筑波実験植物園研報 19 : 19-23, 2000.
Ann. Tsukuba Bot. Gard.

Chromosome Number and Karyotype of Two *Cycas* Species (Cycadaceae, Cycadales)

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國府方吾郎* • Ken D. Hill** • 近藤勝彦***: ソテツ属2種における染色体数と核型

The genus *Cycas* (Cycadaceae, Cycadales; Stevenson 1992) occurs in the Malesian region, Japan and Southeast Asia, extending to Micronesia and Polynesia, Madagascar and East Africa, and is the largest one in the Cycadales (Stevenson *et al.* 1995). Although the taxonomical studies of *Cycas* have been advancing (*e.g.*, Hill 1992, 1994a, 1994b & 1996), its chromosomal studies are lacking. There are two reasons for the lack of chromosomal investigations in *Cycas*: all species of the genus are regarded as endangered species in the world, and identification is quite difficult. The aim of the present study is to investigate chromosome numbers and karyotypes of *C. micronesica* and *C. seemanni*, which have previously unreported, by the standard aceto-orcein staining method.

Materials and Methods

Seeds of *Cycas micronesica* K.D. Hill and *C. seemanni* A. Braun, which were classified to subsection **Rumphiae**, section **Cycas** (Hill 1994b), were obtained from the wild by the second author, and the plants were grown in the Royal Botanic Gardens, Sydney (Table 1). The voucher specimens were deposited in the National Herbarium of New South Wales (NSW).

Young leaflets were pretreated in 3 mM 8-hydroxiquinoline at 4℃ for 20 hr, fixed in acetic ethanol (1:3) at 4℃ for 20 hr, and stored in 70% ethanol at −20℃. The stored leaflets were macerated in 45% acetic acid at 60℃ for 5 min and then stained in 2% aceto-orcein at 20℃ for 5 hr. After squashed with 45% acetic acid, the stained chromosomes at mitotic metaphase were classified with their arm ratio following Levan *et al.* (1964).

Table 1. Plant materials of two *Cycas* species in the present study

Species	Accession number
C. micronesica	RBGS 920530
C. seemanni	RBGS 931245

RBGS: Royal Botanic Gardens, Sydney.

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Three mitotic metaphase plates were measured for each species to obtain mean chromosome lengths and arm ratios. Chromosome length of more than 20 μm was designated as "long", and that of less than 20 μm as "short". Values of asymmetry of arm ratio and chromosome length were calculated following Zarco (1986) with some modifications.

Results and Discussion

Both *Cycas micronesica* and *C. seemanni* showed the chromosome number of 2n=22 (Fig. 1). Their chromosome number corresponded with those of other *Cycas* species in the earlier reports (*e.g.*, Sax and Beal 1934; Kondo *et al.* 1995).

From the 1st to the 4th chromosomes, the two species showed two long subterminal- and two long submedian-centromeric chromosomes (Figs. 2 & 3, Table 2). The two long subterminal-centromeric chromosomes showed a secondary constriction on the interstitial region of the long arm (Fig. 1, arrows). From the 5th to the 22nd chromosomes, they showed twelve subterminal-, two short submedian- and four short median-centromeric chromosomes (Figs. 2 & 3, Table 2). The value of arm ratio asymmetry of *C. micronesica* was 0.62, and that of *C. seemanni* was 0.58. The value of chromosome length asymmetry of *C. micronesica* was 0.24, and that of *C. seemanni* was 0.23. These results indicated that karyomorphologies of the two species were very similar to each other.

Table 2. Chromosomal features of two *Cycas* species studied

Chrom. no.	*C. micronesica*			*C. seemanni*		
	L	R	F	L	R	F
1	24.6	3.2	st	22.1	3.5	st
2	23.1	3.1	st	21.1	3.6	st
3	24.5	2.7	sm	20.5	2.5	sm
4	23.2	2.7	sm	20.1	2.4	sm
5	22.6	4.8	st	16.5	4.4	st
6	20.5	4.9	st	15.9	4.0	st
7	19.8	3.9	st	15.7	4.7	st
8	19.0	5.0	st	15.0	4.1	st
9	18.5	4.0	st	15.0	3.6	st
10	17.6	3.6	st	14.7	3.6	st
11	16.9	4.9	st	14.1	4.3	st
12	16.1	4.3	st	13.8	3.8	st
13	15.8	5.3	st	13.0	4.9	st
14	15.7	4.3	st	12.8	3.4	st
15	15.3	4.4	st	12.4	3.1	st
16	14.1	5.3	st	12.3	3.6	st
17	14.2	2.1	sm	12.5	1.8	sm
18	15.2	1.8	sm	11.2	1.8	sm
19	13.7	1.1	m	12.2	1.1	m
20	12.6	1.3	m	11.8	1.1	m
21	11.7	1.1	m	11.2	1.1	m
22	10.1	1.3	m	10.3	1.1	m

L: chromosome length. R: arm ratio. F: form of chromosome with centromere position. m: median-centromeric chromosome ($1.0<R<1.7$). sm: submedian-centromeric chromosome ($1.8<R<3.0$). st: subterminal-centromeric chromosome ($3.1<R<7.0$). t: terminal-centromeric chromosome ($7.1<R<\infty$).

