



Chromosomal fission accounts for small-scale radiations in *Zamia* (Zamiaceae; Cycadales)

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Zamia is unique among Cycadales in its diversity of morphology, ecology and chromosome numbers. The chromosome numbers in *Zamia* range from 16 to 28, excluding 20, manifest as both interspecific and intraspecific series. It has long been recognized that Robertsonian transformations (chromosomal fission or fusion) probably dominate karyotype evolution in Zamiaceae, although it has been debated whether chromosome numbers are increasing or decreasing. We re-analyse published karyotypes of *Zamia* spp., relating both chromosome forms and sizes to recent phylogenetic data. We show that karyotype evolution is most probably moving towards increased asymmetry, with higher numbers of smaller chromosomes, thus supporting chromosomal fission. We also address additional hypotheses for increasing chromosome numbers, namely pericentric inversions and unequal translocations. Finally, we discuss the role of these chromosomal changes in evolutionary radiations. © 2011 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2011, **165**, 168–185.

ADDITIONAL KEYWORDS: centric fission – gymnosperms – karyotype evolution – linkage disequilibrium – phylogeny – plant diversity.

INTRODUCTION

Chromosomal fission (or centric fission; Robertson, 1916) involves the division of a single chromosome into two smaller chromosomes. The total number of chromosome arms and the amount of genomic material (*C*-value) stay the same, except for duplication of centromeres, associated kinetochore machinery and telomere sequences (Vandel, 1937; Tobias, 1953; Stebbins, 1971; Schubert *et al.*, 1992; Cox *et al.*, 1998; Jones, 1998; Kolnicki, 2000), whereas the number of chromosomes increases. This process can affect chromosomes individually, creating increasing aneuploid series, or the entire karyotype simultaneously, doubling the total chromosome complement (karyotypic fission; Todd, 1970; Kolnicki, 2000). The effects of chromosomal fission have been discussed extensively and include changes to segregation patterns and gene linkage (e.g. Robertson, 1916; Todd, 1970; Stebbins,

1971; Jones, 1977, 1998; Levin, 2002; Guerra, 2008; Raskina *et al.*, 2008).

Chromosomal fission is less recognizable in the evolutionary history of plants than of animals, its action perhaps obscured by chromosome size or whole genome duplication (Jones, 1998). To date, in plants, chromosomal fission is only recognized in flowering plants (e.g. Nordenskiöld, 1951; Marrero, 1992; Cox *et al.*, 1998), although it may have played a part in the macroevolutionary history of ferns (Soltis & Soltis, 1987; Gorelick & Olson, 2011), and has been cited numerous times for the clade containing *Zamia* L. and *Microcycas* A.DC. Morphologically and ecologically diverse throughout the Americas, Zamiaceae contains a substantial portion of the total species richness of the Cycadales (50–60 of the total 100–150 species, following conservative estimates suggested by Scotland & Worley, 2003, although others estimate *c.* 200 *Zamia* spp. among 300 total cycad species; Stevenson, 1992; Rai *et al.*, 2003; Hill, Stevenson & Osborne, 2004, 2007). Mature *Zamia* plants vary from diminutive to massive trees.

