes are characterized by chromosome size and centromeric position in Table 2. At spermatogonial metaphase, the diploid number of chromosomes is 2n=24 (Fig. 1a) and at meiotic diakinesis, haploid number is n=12 (Fig. 1b). Idiogram for this species is displayed in Figure 5. These chromosomes could not be divided into groups according to their lengths. The chromosome complements consists of ten pairs metacentric chromosomes (1, 2, 5, 6, 7, 8, 9, 10, 11,

Table 2. Chromosome measurments and morphology derived male metaphase cells of *Lepidozona albrechtii*.

Chromosome pair No.	Relative length mean	±SD	Arm ratio	Morphology
1	14.86	1.518	1.22	m
2	12.24	1.255	1.39	m
3	10.59	0.888	1.70	m/sm
4	9.71	0.701	1.76	sm
5	8.73	0.726	1.63	m
6	7.47	0.454	1.58	m
7	6.95	0.438	1.38	m
8	6.60	0.315	1.49	m
9	6.19	0.615	1.53	m
10	5.88	0.644	1.28	m
11	5.61	0.796	1.34	m
12	5.42	0.641	1.36	m

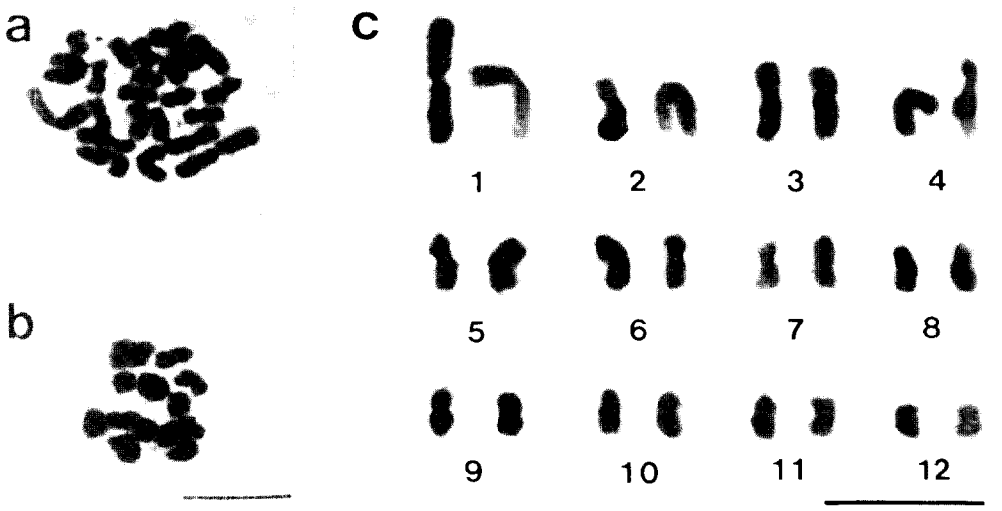

Fig. 1. The chromosomes and karyotype of *Lepidozona albrechtii*.
a, Spermatogonial metaphase; b, Diakinetic bivalents in male gonads. c, Karyotype constructed from spermatogonial metaphase. (Scale bars indicate 10 μm)

12), one pair metacentric/submetacentric (3) and one pair submetacentric (4). The karyological data for this species can be expressed as 10m, 1m/sm, 1sm.

2. *Lepidozona coreanica* (Reeve, 1847)

Forty-six metaphases were employed for chromosome analysis. The relative lengths of the chromosomes and the arm ratios were calculated (Table 3). At spermatogonial metaphase, the diploid number of chromosomes is 2n=24 (Fig. 2a) and at meiotic diakinesis, haploid number is n=12 (Fig. 2b). These chromosomes could not be divided into groups according to their lengths. The chromosome complements consists of eight pairs metacentric (1, 6, 7, 8, 9, 10, 11, 12), one pair metacentric/submetacentric (5) and three pairs submetacentric (2, 3, 4) chromosomes. The karyological data for this species can be expressed as 8m, 1m/sm, 3sm.