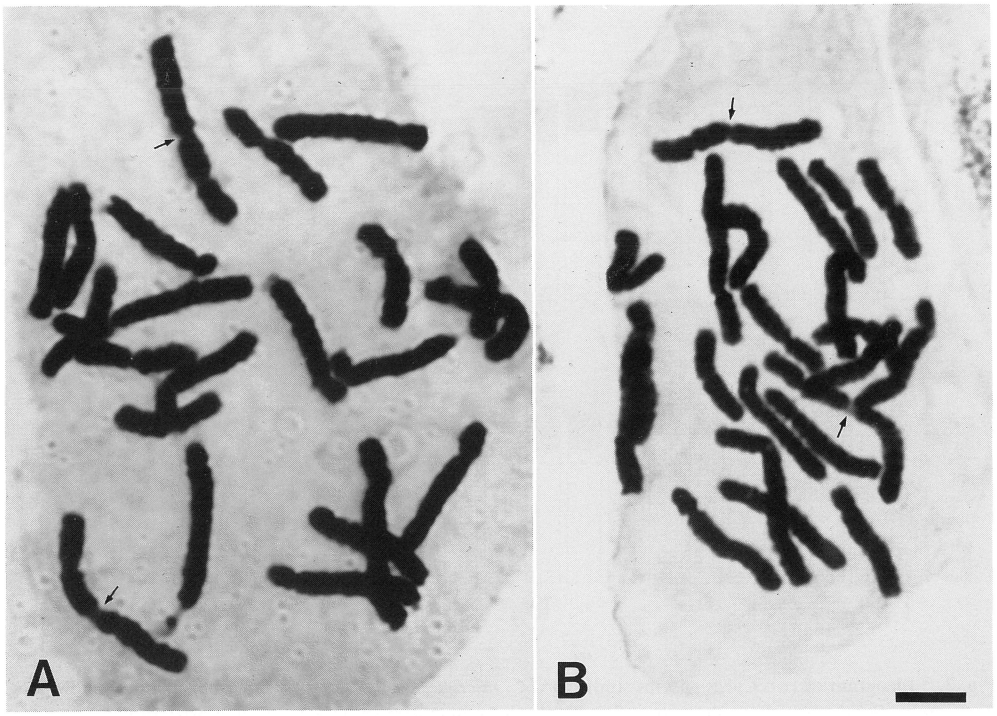

Fig. 1. Chromosomes at mitotic metaphase of two *Cycas* species studied. **A.** *C. micronesica*. **B.** *C. seemanni*. Arrows show secondary constriction. Bar shows 10 μ m.

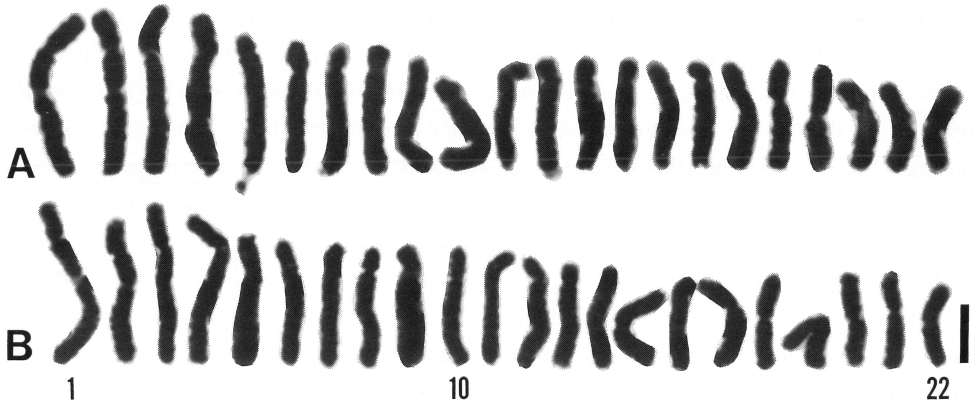

Fig. 2. Karyotypes of two *Cycas* species studied. **A.** *C. micronesica*. **B.** *C. seemanni*. Bar shows 10 μ m.

