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Comparative Karyomorphological Study between Male and Female Plants of Some *Cycas* and *Zamia* Species

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ABSTRACT

This paper presents the karyotype and the idiogram of known sex plants for five species of *Cycas* (Cycadaceae) and seven species of *Zamia* (Zamiaceae). All five species of the genus *Cycas* had the same chromosome number (2n = 2x = 22) but their karyotype formulae varied into three groups: 10m+10sm+2st, 12m+8sm+2st and 14m+8sm. The karyotype of male and female *Cycas* plants could be clearly distinguished by the satellite chromosomes. All seven species of the genus *Zamia* varied in both chromosome numbers (2n = 2x = 16 up to 2n = 2x = 24) and karyotype formulae. It was difficult to differentiate between the male and female plants of the *Zamia* spp. by karyotyping because of the lack of satellite chromosomes in both sexes.

Key words: Cytogenetics, sex determination, karyotype, idiogram, satellite chromosome

INTRODUCTION

Cycas (Cycadaceae) consists of three sections and eighty species (Schuster, 1932). It is considered the most primitive genus among the cycads (Stevenson, 1990), since the genus seems to form a homoploid series with a chromosome number of 2n = 2x = 22 and to possess similar karyotypes in all species with only slight variation (Sax and Beal, 1934; Kondo *et al*., 1995). Among the genera of cycads (Cycadales) (Stevenson, 1990), *Zamia* (Zamiaceae) is unique in showing interspecific karyotype variation (Marchant, 1968; Norstog, 1980; Moretti, 1990). Moreover, six species of *Zamia* have been reported to have intraspecific variation (Norstog, 1980; Moretti and Sabato, 1984; Moretti, 1990). The chromosome numbers known for the genus are 2n = 16, 18, 21, 22, 23, 24, 25, 26, 27 and 28. The diploid, odd chromosome numbers occur in species with intraspecific chromosome variation.

In dioecious gymnosperms, very little work has been carried out on the cytology of sex determination. This is probably due to the fact that in many of the dioecious gymnosperms, the male and female individuals are identical in their external vegetative features, and as they normally take many years to become reproductively mature, one has to wait for a long period to discern the sex of a particular plant (Abraham and Mathew, 1962). The first report of chromosome-based sex determination in plants was made by Allen (1917) on *Sphaerocarpos*–liverwort. Subsequently, chromosomes associated with sex have been discovered in a number of dioecious plants, in lower orders as well as higher. Eichhorn (1928) was the first to report the existence of chromosome satellites in *Ginkgo biloba* and suggested that this might be related to sex determination. However, Lee (1954) and Pollock (1957) presented evidence

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of the association of satellites based on a heteromorphic chromosome pair linked to sex. Newcomer (1954) reported the karyotype and sex chromosome of *Ginkgo biloba* Linn. Similar observations were made in *Cycas pectinata* by Abraham and Mathew (1962). An XY type of sex mechanism has been assumed to be present in these plants. Segawa *et al*. (1971) reported sex chromosomes of *Cycas revoluta*. Sangduen *et al*. (2007) reported the karyotype and idiogram of three species of *Cycas* (*C. elephantipes, C. siamensis and C. tansachana)* and of *Zamia (Z. integrifolia, Z. pumila* and *Z. pygmeae*). Kanchanaketu *et al*. (2007) presented the analysis of sex determination in cycads using the MSAP (methylation sensitive amplification and polymorphism) technique. They suggested that sex in cycads may be associated with DNA methylation. The current work based on a simple technique was undertaken to study the general morphology of chromosomes in the male and female plants of *Cycas* and *Zamia* that could be correlated with any corresponding chromosomal difference.

MATERIALS AND METHODS

Plant materials

The present study used five species of *Cycas* and seven species of *Zamia* collected from different geographical areas throughout the world and maintained from mature plants of known external sex determination in a living germplasm bank at Nong Nooch Tropical Botanical Garden, Thailand (Table 1).

Chromosome preparation

Immature still-folded leaflets of known sex plants were harvested and cut into small pieces of about two mm. They were pretreated in 5.5 umole/l isopropyl-N-phenyl-carbamate for 24 h then fixed in a Carnoy's fluid (ethanol:glacial acetic acid:chloroform in the ratio 2:1:1) for 24 h twice and incubated in 1 N HCl for 5 min. Chromosome preparation involved enzyme maceration of 2% cellulose Onozuka R-10 (Yakult) and 1% pectolyase Y-23 (Kikkoman) in distilled water (W/V) at 37°C for 20 min. Then trichomes were removed, stained and macerated in 2% aceto–orcein. For identification of the chromosomes, specimens with well spread chromosomes were photographed. Chromosome lengths and arm ratios were then measured from the pictures taken and converted into actual lengths according to their magnification. The number and size of chromosomes and satellite chromosomes were recorded.

