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Karyotypes of Four *Acanthochitona* Species (Acanthochitonidae, Polyplacophora) in Korea

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= 국문요약 =

한국산 가시군부속 (가시군부과: 다판강) 4종의 핵형

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한국산 가시군부속(*Acanthochitona*)의 좀털군부(*A. achates*), 참털군부(*A. circellata*), 털군부(*A. defilippii*) 및 애기털군부(*A. rubrolineata*)등 4종의 정소를 재료로 warm drying method를 통해 염색체를 관찰하였다. 염색체의 수는 4종 모두 2n=16, n=8로 확인되었다. 핵형 분석결과 형태적으로 좀털군부는 다섯 쌍의 중부염색체, 한 쌍의 차중부염색체, 두 쌍의 차단부염색체로; 참털군부는 한쌍의 중부염색체, 네 쌍의 차중부염색체, 두 쌍의 차단부염색체, 한 쌍의 말단부염색체로; 털군부는 세 쌍의 중부염색체, 세 쌍의 차중부염색체, 한 쌍의 차단부염색체, 한 쌍의 말단부염색체로; 애기털군부는 다섯 쌍의 중부염색체, 한 쌍의 차중부염색체, 한 쌍의 차중부 또는 차단부염색체로 구성되어 있었다. 외부 형태적으로 매우 유사한 애기털군부와 좀털군부의 경우, 특히 4번 염색체에서 현저한 형태적 차이를 보였다. 8번 염색체의 경우, 애기털군부와 좀털군부는 중부염색체인 반면 참털군부와 털군부는 말단부염색체로 나타났다. 동북아시아와 유럽에 분포하는 가시군부류 사이에 나타나는 염색체의 속내 종간 숫적변이를 보면 동북아시아산(2n=16)이 유럽산(2n=24, 18) 보다 분화된 것으로 보인다.

Keywords : Karyotype, Polyplacophora, *Acantochiton*

INTRODUCTION

The karyological studies on a total of seventeen chiton species have been performed so far by several authors. Of these species, *Acanthochitona discrepans* (Acantochitonidae) was studied by Certain (1951, cited from Nishikawa and Ishida, 1969); eight species, namely, *Lepidozona coreanica*, *Ischnochiton comptus*, *I. boninensis* (Ischnochitonidae), *Placiphoralla stimpsoni* (Mopaliidae), *Liolophura japonica*, *Onithochiton hirasei* (Chitoniidae), *A. defilippii*, *A. rubrolineata*

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(Acanthochitonidae), by Nishikawa and Ishida (1969); *Katharina tunicata* (Mopaliidae) by Dolph and Humphrey (1970); *Chiton olivaceus* (Chitonidae) by Vitturi (1982); two species, namely, *Lepidochitona corrugata* (Ischnochitonidae), *Acanthochitona communis* (Acanthochitonidae)], by Vitturi *et al.* (1982); *Acanthochitona crinitus* (Acanthochitonidae) by Colombera and Tagliaferri (1983). Besides the above mentioned reports, Kawai (1976) made reconfirmation of the karyotype of two species, namely, *L. japonica* and *A. defilippii* which were earlier reported by Nishikawa and Ishida (1969). Recently, new karyological data of three species (*Ischnochiton hakodadensis*, *Lepidozona albrechtii* (Ischnochitonidae) and *Chiton kurodai* (Chitonidae)) have been reported by Choe *et al.* (1995), and Yum and Choe (1996).

The diploid chromosome number of 16 was shown in *Acanthochitona defilippii* (Nishikawa and Ishida, 1969; Kawai, 1976) and *A. rubrolineata* (Nishikawa and Ishida, 1969), and that of *A. crinitus* (Colombera and Tagliaferri, 1983) and *A. discrepans* (Certain, 1951, cited from Nishikawa and Ishida, 1969)as 18, and 24 in *A. communis* (Vitturi *et al.*, 1982).