3. *Placiphorella stimpsoni* (Gould, 1859)

In this species, four metaphase chromosomal spreads were studied. Table 4 gives the mea- rments and classification of metaphase spreads. At meiotic diakinesis, haploid number is n=12 and at spermatogonial metaphase, the diploid number of

Table 3. Chromosome measurments and morphology derived male metaphase cells of *Lepidozona coreanica*.

Chromosome pair No.	Relative length		Arm ratio	Morphology
	mean	±SD		
1	14.79	1.418	1.17	m
2	12.03	1.081	1.72	sm
3	10.39	0.649	1.80	sm
4	9.47	0.525	1.82	sm
5	8.68	0.454	1.66	m/sm
6	7.73	0.454	1.34	m
7	7.13	0.427	1.34	m
8	6.70	0.431	1.31	m
9	6.30	0.424	1.27	m
10	6.00	0.418	1.34	m
11	5.60	0.495	1.34	m
12	5.19	0.589	1.36	m

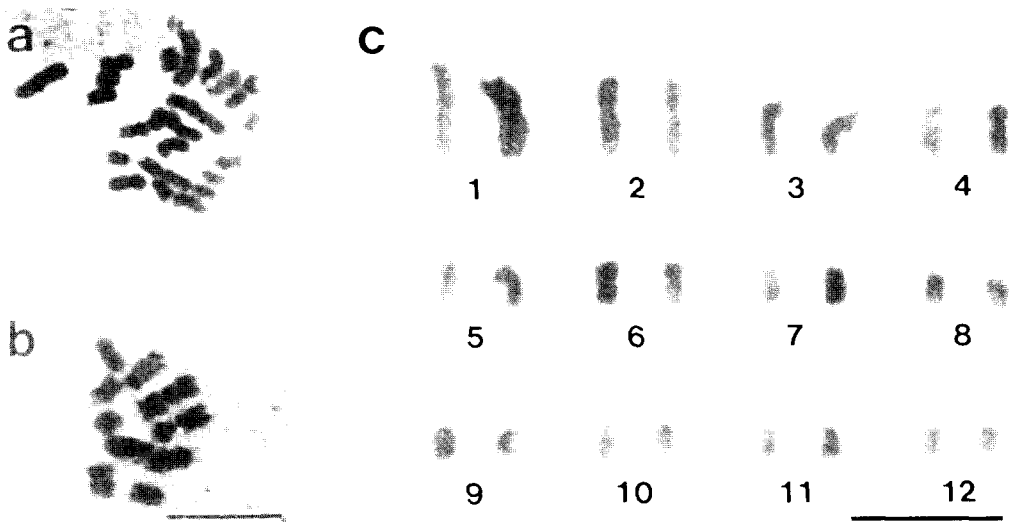

Fig. 2. The chromosomes and karyotype of *Lepidozona coreanica*.
a, Spermatogonial metaphase; b, Diakinetic bivalents in male gonads. c, Karyotype constructed from spematogonial metaphase. (Scale bars indicate 10 μm)

chromosomes is 2n=24 (Fig. 3b). Karyotype shows two large elements (1, 2) and small elements (3, 4, 5, 6, 7, 8, 9, 10, 11, 12) according to their lengths. Six pairs are metacentric (1, 2, 3, 4, 5, 10), one pair is subtelocentric (7) and five pairs are telocentric (6, 8, 9, 11, 12). The overall appearance of the karyotype is better visualized in the idiogram (Fig. 5). The karyological data for this species can be expressed as 6m, 1st, 5t.

4. *Chiton kurodai* Taki & Taki, 1929

Karyological data were obtained from four metaphase chromosomal spreads. The relative lengths of the chromosomes and the arm ratios were calculated (Table 5). At meiotic diakinesis, haploid number is n=12 (Fig. 4a) and at spermatogonial metaphase, the diploid number of chromosomes is 2n=24 (Fig. 4b). The first chromosome pair can be distinguished from the other chromosome pairs according to their lengths. Pairs 1, 2, 3, 6, 7, 11 and 12 are metacentric, four pairs 4, 5, 8 and 10 are submetacentric, pair 9 is subtelocentric. The idiogram (Fig. 5) constructed from relative length and centromeric values visualizes the karyotype. The karyological data for

Table 4. Chromosome measurments and morphology derived male metaphase cells of *Placiphorella stimpsoni*.