Chromosome identification followed Levan *et al*. (1964): Median–centromere chromosome (= m–chromosome; arm ratio = long arm/ short arm = 1.0-1.7), submetacentric – centromeric chromosome (= sm–chromosome; arm ratio = 1.8-3.0), subterminal – centromeric chromosome (= st–chromosome; arm ratio = 3.1–5.0), and terminal–centromeric chromosome, and "t – chromosome" means telocentric chromosome.

RESULTS AND DISCUSSION

Karyotype of *Cycas*

The five species of *Cycas* for which the chromosome number was determined were *Cycas clivicola* subsp. *lutea* and *C. edentata* with the first report of chromosome number of 2n = 2x = 22; *C. elephantipes, C. tansachana* and *C. simensis* showed a chromosome number of 2n = 2x = 22. Three species of *Cycas* agreed with the findings of Toahsakul *et al*. (2002) and Sangduen *et al*. (2007). Chromosomes of the first and second pairs were observed to possess secondary constriction in all preparations.

C. clivicola subsp. *lutea* had a karyotype consisting of 14 median (m-chromosome) and 8 submedian (sm-chromosome). In haploid complement, a satellite chromosome was found

Table 1 Geographical distribution, number of plants and chromosome number of five species of *Cycas* and seven species of *Zamia* used in the study.

Species	Geographical distribution	Number of plants (sex) used		Chromosome number		References
		♂	♀	Present count	Previous count	
C. clivicola subsp. *Lutea* K.D.Hill	Aranyaprathet, Thailand	23	30	2n = 2x = 22*	N/A	
C. edentata De Laubent	Philippines	17	31	2n = 2x = 22*	N/A	
C. elephantipes Lindstrom & Hill	Chaiyaphum, Thailand	18	20	2n = 2x = 22	2n = 2x = 22	Sangduen *et al.*, 2007
C. siamensis Miquel	Kanchanaburi, Thailand	17	25	2n = 2x = 22	2n = 2x = 22	Sangduen *et al.*, 2007
C. tansachana K.D. Hill & S.L. Yang	Saraburi, Thailand	12	14	2n = 2x = 22	2n = 2x = 22	Sangduen *et al.*, 2007
Z. herrerae Calderon & Standley	Mexico	15	18	2n = 2x = 24*	N/A	
Z. integrifolia Linnaeus fil in Aiton	Florida, USA	33	33	2n = 2x = 16*	2n = 2x = 16	Johnson, 1963; Tagashira and Kondo, 1999; Sangduen *et al.*, 2007
Z. monticola Chamberlain	Guatemala	14	13	2n = 2x = 21*	N/A	
Z. muricata Willdenow	Venezuela	24	29		2n = 2x = 23	Moretti, 1990; Norstog, 1980; Tagashira and Kondo, 1999
Female plants				2x = 18, 24*	N/A	
Male plants				2n = 2x = 23	2n = 2x = 23	Tagashira and Kondo, 2001
Z. pumila Linnaeus	Dominican Republic	26	31	2n = 2x = 16	2n = 2x = 16	Sax and Beal, 1934; Moretti, 1990
Z. pygmeae Sims	Cuba	27	26	2n = 2x = 16	2n = 2x = 16	Moretti, 1990; Tagashira and Kondo, 1999; Sangduen *et al.*, 2007
Z. vazquezii D.W.Stevenson, Sabato & Moretii	Mexico	12	18	2n = 2x = 18	2n = 2x = 18	Moretti *et al.*, 1991; Tagashira and Kondo, 1999

* first report
N/A = not available

on the short arm of the 4th chromosome in male plants (Figure 1A-B) and 3 satellite chromosomes on the long arm of the 5th, 6th and 7th chromosome were observed in female plants (Figure 1C-D). The number of satellite chromosomes in the diploid genome was used to identify sex; male plants had two satellite chromosomes on the short arm, while female plants had six satellite chromosomes on the long arm.