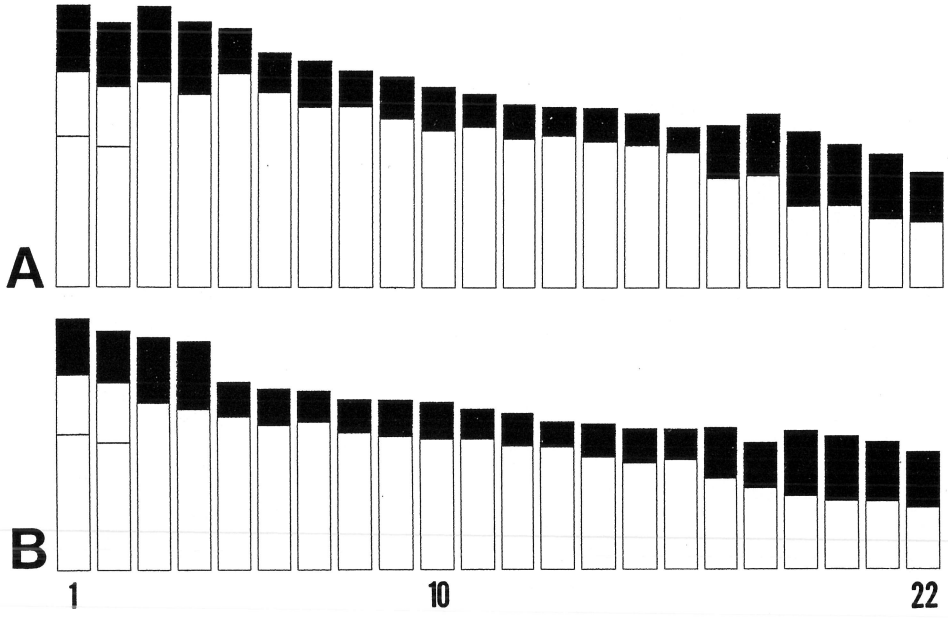

Fig. 3. Ideogram of two *Cycas* species studied. **A.** *C. micronesica*. **B.** *C. seemanni*. Opened areas show long arm. Solid areas show short arm. Straight lines on long arm show secondary constriction.

It has previously been reported that karyotypes were very stable in the genus *Cycas*, and consisted of two median-, eight submedian- (or subterminal-) and twelve terminal-centromeric chromosomes (*e.g.*, Kondo *et al.* 1995). The differences of *C. micronesica* and *C. seemanni* from the other species in the previous reports are follows, 1) a secondary constriction appear at the interstitial region of the long arm of two long subterminal-centromeric chromosomes (the 1st and 2nd in Figs. 2 & 3) unlike the other *Cycas* species which show two submedian-centromeric chromosomes without any secondary constrictions; 2) the two species show twelve subterminal-centromeric chromosomes (the 5th to the 16th in Figs. 2 & 3) unlike the other *Cycas* species which show twelve terminal-centromeric chromosomes; and 3) the 21st and the 22nd chromosomes of the two species are median-centromeric (in Figs. 2 & 3) unlike the other *Cycas* species are submedian-centromeric.

In morphological features, the two species were quite similar to each other, and they were taxonomically classified into subsection ***Rumphiae***, section ***Cycas*** (Hill 1994b). In the present study, there is not enough data to reveal that karyomorphological differences reflect the interspecific relationship in the genus, but it is quite plain that *C. micronesica* and *C. seemanni* show not only morphological similarities but also karyomorphological ones.

Summary

Somatic chromosomes of *Cycas micronesica and C. seemanni*, which were classified into subsection **Rumphiae**, section **Cycas**, were observed by standard aceto-orcein staining method. The two species showed chromosome number of 2n=22, and karyotype consist of two long subterminal-, two

long submedian-, twelve subterminal-, two short submedian- and four short median-centromeric chromosomes. The karyotypes of the two species were different from those of the other *Cycas* species as follows: 1) a secondary constriction appeared at the interstitial region of the long arm of two long subterminal-centromeric chromosomes; 2) the two species showed twelve subterminal-centromeric chromosomes; and 3) the 21st and 22nd chromosomes of the two species were median-centromeric.

摘　要

ソテツ属（ソテツ科，ソテツ目）の **Cycas** 節 **Rumphiae** 亜節2種，*Cycas micronesica* と *C. seemanni* の2種の染色体と核型をアセトオルセイン染色法により観察した。2種ともに染色体数2n＝22を示し，核型は2個の長い次端部動原体型染色体，2個の長い次中部動原体型染色体，12個の次端部動原体型染色体，2個の短い次中部動原体型染色体，および4個の短い中部動原体型染色体からなっていた。これら2種の核型は，過去に報告されたソテツ属数種の核型と次の3つの点が異なっていた。1）第1番目と第2番目の次端部動原体型染色体の長腕介在部に2次狭窄がみられること；2）第5番目から第16番目が次端部動原体型染色体であること；3）第21番目と第22番目が中部動原体型染色体であること。また，本研究により，この2種は外部形態が類似しているのに加え，核型も類似していることが明らかとなった。

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