Chromosome pair No.	Relative length		Arm ratio	Morphology
	mean	±SD		
1	16.61	1.484	1.51	m
2	14.70	0.665	1.32	m
3	10.18	2.103	1.02	m
4	8.72	0.382	1.42	m
5	7.79	0.304	1.16	m
6	7.20	0.258		t
7	6.70	0.747	4.42	st
8	6.29	0.804		t
9	5.73	0.999		t
10	5.46	0.745	1.21	m
11	4.83	0.690		t
12	4.14	0.730		t

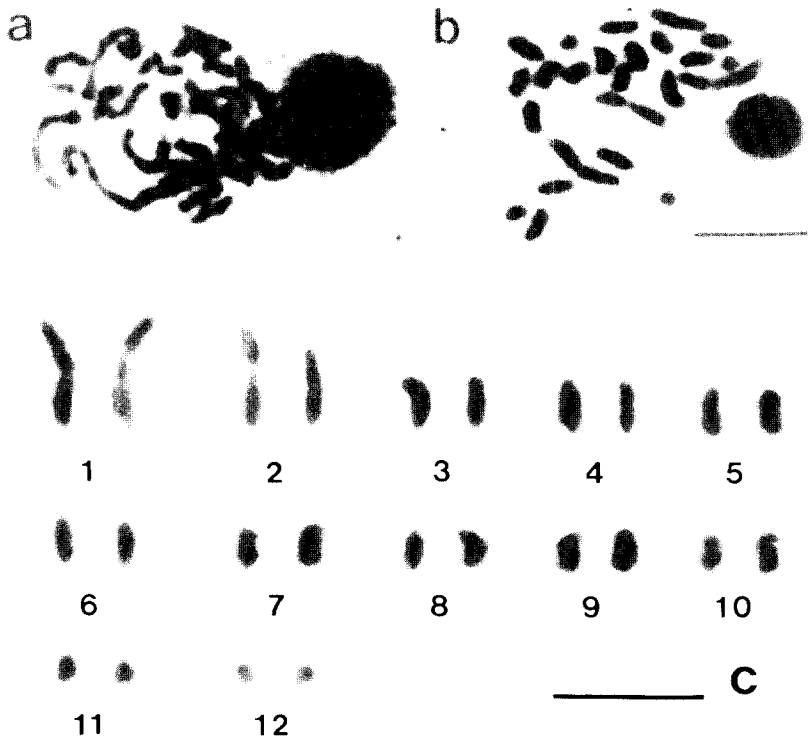

Fig. 3. The chromosomes and karyotype of *Placiphorella stimpsoni*.
a, Leptotene chromosomes in male gonads; b, Spermatogonial metaphase; c, Karyotype constructed from spermatogonial metaphase. (Scale bars indicate 10 μm)

this species can be expressed as 7m, 4sm, 1st.

DISCUSSION

Until now, only five species have been studied karyologically in the family Ischnochtionidae, Lepidochitona corrugata (2n=24, n=12, metacentric or slightly submetacentric chromosomes) (Vitturi *et al.*, 1982); *Lepidozona coreanica* (2n=24, n=12, metacentric, submetacentric and telocentric chromosomes) (Nishikawa & Ishida, 1969); *Ischnochiton boninensis* (2n=24, n=12, metacentric submetacentric and telocentric chromosomes) (Nishikawa & Ishida, 1969); I. comptus (2n=24, n=12, metacentric, submetacentric and telocentric chromosomes, Nishikawa & Ishida, 1969; 2n=24, n=12, 12m, Choe *et al.*, 1995); *I. hakodadensis* (2n=24, n=12, 12m) (Choe *et al.*, 1995). In this paper, new data for two Ischnochitonidae species, *Lepidozona albrechtii* and *L. coreanica* are presented. In *L. coreanica*, the chromosome number coincides with the report of Nishikawa & Ishida (1969) but our results show a morphological difference not observed in their study. They mentioned the presence of telocentric chromosome pairs from the Japanese species. It seems that they may have made an error, perhaps as a result of the extremely constricted small elements. The same phenomenon was found in *I. comptus* (Choe *et al.*, 1995). The species belonging to this family studied up to now have the same

Table 5. Chromosome measurments and morphology derived male metaphase cells of *Chiton kurodai*.