C. edentata had a karyotype consisting of 12-m chromosome, 8 sm-chromosome and 2 subterminal (st – chromosome). A satellite chromosome was found on the 2nd and 3rd chromosomes (1 satellite chromosome on the long arm of 2nd chromosome, 2 satellite chromosomes on the short arm of 3rd chromosome) in male plants, which gave the two patterns of the male gametes (Figure 1E-F). However, the female plants were found to have 6 satellite chromosomes on the long arm of 2nd, 7th and 8th chromosome (Figure 1G-H). It is interesting to recall here the work of Abraham and Mathew (1962) on the karyotype and sex chromosome of *C. pectinata*. The only difference between the male and female plants was the two chromosomes of the somatic complement-bearing satellites observed in females, while the male had the same heteromorphic pair with only one of its members bearing a satellite.

C. elephantipes, a newly described species from Thailand (Lindstrom and Hill, 2002), had a karyotype consisting of 12m - chromosome, 8sm – chromosome and 2st – chromosome (Sangduen *et al.*, 2007). A satellite chromosome was located on the long arm of the 1st, 4th and 5th chromosome in male plants (Figure 1I-J). Two satellite chromosomes on the long arm of the 5th chromosome were found in female plants (Figure 1K-L).

C. siamensis had a karyotype consisting of 10 m–chromosome, 10 sm-chromosome and 2 st –chromosome (Sangduen *et al.,* 2007). A satellite chromosome was found on the long arm of the 1st, 4th, 5th and 6th chromosome in male plants and represented two patterns of male gametes (Figure 1M-N). Only 2 satellite chromosomes on the long arm of 3rd chromosome were found in female plants (Figure 1O-P).

C. tansachana had a karyotype consisting of 12 m-chromosome, 8 sm-chromosome and 2 st-chromosome (Sangduen *et al.*, 2007). The satellite chromosome was found on the long arm of the 1st and 8th chromosome with various numbers of satellite chromosomes in male plants which gave two patterns of male gametes (Figure 1 Q-R). Only 2 satellite chromosomes on the long arm of the 8th chromosome were found in female plants (Figure 1 S-T).

Sex determination in *Cycas*

The difference in sex determination in the species of *Cycas* in the current study could be categorized in terms of the number of satellite chromosomes and their heteromorphic or homomorphic pattern as follows:

C. siamensis and *C. tansachana* showed a heteromorphic chromosome on the 1st chromosome in male plants. A homomorphic chromosome pattern was found in female plants. This chromosome pattern was also observed in *C. edentata* except for heteromorphic chromosomes located on the 2nd chromosome.

C. clivicola showed homomorphic satellite chromosomes on different chromosomes in both male and female plants.

C. elephantipes showed homomorphic satellite chromosomes on different arms in male plants and a homomorphic chromosome pattern on one of the chromosomes in female plants.

Karyotype of *Zamia*

Seven species of *Zamia*: *Zamia herrerae*, *Z. integrifolia*, *Z. monticola*, *Z. muricata*, *Z. pumila*, *Z. pygmeae* and *Z. vazquezii*, varied in chromosome number from 2n = 2x = 16 up to 2n

