

Comparison of Chromosome Number and Karyotype of Two *Lepidozamia* Species (Zamiaceae, Cycadales)

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國府方吾郎*・近藤勝彦**・Lou M. Randall***: *Lepidozamia* 属 (ザミア科, ソテツ目)
2種における染色体数及び核型の比較研究

Lepidozamia (Zamiaceae, Cycadales; Stevenson 1992) is endemic to Australia, and consists of *L. hopei* Regel and *L. peroffskyana* Regel. Two species of *Lepidozamia* are enable to be recognized with the width of leaflet; *i. e.* the former has wider leaflet than the latter (Jones 1993).

The chromosome number and karyotype of *L. hopei* and *L. peroffskyana* was previously reported by Marchant (1968) and Moretti (1990). In certain cycad-taxa, however, there are some mistakes of their karyotype identification for the previous reports, and it is necessary to reinvestigate their of the chromosomal characters (Kokubugata *et al.* 1999). The aim of the present study is investigation of the chromosome number and karyotype of *L. hopei* and *L. peroffskyana* by the standard aceto-orcein staining method for the cytotaxonomical accumulations of *Lepidozamia*.

Materials and Methods

The taxonomical treatment follows Jones (1993). The plants investigated in the present study were collected in the each habit and exported under a CITES permit before cultivation in the greenhouse of the Tsukuba Botanical Garden (Table 1).

Root tips were harvested and pretreated in 4 mM 8-hydroxyquinoline at 4°C for 10 hour. They were fixed in acetic ethanol (3:1) at 4°C for 24 hour, and were transferred and stored in 70% ethanol at -20°C.

Fixed Root tips were macerated in a mixture of 1N HCl and 45% acetic acid (2:1) at 60°C for 10 sec., put on glass slides, and then stained in 2% aceto-orcein at room temperature for 4 hour. Orcein-stained chromosomes were classified according to centromeric position defined using arm ratios (Levan *et al.* 1964).

Table 1. *Lepidozamia* species used in this study

Species	Origin	Accession no.
<i>L. hopei</i>	Australia, State of Queensland: El Arish	TBG126733
<i>L. perffoskyana</i>	Australia, State of Queensland: Caloundra	TBG122893

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Fig. 1. Chromosome complement of two *Lepidozamia* species at mitotic metaphase. A. *L. hopei*. B. *L. peroffskyana*. Bar indicates 10 μm .



Fig. 2. Somatic chromosome of two *Lepidozamia* species at mitotic metaphase. A. *L. hopei*. B. *L. peroffskyana*. Bar indicates 10 μm .

Table 2. Arm ratio and chromosome length in the complement of two *Lepidozamia* species at mitotic metaphase

Chrom. No.	Long arm (μm)		Long arm (μm)		Long arm (μm)		Arm ratio		Chromosomal classification	
	H	P	H	P	H	P	H	P	H	P
1	15.1	23.0	13.0	19.0	28.1	42.0	1.2	1.2	m	m
2	13.8	22.5	13.5	17.8	27.3	40.3	1.0	1.3	m	m
3	13.2	20.0	13.0	18.8	26.2	38.8	1.0	1.1	m	m
4	14.5	20.0	11.6	18.0	26.1	38.0	1.3	1.1	m	m
5	13.0	19.0	13.0	15.5	26.0	34.5	1.0	1.2	m	m
6	12.6	17.0	11.5	15.2	24.1	32.2	1.1	1.1	m	m
7	9.1	15.0	8.8	12.0	17.9	27.0	1.0	1.3	m	m
8	8.2	13.0	7.8	11.4	16.0	24.4	1.1	1.1	m	m
9	13.5	19.5	5.0	4.5	18.5	24.0	2.7	4.3	sm	st
10	12.8	18.5	5.1	5.0	17.9	23.5	2.5	3.7	sm	st
11	14.0	17.5	3.8	5.1	17.8	22.6	3.7	3.4	st	st
12	12.3	15.5	3.5	5.8	15.8	21.3	3.5	2.7	st	sm
13	12.2	16.9	3.4	3.8	15.6	20.7	3.6	4.4	st	st
14	12.1	17.0	3.0	3.5	15.1	20.5	4.0	4.9	st	st
15	14.9	20.5	0.6	0.5	15.5	21.0	24.8	41.0	t	t
16	14.5	20.0	0.7	0.5	15.2	20.5	20.7	40.0	t	t
17	10.0	12.8	4.2	4.2	14.2	17.0	2.4	3.0	sm	sm
18	10.0	12.7	3.6	4.1	13.6	16.8	2.8	3.1	sm	st

H: *L. hopei*. P: *L. peroffskeyana*. m: median-centromeric chromosome. sm: submedian-centromeric chromosome. st: subterminal-centromeric chromosome. t: terminal-centromeric chromosome.

Results and Discussion

Lepidozamia hopei and *L. peroffskeyana* showed the chromosome number of $2n=18$ (Fig. 1), which was consistent with those of the previous reports (Marchant 1964; Moretti 1990).