Chromosome pair No.	Relative length mean	±SD	Arm ratio	Morphology
1	13.74	1.779	1.24	m
2	11.04	0.875	1.42	m
3	10.18	1.096	1.42	m
4	9.54	0.107	2.65	sm
5	9.01	0.437	2.36	sm
6	8.37	0.554	1.52	m
7	7.79	0.423	1.18	m
8	7.15	0.703	2.33	sm
9	6.58	0.689	4.31	st
10	6.07	0.467	1.89	sm
11	5.48	0.500	1.33	m
12	4.95	0.419	1.16	m

chromosome number of 2n=24, n=12, and consist mainly metacentric or submetacentric chromosomes.

In the family Mopaliidae, two species have been investigated karyologically, *Katharina tunicata* (2n=12, 4m, 2t) (Dolph & Humphrey, 1970) and *P. stimpsoni* (2n=24) (Nishikawa & Ishida, 1969). In chromosome morphology of *P. stimpsoni*, the presence of subtelocentric and telocentric are observed, and in the chromosome number of this species, agrees with the previously reported.

In the family Chitonidae, three species have been investigated karyologically, *Chiton olivaceus* (2n=26, n=13, 12m, 1sm) (Vitturi, 1982), *Liolophura japonica* (2n=24, n=12, metacentric, submetacentric and telocentric chromosomes, Nishikawa & Ishida, 1969; 2n=24, 12m/sm, Kawai, 1976), Onithochiton hirasei (2n=24, n=12, metacentric, submetacentric and telocentric chromosomes) (Nishikawa & Ishida, 1969). When compared, the karyotype of *C. olivaceus* and *C. kurodai* showed variation in chromosome number is found within the genus *Chiton*.

Karyotypes of the family Acanthochitonidae, showing a variation in chromosome number (2n=18, 16) within the genus even in the family level of Polyplacophora, would be reviewed in another paper now in preparation.

Unfortunately, we can not discuss the evolutionary scheme of the chiton using the karyological data studied to date. It will be necessary not only to employ various chromosome banding techniques but also to study a wider range of species belonging to the different categories in order to accurately estimate the karyological evolution within the class Polyplacophora.

SUMMARY

Chromosomes of four chiton species were studied from the spermatogonia at metaphase stage using a warm drying method. In the Ischnochitonidae, *Lepidozona albrechtii* has 2n=24 and n=12 with 10m, 1m/sm, 1sm; *Lepidozona coreanica* with 8m, 1m/sm, 3sm. All of the chromosomes of the two species are characterized by metacentric/submetacentric and can not be grouped into different classes according to their lengths. In the Mopaliidae, *Placiphorella stimpsoni* has 2n=24, n=12 with 6m, 1st, 5t. The first two chromosome pairs can be classified as large elements when compared to the remaining elements. In the Chitonidae, *Chiton kurodai* has 2n=24, n=12 with 7m, 4sm, 1st. The first chromosome pair can be distinguished as a large element from the other chromosome pairs according to their lengths.

Compared with this result to previous reported, Ischnochitonidae has a diploid chromosome number of 2n=24; 2n=12 and 24 for Mopaliidae; 2n=24 and 26 for Chitonidae. Variation in chromosome number is found in the genus *Chiton*.