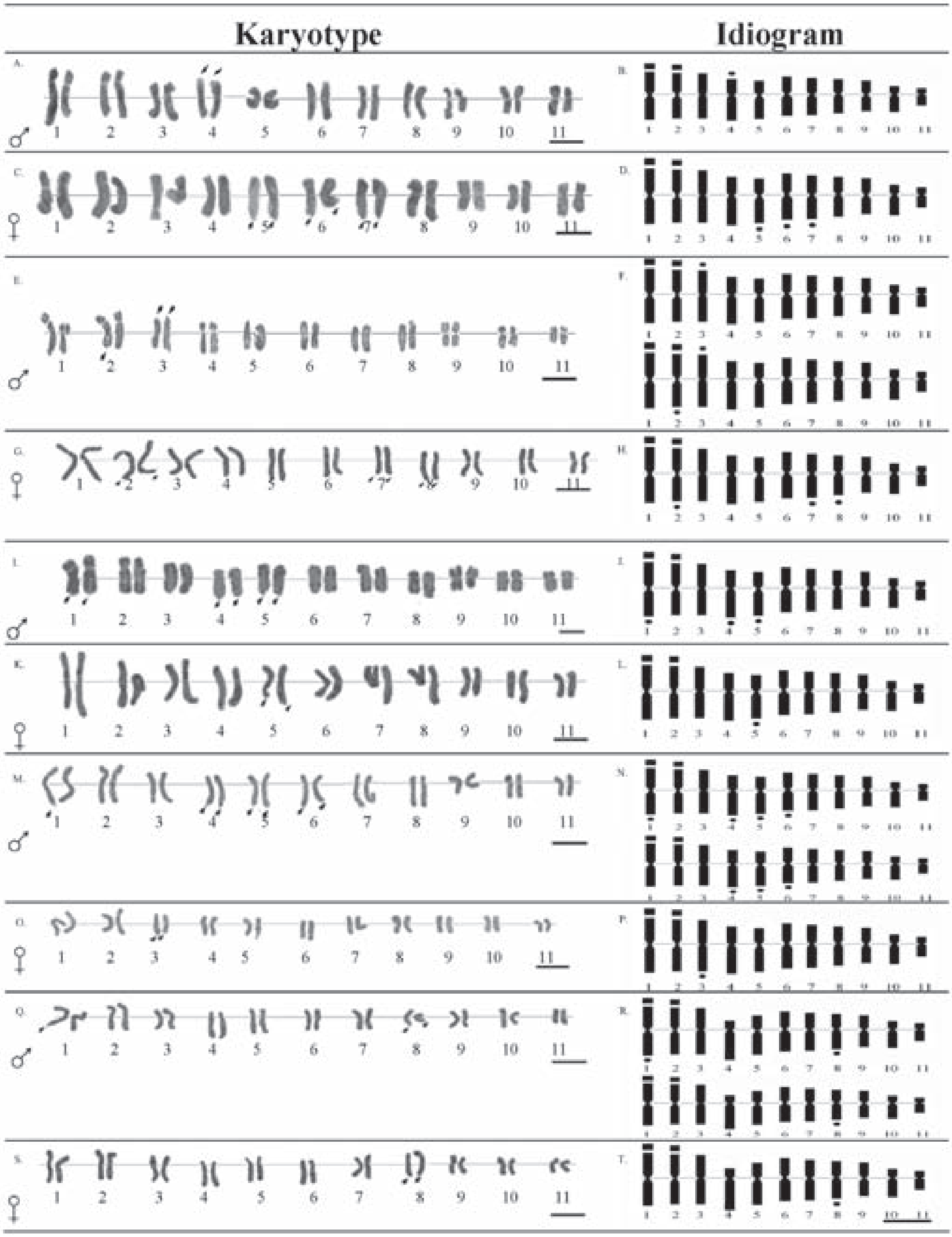

Figure 1 Karyotypes and idiograms of five species of *Cycas* (2n = 2x = 22). A-B. *Cycas clivicola* (male). C-D. *C. clivicola* (female) . E-F. *C. edentata* (male). G-H. *C. edentata* (female). I-J. *C. elephantipes* (male). K-L. *C. elephantipes* (female). M-N. *C. siamensi*s (male). O-P. *C. siamensi*s (female). Q-R. *C. tansachana (male).* S-T. *C. tansachana (femal*e). (▬ = 10 µm).

▬ = secondary constriction ; • = satellite

= 2x = 24. This was the first report of the chromosome number in *Z. herrerae* (2n = 2x = 24), *Z. monticola* (2n = 2x = 21), and *Z. muricata* for both male (2n = 2x = 23) and female (2n = 2x = 18 and 2n = 2x = 24) plants.

Z. herrerae showed a chromosome number of 2n = 2x = 24 and the karyotype consisted of 4m-chromosome, 14 sm-chromosome and 6t – chromosome. No satellite chromosomes were observed in either male or female plants of *Z. herrerae* (Figure 2A-B, 2C-D).

Z. integrifolia showed a chromosome number of 2n = 2x = 16 (Moretti, 1990; Caputo *et al*., 1996; Tagashira and Kondo, 1999, 2001; Sangduen *et al*., 2007) and the karyotype consisted of 12m-chromosome and 4 sm-chromosome (Moretti and Sabato, 1984; Moretti, 1990; Sangduen *et al*., 2007). No satellite chromosomes were found in either male or female plants of *Z.integrifolia* (Figure 2E-F, 2G-H).

Z. monticola showed a chromosome number of 2n = 2x = 21 and the karyotype consisted of 10m-chromosome, 7sm-chromosome and 4t-chromosome. This was the first record of the chromosome number of this species. No satellite chromosomes were observed in either male or female plants of *Z. monticola* (Figure 2I-J, 2K-L).