The present study was carried out to determine the number and morphological characteristics of chromosomes of four species of *Acanthochitona* (Acanthochitonidae).

MATERIALS AND METHODS

Four *Acanthochitona* species, *A. achates* (Gould, 1859), *A. circellata* (Reeve, 1847), *A. defilippii* (Tapparone-Canefri, 1874), and *A. rubrolineata* (Lischke, 1873) were collected from the rocky shore in the Kotchi beach, western coast of Korea during May to June in 1993 and 1994. Chromosomes were prepared from testes during the stage of spermatogenesis by means of Nakamura's method (1986) with minor modifications as per the method of Choe *et al.* (1995). Abbreviations of chromosome morphology, as used in the tables were adopted from Levan *et al.* (1964). They are as follows: M - metacentric, arm ratio 1 to 1.7; Sm - submetacentric, 1.7 to 3; St - subtelocentric, 3 to 7; T - telocentric, 7 to ∞. Relative length and arm ratio were derived as follows;

$$\text{relative length} = \frac{\text{length of each chromosome}}{\text{total haploid length of chromosomes}} \times 100$$

arm ratio (r) = l/s (l: length of the long arm, s: length of the short arm).

RESULTS

Acanthochitona achates (Gould, 1859)

Karyological data were obtained from twenty five chromosomal spreads from 3 individuals. The relative lengths of the chromosomes and the arm ratios were calculated (Table 1). At spermatogonial metaphase, the diploid number of chromosomes was found to be 2n=16 (Fig. 1b), and at meiotic diakinesis, haploid number was n=8 (Fig. 1c). The

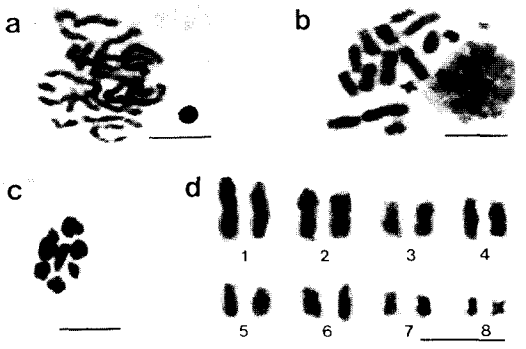

Fig. 1. The chromosomes and karyotype of *Acanthochitona achates*. a, Leptotene chromosomes in male gonads; b, Spermatogonial metaphase spread; c, Diakinetic bivalents in male gonads; d, Karyotype constructed from spermatogonial metaphase. Scale bars represent 10 μm.

Table 1. Relative lengths and morphological characters of chromosomes derived from male metaphase cells of *Acanthochitona achates*.

Chromosome pair No.	Relative length*		Arm ratio**	Morphology***
	mean	±SD		
1	18.01	±1.659	1.33	M
2	16.07	±1.150	1.34	M
3	14.15	±1.096	1.28	M
4	12.54	±0.798	3.20	St
5	12.06	±0.728	4.54	St
6	10.91	±0.868	1.73	Sm
7	8.60	±1.392	1.40	M
8	7.23	±1.462	1.28	M

* = $\frac{\text{length of each chromosome}}{\text{total haploid length of chromosomes}}$ X 100

** = l/s (l: length of the long arm, s: length of the short arm).

*** M - metacentric, arm ratio 1 to 1.7; Sm - submetacentric, 1.7 to 3; St - subtelocentric, 3 to 7; T - telocentric, 7 to ∞.

chromosome length decreased progressively from pair 1 to pair 8. Five pairs of chromosomes were metacentric (1, 2, 3, 7 and 8), one pair was submetacentric (6) and two pairs were subtelocentric (4 and 5).