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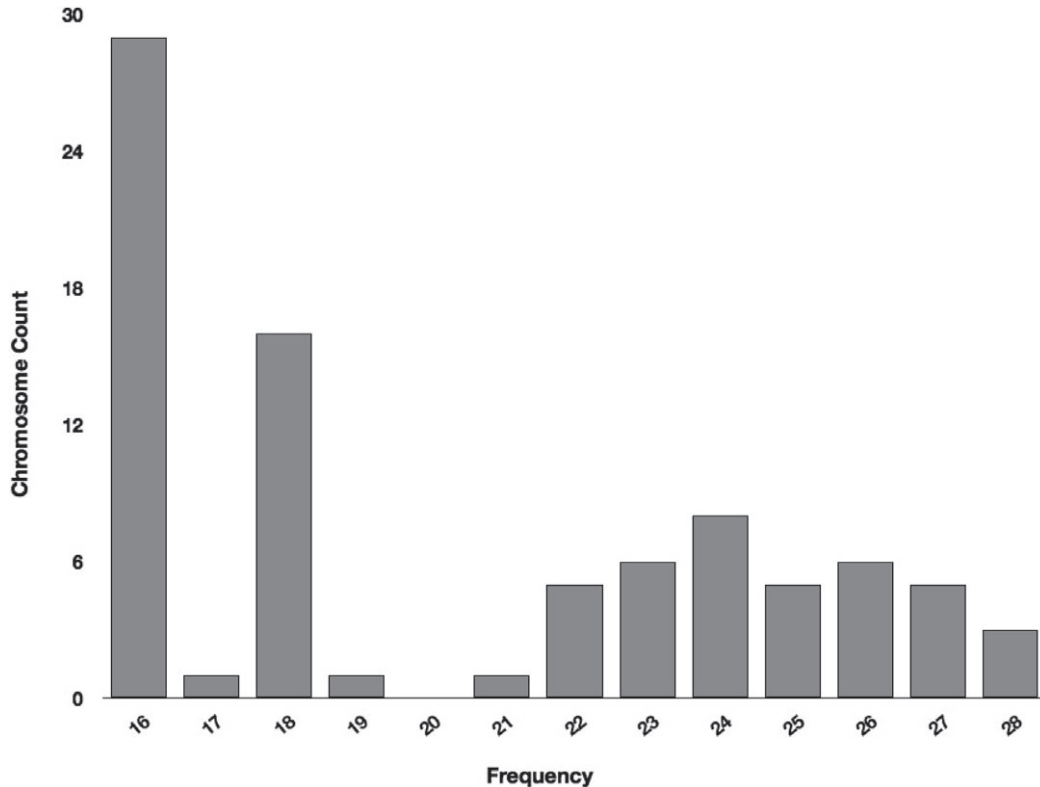


Figure 1. Frequency of chromosome counts of *Zamia* spp. from published records and personal communication. Note that many chromosome counts in the upper range are from the same species (e.g. *Z. prasina*, *Z. paucijuga*, *Z. roezlii*). Refer to Appendix 1 for chromosome numbers for each species examined.

Habitats range from coastal to montane, in both open and heavily forested environments. The genus even contains epiphytes (*Z. pseudoparasitica* Yates) and mangroves (*Z. nesophila* A.S.Taylor, J.L.Haynes & Holzman), which are unique in Cycadales. *Zamia* is unique among cycads in that both inter- and intraspecific chromosome numbers range from 16 to 28, excluding 20 (Moretti, Caputo & Cozzolino, 1993; Goldblatt & Johnson, 2006, 2008), with varied karyotype composition (refer to Figs 1, 2, Appendix 2). In cases of intraspecific karyotype differences in *Zamia*, a one-to-two relationship exists between changes in the numbers of metacentric and telocentric (including 'acrocentric') chromosomes. Losing one metacentric chromosome corresponds to gaining two telocentric chromosomes, or vice versa. This relationship can be explained equally well by chromosome fission or chromosome fusion, raising the question of karyotypic plesiomorphy in the genus. Here, we present a re-examination of published *Zamia* karyotypes in the light of recent molecular phylogenetic data, providing additional evidence for chromosomal fission in the evolution of *Zamia*. We also address the implications of chromosomal fission at both large and small evolutionary scales.

METHODS

We compiled a comprehensive collection of peer-reviewed articles containing chromosome images for *Zamia*, since Marchant (1968), in PDF format. We cross-referenced all articles for taxonomic revisions and used the most current taxonomy (refer to Appendix 1). Using Preview version 4.2 in Mac OS X, we created graphics interchange format (GIF) images at 300 pixels per inch of each chromosome representation. We gave order of precedence to karyograms, followed by karyotypes and idiograms, to ensure accuracy of measurement. Images were imported to OsiriX version 3.5 dicom viewer and analysed using the two-dimensional viewer and region of interest (ROI) length tool. Chromosome arms were measured to the primary constriction point. Lengths were recorded in pixels and converted to micrometres if scale bars were available. If scale bars were absent, we did not report chromosome sizes. We then divided the length of the long arm by the short arm to obtain the *r*-value (the ratio of the long to short arm), as described by Levan, Fredga & Sandberg (1964). We then classified the chromosomes into one of six categories based on the *r*-value, as outlined by Levan