The karyotype of *L. hopei* consisted of eight median-, four submedian-, four subterminal- and two terminal-centromeric chromosomes (Figs. 2A and 3A, Table 2). On the other hand, those of *L. peroffskeyana* consisted of eight median-, two submedian-, six subterminal- and two terminal-centromeric chromosomes (Figs. 2B and 3B, Table 2). The arm ratio value of four submedian-centromeric chromosomes of *L. hopei* and two submedian-centromeric chromosomes of *L. peroffskeyana* were very close to 3.1, the lowest extreme of the arm ratio range of the subterminal-centromeric chromosome according to Levan *et al.* (1964). Thus, the karyotypes of two *Lepidozamia* species in the present investigation were very similar to each other, and was very similar to the karyotype of eight median-, four submedian- (or subterminal-) and two terminal-centromeric chromosomes of two *Lepidozamia* species reported by Marchant (1968) and Moretti (1990).

It has been reported that members of Cycadales show the stable karyotype within the each genus (Marchant 1968; Moretti 1990), excepting *Zamia* (Norstog 1980; Vovides 1983; Moretti and Sabato 1984) and *Bowenia* (Kokubugata *et al.* 1999). The present investigation indicated that the two *Lepidozamia* species are the same chromosome number, $2n=18$ and similar karyotype. These results

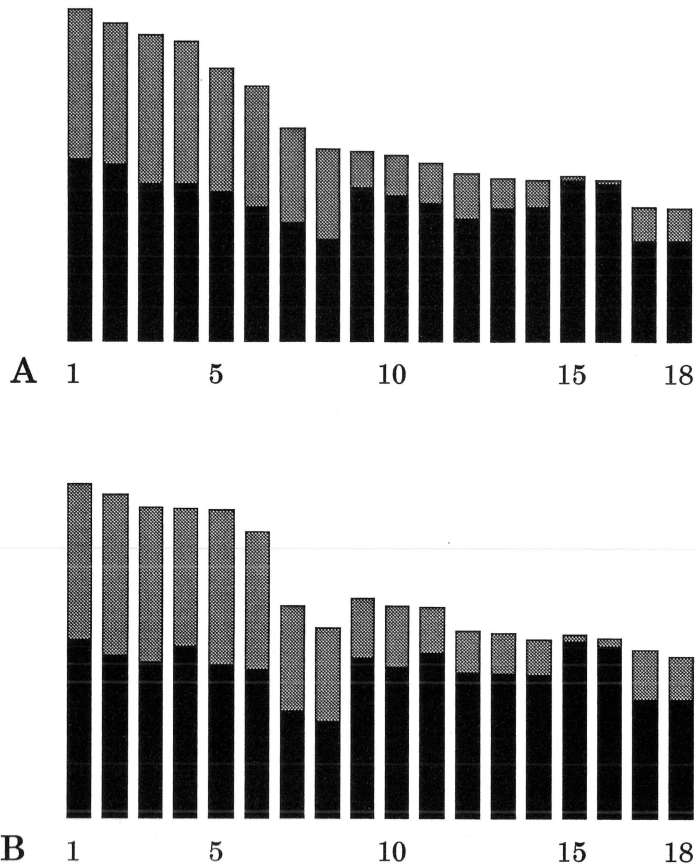


Fig. 3. Ideogram of two *Lepidozamia* species **A.** *L. hopei*. **B.** *L. paroffskyana*. Solid areas show long arm. Stippled areas show short arm.

intimate that there must be no karyomorphological arrangements in the speciation between two *Lepidozamia* species.

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Summary

Somatic chromosomes at mitotic metaphase of *Lepidozamia hopei* and *L. paroffskyana* were investigated by the standard aceto-orcein staining method. They showed the chromosome number of $2n=18$ and the karyotype consisted of eight median-, eight subterminal- (or submedian-) and two terminal-centromeric chromosomes.

摘 要

Lepidozamia 属 2 種, *L. hopei* と *L. peroffskyana* の体細胞染色体をアセトオルセイン染色法により観察した。*Lepidozamia* 2 種は, ともに染色体数が $2n=18$ であり, 核型は 8 個の中部動原体型染色体, 8 個の次端部 (或いは次中部) 動原体型染色体, 2 個の端部動原体型染色体から構成されていた。

References

- Jones, D. L., 1993. *Cycads of the world*, Smithsonian Institution Press, Washington, D. C.
- Kokubugata G., K. Kondo, G. W. Wilson, L. M. Randall, A. van der Schans and D. K. Morris, 1999. Comparison of karyotype and rDNA-distribution in somatic chromosomes of *Bowenia* species (Stangeriaceae, Cycadales). *Aust. Pl. Syst. Bot.* **13**: in press.
- Levan, A., K. Fredga and A. A. Sandberg, 1964. Nomenclature for centromeric position on chromosomes. *Hereditas* **52**: 201-220.
- Marchant, C. J., 1968. Chromosome patterns and nuclear phenomena in the cycad families Stangeriaceae and Zamiaceae. *Chromosoma (Berl.)* **24**: 100-134.
- Moretti, A., 1990. Cytotaxonomy of cycads. *Mem. New York Bot. Gard.* **57**: 114-122.
- , and S. Sabato, 1984. Karyotype evolution by centromeric fission in *Zamia* (Cycadales). *Pl. Syst. Evol.* **146**: 215-223.
- Norstog, K., 1980. Chromosome numbers in *Zamia* (Cycadales). *Caryologia* **33**: 419-428.
- Stevenson, D. W., 1992. A formal classification of the extant cycads. *Brittonia* **44**: 220-223.
- Vovides, A., 1983. Systematic studies on the Mexican Zamiaceae. I. Chromosome numbers and karyotypes. *Amer. J. Bot.* **70**: 1002-1006.