Acanthochitona circellata (Reeve, 1847)

Karyological data were obtained from twenty seven chromosomal spreads from 4 individuals. The relative lengths of the chromosomes and the arm ratios were calculated (Table 2). At spermatogonial metaphase, the diploid number of chromosomes was found to be 2n=16 (Fig. 2a), and at meiotic diakinesis, haploid number was n=8 (Fig. 2b). The chromosome length decreased progressively from pair 1 to pair 8. One pair of chromosomes was metacentric (1), four pairs were submetacentric (2, 3, 4 and 7), two pairs were subtelocentric (5 and 6) and one pair was telocentric (8).

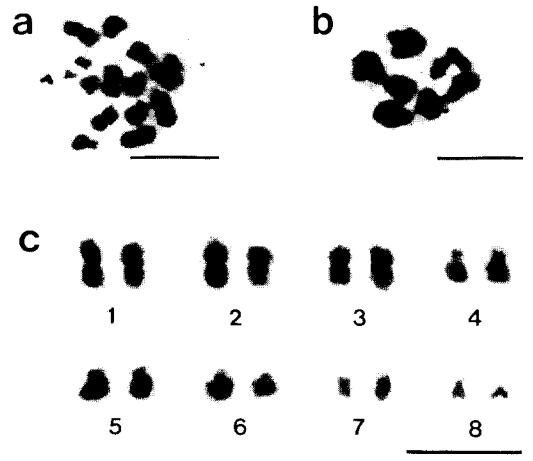

Fig. 2. The chromosomes and karyotype of *Acanthochitona circellata*. a, Spermatogonial metaphase spread; b, Diakinetic bivalents in male gonads; c, Karyotype constructed from spermatogonial metaphase. Scale bars represent 10 μm.

Table 2. Relative lengths and morphological characters of chromosomes derived from male metaphase cells of *Acanthochitona circellata*.

Chromosome pair No.	Relative length*		Arm ratio**	Morphology***
	mean	±SD		
1	17.54	±1.239	1.24	M
2	15.71	±1.078	1.57	Sm
3	14.51	±1.124	1.72	Sm
4	13.12	±0.654	2.83	Sm
5	12.32	±0.544	3.45	St
6	11.26	±0.737	3.77	St
7	8.66	±1.039	1.59	Sm
8	6.96	±1.177	-	T

*, **, and *** experimental details same as described in Table 1.

Acanthochitona defilippii (Tapparone-Canefri, 1874)

Karyological data were obtained from three chromosomal spreads from 1 individual. The relative lengths of the chromosomes and the arm ratios were calculated (Table 3). At spermatogonial metaphase,

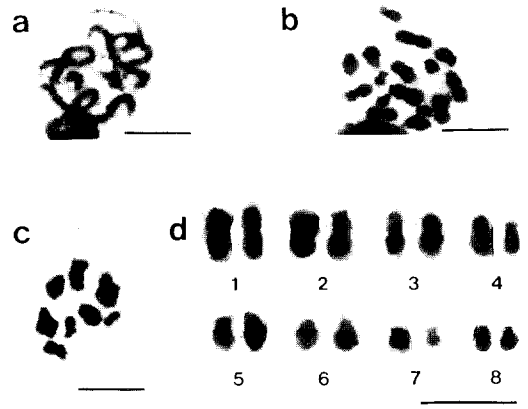

Fig. 3. The chromosomes and karyotype of *Acanthochitona defilippii*. a, Pachytene chromosomes in male gonads; b, Spermatogonial metaphase spread; c, Diakinetic bivalents in male gonads; d, Karyotype constructed from spermatogonial metaphase. Scale bars represent 10 μm.

Table 3. Relative lengths and morphological characters of chromosomes derived from male metaphase cells of *Acanthochitona defilippii*.

Chromosome pair No.	Relative length*		Arm ratio**	Morphology***
	mean	±SD		
1	18.72	±1.703	1.29	M
2	16.70	±0.444	1.22	M
3	15.03	±0.732	1.33	M
4	12.92	±0.886	2.69	Sm
5	12.31	±0.993	5.09	St
6	10.76	±1.001	1.82	Sm
7	7.44	±0.625	2.13	Sm
8	6.14	±0.522		T

*, **, and *** experimental details same as described in Table 1.

the diploid number of chromosomes was found to be 2n=16 (Fig. 3b) and at meiotic diakinesis, haploid number was n=8 (Fig. 3c). The chromosome length decreased progressively from pair 1 to pair 8. Three pairs of chromosomes were metacentric (1, 2 and 3), three pairs were submetacentric (4, 6 and 7), one pair was subtelocentric (5) and one pair was telocentric (8).