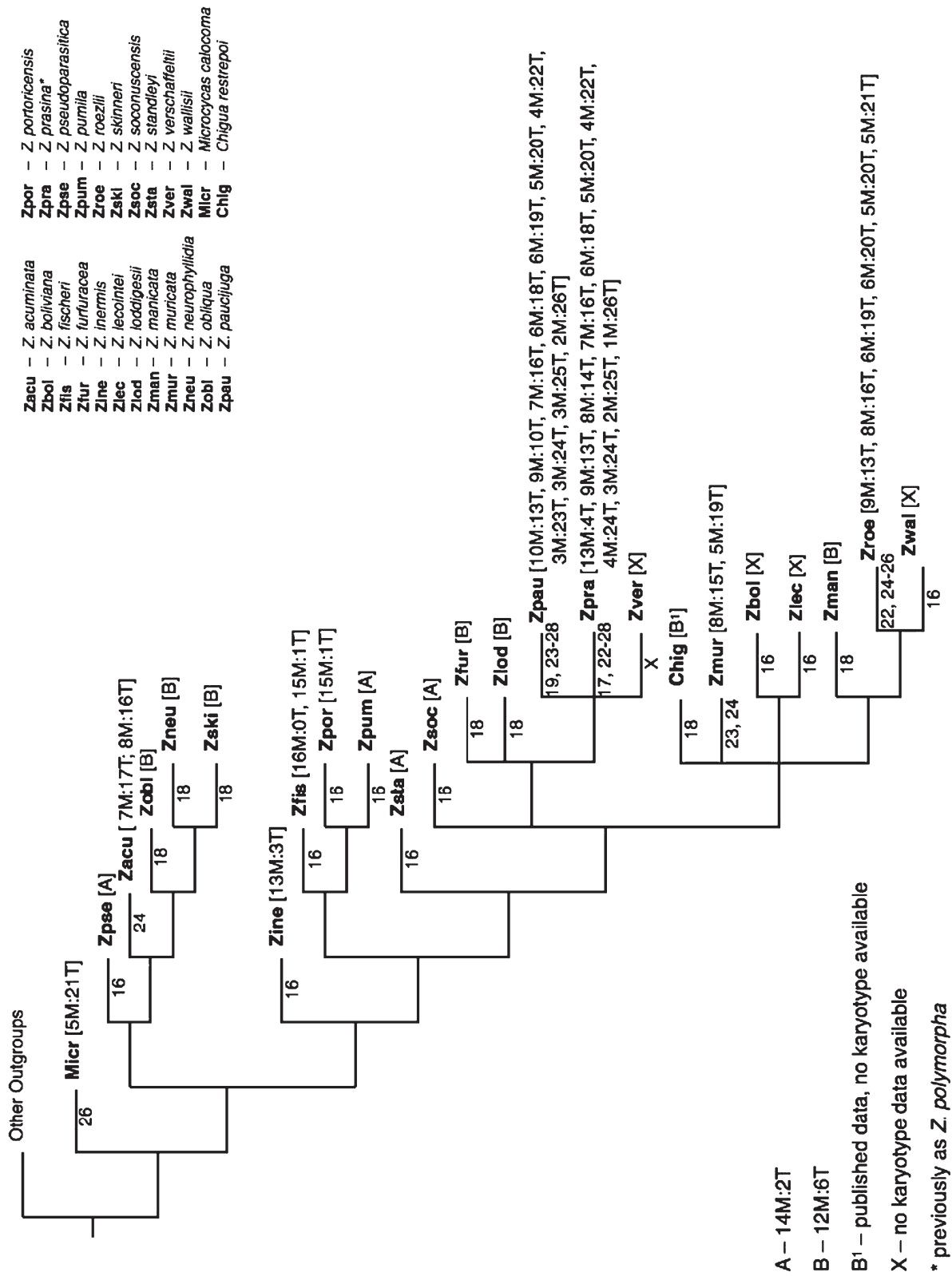


Figure 2. Molecular phylogenetic tree based on internal transcribed spacer 2 (ITS2) sequences (reproduced with permission from Caputo *et al.*, 2004), with superimposed chromosome numbers and karyotype formulae. Refer to Appendices 1 and 2 for citations and full chromosome counts.

Table 1. Chromosome classification scheme based on arm length ratios (*r*-values)

Term	Centromeric location	<i>r</i> value
M	Median point	1
m	Median region	> 1.0–1.7
sm	Submedian region	> 1.7–3.0
st	Subterminal region	> 3.0–7.0
t	Terminal region	> 7.0– ∞
T	Terminal point	∞

Categories based on Levan *et al.* (1964), modified by Walker (1985).