Z. pumila showed a chromosome number of 2n = 2x = 16 (Moretti, 1990; Caputo *et al*., 1996; Tagashira and Kondo, 1999, 2001; Sangduen *et al*., 2007) and the karyotype consisted of 12m-chromosome and 4 sm-chromosome (Moretti and Sabato, 1984; Moretti, 1990; Sangduen *et al*., 2007). Three satellite chromosomes and 4 satellite chromosomes on the 1^{st} and 8^{th} chromosome, respectively were visible in male and female plants of *Z. pumila* (Figure 2S-T, 2U-V).

Z. muricata was reported to have a chromosome number of 2n = 2x = 23 but sex differences in this species were not investigated (Caputo *et al*., 1996). The karyotype consisted of 8m – chromosome, 9sm-chromosome and 6t-chromosome in males. Two idiograms were observed in male haploid complements, n = x = 11 and n = x = 12, respectively (Figure 2M-N). Female plants had distinctly and uniformly different chromosome numbers of 2n = 2x = 18 and 24, along with an 18-chromosome karyotype consisting of a 10m-chromosome, 6sm-chromosome and 2t-chromosome and a 24-chromosome karyotype consisting of an 8m-chromosome, 10sm-chromosome and 6t-chromosome, respectively. This was also the first record of chromosome-number differences in female plants (2n = 2x = 18 and 2n = 2x = 24) of *Z. muricata*. Two idiograms were observed in female haploid n = x = 9 and n = x = 12, respectively (Figure 2O-P, 2Q-R). No satellite chromosomes that could be related to sex were observed in either male or female plants of *Z. muricata*.

Z. pygmeae showed a chromosome number of 2n = 2x = 16 (Moretti, 1990; Caputo *et al*., 1996; Tagashira and Kondo, 1999, 2001; Sangduen *et al*., 2007) and the karyotype consisted of 12m-chromosome and 4 sm-chromosome (Moretti and Sabato, 1984; Moretti, 1990; Sangduen *et al*., 2007). Two satellite chromosomes on the 8^{th} chromosome were observed in both male and female plants of *Z. pygmeae* (Figure 3A-B, 3C-D).

Z. vazquezii showed a chromosome number of 2n = 2x = 18 (Moretti, 1990; Caputo *et al*., 1996; Tagashira and Kondo, 1999, 2001) and the karyotype consisted of 10m-chromosome, 2sm-chromosome and 6t-chromosome (Tagashira and Kondo, 1999, 2001). One satellite chromosome on the 4^{th} chromosome in male and no satellite chromosomes in female plants of *Z. vazquezii* were observed (Figure 3E-F, 3G-H).

Sex determination in *Zamia*

The difference in chromosomes related to sex determination in *Zamia* in this study could be categorized in terms of the number of satellite

Figure 2 Karyotypes and idiograms of five spp. of. *Zamia* (2n = 2x = 16 up to 24). A-B. *Z. herrerae* (male). C-D. *Z. herrerae* (female). E-F. *Z. integrifolia* (male). G-H. *Z. integrifolia* (female). I-J. *Z. monticola* (male). K-L . *Z. monticola* (female). M-N. *Z. muricata* (male). O-P. Z. *muricata* (female). Q-R. *Z. muricata* (female). S-T. *Z. pumila* (male). U-V. Z. *pumila* (female). (▬ = 10 µm).