Acanthochitona rubrolineata (Lischke, 1873)

Karyological data were obtained from fourteen chromosomal spreads from 5 individuals. The relative lengths of the chromosomes and the arm ratios were calculated (Table 4). At spermatogonial metaphase, the diploid number of chromosomes was found to be 2n=16 (Fig. 4b) and at meiotic diakinesis, haploid number was n=8 (Fig. 4c). The chromosome length decreased progressively from pair 1 to pair 8. Five pairs of chromosomes were metacentric (1, 2, 3, 4, 8), one pair was submetacentric (7), one pair was submetacentric or subtelocentic (6) and one pair was subtelocentric (5).

DISCUSSION

All four species studied in the present study are characterized by having the same chromosome number, i.e., 2n=16. However, each species showed

Table 4. Relative lengths and morphological characters of chromosomes derived from male metaphase cells of *Acanthochitona rubrolineata*.

Chromosome pair No.	Relative length*		Arm ratio**	Morphology***
	mean	±SD		
1	18.16	±0.861	1.15	M
2	15.99	±1.062	1.14	M
3	14.44	±0.743	1.16	M
4	12.66	±0.806	1.16	M
5	11.82	±0.513	5.34	St
6	10.92	±0.618	3.08	Sm/St
7	8.97	±0.977	2.34	Sm
8	7.04	±0.632	1.29	M

*, **, and *** experimental details same as described in Table 1.

quite distinct characteristics in their chromosome morphology. The main differences were found at the fourth chromosome pair between *A. achates* which had subtelocentric and *A. rubrolineata* with metacentric, and at the eighth chromosome pair which showed metacentric in *A. achates* and *A. rubrolineata* while telocentric in *A. circellata* and *A. defilippii*.

Diploid chromosome numbers of *Acanthochitona* species were determined to be 24 by Vitturi *et al.* (1982), as 18 by Colombera and Tagliaferri (1983) and Certain (1951; cited from Nishikawa and Ishida, 1969), and as 16 by Nishikawa and Ishida (1969) and Kawai (1976). While the European species (*A. communis*, *A. crinatus* and *A. discrepans*) were shown to have 24 or 18 chromosomes (Vitturi *et al.*, 1982; Colombera and Tagliaferri, 1983; Certain, 1951 [cited from Nishikawa and Ishida, 1969]), north-east Asian species (*A. achates*, *A. circellata*, *A. defilippii* and *A. rubrolineata*) were shown to have 16 as their chromosome number (Nishikawa and Ishida,

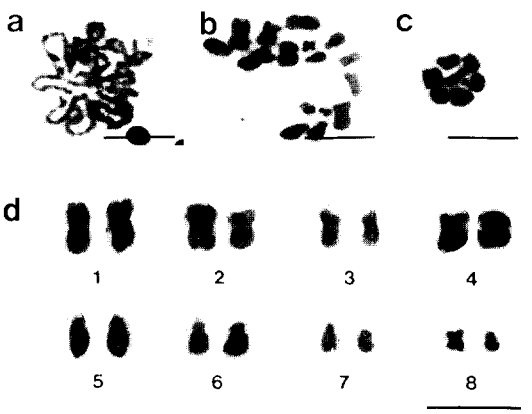

Fig. 4. The chromosomes and karyotype of *Acanthochitona rubrolineata*. a, Leptotene chromosomes in male gonads; b, Spermatogonial metaphase spread; c, Diakinetic bivalents in male gonads; d, Karyotype constructed from spermatogonial metaphase. Scale bars represent 10 μm.