ACKNOWLEDGEMENTS

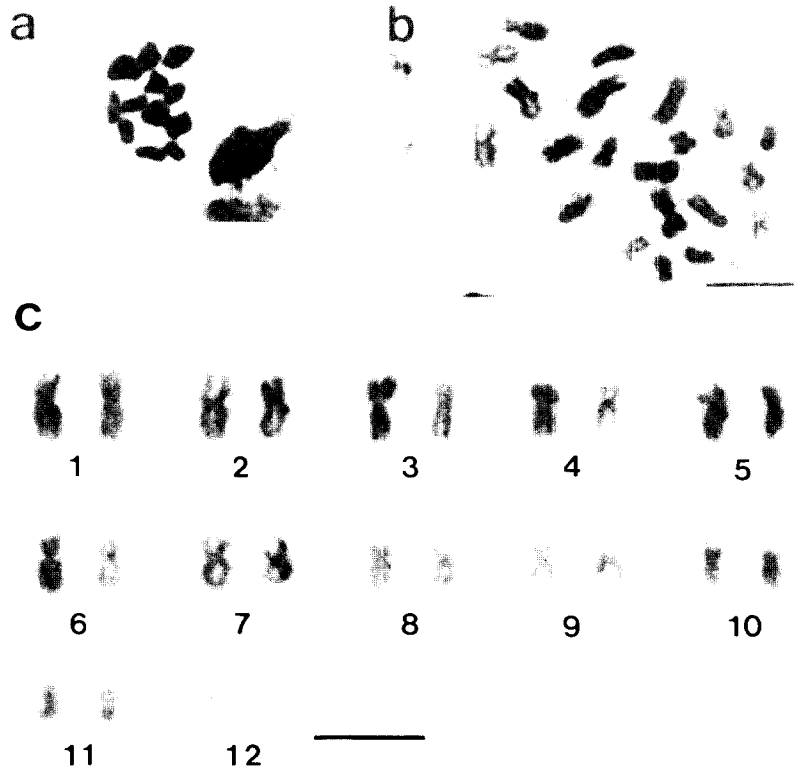

Fig. 4. The chromosomes and karyotype of *Chiton kurodai*.
a, Diakinetic bivalents in male gonads; b, Spermatogonial metaphase; c, Karyotype constructed from spermatogonial metaphase. (Scale bars indicate 10 μm)

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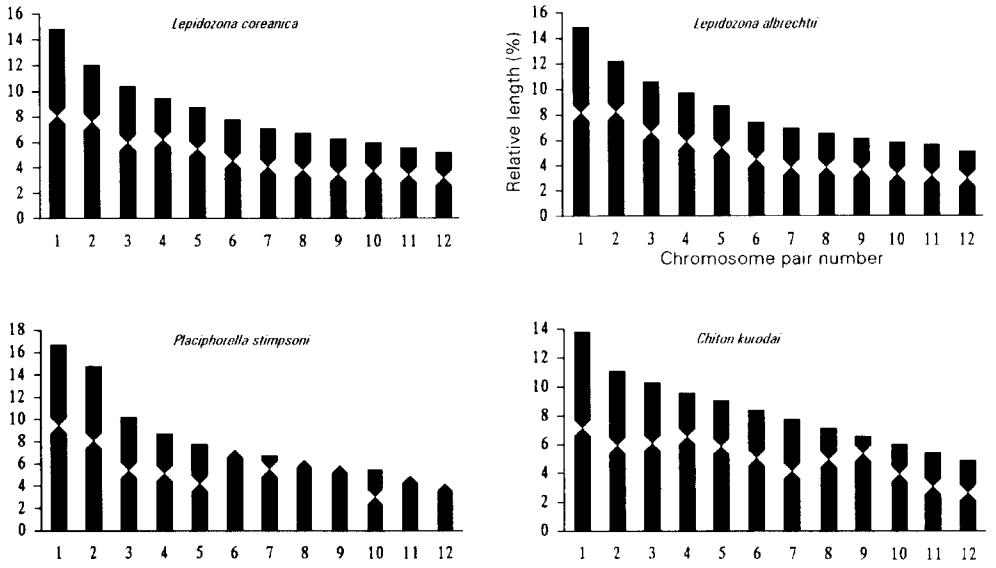

Fig. 5. Idiograms of the four chiton species constructed from relative length and arm ratio.

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