▬ = secondary constriction ; • = satellite

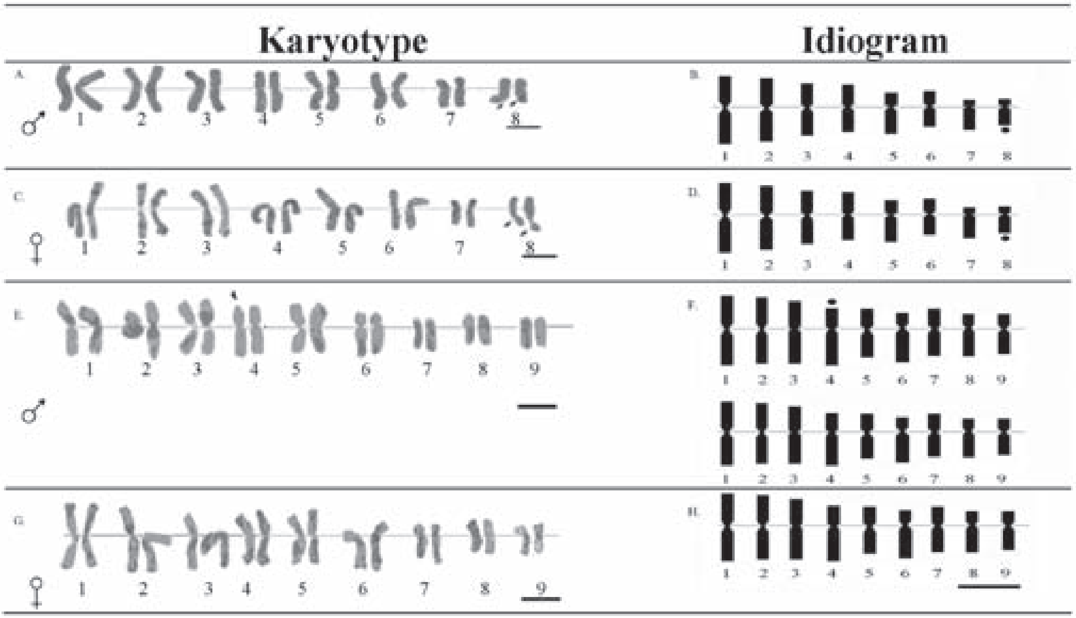

Figure 3 Karyotypes and idiograms of 2 spp. of *Zamia* (2n = 2x = 16 up to 18). A-B. *Z. pygmeae* (male). C-D . *Z. pygmeae* (female). E-F. *Z. vazquezii* (male). G-H. *Z. vazquezii* (female). (▬ = 10 μm).

chromosomes and the heteromorphic and homomorphic pattern as follows:

1. A satellite chromosome was not found in male and female plants e.g. *Z. integrifolia*, *Z. herrerae*, *Z. monticola* and *Z. muricata*. These four species of *Zamia* showed no chromosomal difference between male and female plants. Thus, other techniques need to be derived for more detailed chromosome banding such as C-banding, G-banding, fluorescence *in situ* hybridization (FISH), DNA markers and the MSAP technique.

2. A heteromorphic pattern of chromosomes in male and a homorphic pattern in female plants were observed in *Z. pumila* and *Z. vazquezii*.

3. A homomorphic pattern of chromosomes in both male and female plants was observed in *Z. pygmeae*. Male and female plants could not be differentiated in this species.

Zamia has previously been recorded as being heterogenome in chromosome number from 2n = 2x = 16 up to 24 and this was supported by the current study. The suborder *Zamiineae* shows the largest chromosome number range from 2n = 2x = 16 to 18 and also the only recorded intraspecific variation in chromosome number from 2n = 2x = 16 to 26 (Moretti and Sabato, 1984). A previous theory suggested that species of *Zamia* found in a uniform and fairly geologically stable environment had a chromosome number of 2n = 2x = 26, while species found in a wide range of environments had a chromosome number of 2n = 2x = 18 or more (Moretti *et al.*, 1991; Caputo *et al.*, 1996; Vovides and Olivares, 1996). Schutzman *et al.* (1988) reported that karyotypes with high chromosome numbers in *Zamia* were commonly found in inhospitable environments. Vovides and Olivares (1996) also postulated that the onset of drier ecological conditions could have exerted selective pressure, resulting in chromosomal change in the genus *Zamia*.

Thus, chromosomal evolution in the genus *Zamia* has been hypothesized to follow the process of Robertsonian events (Marchant, 1968; Moretti and Sabato, 1984; Moretti, 1990). The mechanisms of karyotype variation in *Zamia* might have occurred by centric fission (Marchant, 1968; Caputo *et al*., 1996), pericentric inversions and unequal translocation (Vovides and Olivares, 1996). However, whether chromosome number in *Zamia* has increased or decreased was difficult to determine since the progress of centric fission and fusion might occur over a timespan of karyotypic evolution (Jones, 1977).

CONCLUSION

The karyotype comparison of *Cycas* and *Zamia* was studied in immature still-folded leaflets of known sex plants. All five species of *Cycas* had a common chromosome number of 2n = 2x = 22 but varied in karyotype formulae. *Cycas clivicola* and *C. edentata* showed a chromosome number of 2n = 2x = 22 for the first time. Sex determination in male and female plants of *Cycas* appeared to be related to satellite chromosomes. All seven species of *Zamia* studied varied in chromosome number from 2n = 2x = 16 up to 2n = 2x = 24 as well as in karyotype formulae. *Zamia herrerae* and *Z. monticola* showed a chromosome number of 2n = 2x = 24 and 2n = 2x = 21, respectively, for the first time. Male and female plants were difficult to distinguish due to the lack of satellite chromosomes in both sexes.

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