et al. (1964), but followed the modifications suggested by Walker (1985) to eliminate redundancy (refer to Table 1). The application of these definitions of chromosome forms allows for greater comparison across taxa in future studies and removes ambiguity associated with the term ‘acrocentric’ used in many of the papers cited here. Levan *et al.* (1964) cautioned against the category acrocentric because it is subject to individual interpretation and is insufficiently descriptive. The impetus for us measuring images of chromosomes in these publications, even where lengths or chromosome categories were included, was twofold. First, many authors of the karyotype source papers did not describe the methods used for the classification of chromosomes, and a consistent method of measurement and classification was necessary for all available chromosome images. Second, many authors held the number of submetacentric and acrocentric chromosomes constant in their analyses, citing the work of Lima de Faria (1983) regarding the heterochromatic nature of the short arms on some acrocentric chromosomes (Moretti, 1990; Moretti *et al.*, 1991; Caputo *et al.*, 1996; Napolitano, Caputo & Moretti, 2004). We recognize the validity of this decision, but nevertheless chose to report the chromosome measurements directly from the figures, ensuring consistency across publications. With regard to sample size, we note that all cited authors presented values for only one specimen or an average of all specimens, and so the sample sizes presented here are probably within the ranges of the original publications.

For clarity of presentation, we consolidated median point (M), median region (m) and submedian region (sm) chromosomes as ‘median/metacentric’ (‘M’) and subterminal region (st), terminal region (t) and terminal point (T) chromosomes as ‘terminal/telocentric’ (‘T’) when reporting ratios. The values for each category can be found in Appendices 2 and 3.

Chromosome numbers and consolidated karyotype values were superimposed on two recent phylogenetic

trees, one solely molecular (Caputo *et al.*, 2004; Fig. 2) and the other a combination of molecular and morphological data (Meerow *et al.*, 2007; Fig. 3), following published methods (Todd, 1970; Giusto & Margulis, 1981; Cox *et al.*, 1997; Kolnicki, 2000). Although these two phylogenetic trees are based on the same molecular data, together they provide greater species coverage. Appendix 4 addresses the correspondence between the idiograms and karyotypes published in Vovides & Olivares (1996).

RESULTS

We examined 83 published karyotypes of *Zamia* using the described methods, covering 35 species. Chromosome numbers for *Z. wallisii* A. Braun were included, although no karyotype was available for analysis. In addition, chromosome numbers for *Z. lacandona* Schutzman & Vovides and *Z. herrerae* S. Calderón & Standl. were kindly supplied by A. Vovides (Instituto de Ecología, A.C., Xalapa, personal communication). The most common chromosome numbers among *Zamia* spp. are 16 (20 of 38 species) and 18 (nine of 38 species; note that *Z. lacandona* has records of both numbers and is therefore included here only once), with little variation in chromosome form. Higher chromosome numbers were less common interspecifically, although certain taxa showed considerable intraspecific chromosome ranges, coupled with variation in chromosome form (e.g. *Z. prasina* W. Bull., *Z. paucijuga* Wieland, *Z. roezlii* Regel; refer to Fig. 3). Within taxa, ‘T’ chromosomes were consistently shorter than ‘M’ chromosomes. (Refer to Figs 2 and 3 for relationships between chromosome form and phylogeny.) Full chromosome form and size data can be found in Appendices 2 and 3.

DISCUSSION

REVISING VIEWS OF KARYOTYPIC EVOLUTION BASED ON PHYLOGENIES

Chromosomal fission and fusion are the two most regularly cited mechanisms of karyotypic change in *Zamiaceae*. Norstog (1980), like his predecessor Marchant (1968), believed that highly asymmetrical karyotypes dominated by telocentric chromosomes were ‘primitive’, progressing towards ‘chromosomal stability’ and lower chromosome numbers via chromosomal fusion. His hypothesis was largely based on work with *Z. roezlii* (as *Z. chigua* Seem.; Norstog, 1986). Based on its stable Colombian rainforest habitat and putatively primitive morphological characters, Norstog believed this species to be the most ‘basal’ in the genus, meaning that high chromosome numbers ($2n = 22, 24–26$) are plesiomorphic and chro-