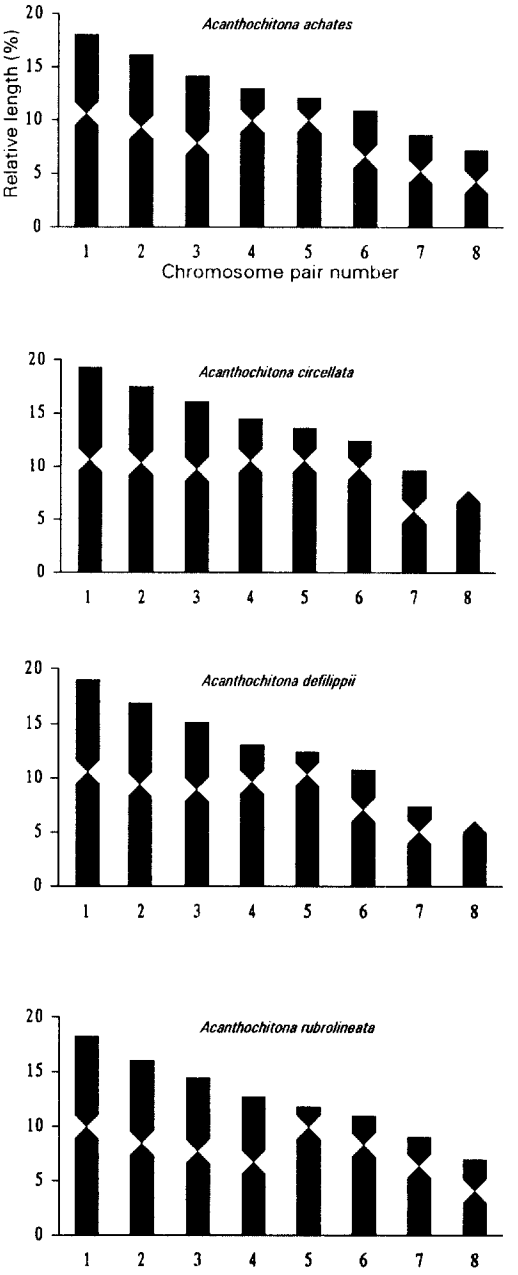

Fig. 5. Idiograms of the four *Acanthochitona* species constructed from relative length and arm ratio of the chromosomes.

1969; Kawai, 1976; the present study). According to the hypothesis of Vitturi *et al.* (1982), these numerical variations in chromosome number indicate that the *Acanthochitona* species of north-east Asia are more specialized than those of Europe. Yum and Choe (1996) reported comparable results for the genus *Chiton*.

SUMMARY

Chromosome numbers and morphology of four Korean species belonging to the genus *Acanthochitona* (*A. achates*, *A. circellata*, *A. defilippii* and *A. rubrolineata*) were studied during the spermatogenesis using warm drying method.

All the four species exhibited an identical diploid chromosome number of 16. However, the chromosomes morphology of each species was different: *A. achates* showed 5 metacentric, 1 submetacentric, 2 subtelocentric; *A. circellata* showed 1 metacentric, 4 submetacentric, 2 subtelocentric, 1 telocnetric; *A. defilippii* showed 3 metacentric, 3 submetacentric, 1 subtelocentric, 1 telocentric; *A. rubrolineata* showed 5 metacentric, 1 submetacentric, 1 submetacentric or subtelocentric, 1 subtelocentric. The closely related species, *A. achates* and *A. rubrolineata* could be distinguished using the morphological differences at the fourth chromosome pair. At the eighth chromosome pair, *A. achates* and *A. rubrolineata* exhibited metacentric, whereas *A. circellata* and *A. defilippii* showed telocentric in their chromosome morphology.

The variable chromosome numbers of this genus (2n=24, 18 and 16) indicate that the north-east Asian *Acanthochitona* are more specialized than their European counterparts.

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