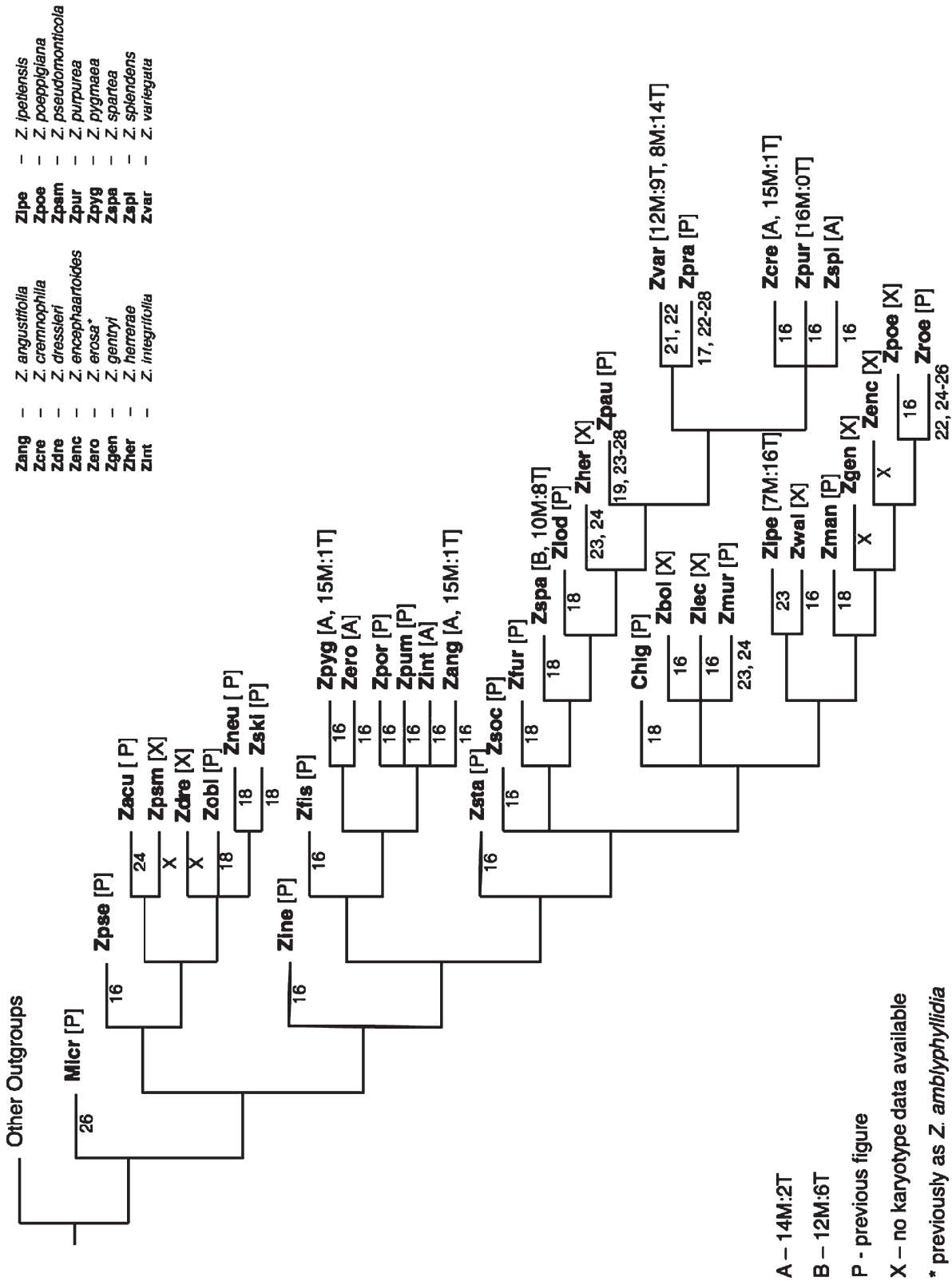


Figure 3. Combined molecular [based on internal transcribed spacer 2 (ITS2) sequences] and morphological phylogenetic tree (reproduced with permission from Meerow *et al.*, 2007), with superimposed chromosome numbers and karyotype formulae. Species' abbreviations and karyotype data for taxa included in the molecular analysis are detailed in Figure 2. Refer to Appendices 1 and 2 for citations and full chromosome counts.

mosome fusion produced the lower apomorphic chromosome numbers found in the genus. Norstog's hypothesis is regularly cited as a counterpoint to chromosome fission (Moretti & Sabato, 1984; Moretti, 1990; Moretti *et al.*, 1991, 1993; Caputo *et al.*, 1996; Kondo & Tagashira, 1998; Tagashira & Kondo, 1999, 2001; Napolitano *et al.*, 2004), but *Z. roezlii* is now thought to be among the most derived species in the genus.

Morphological cladistics combined with karyotype data were first used to challenge Norstog's fusion hypothesis, in part by re-examining his treatment of *Z. roezlii* (Caputo *et al.*, 1996). *Zamia* was divided into two major clades, the *inermis* clade and the *pseudoparasitica* clade. Contrary to Norstog (1980), *Z. roezlii* was ultimately placed among the most derived taxa within the *pseudoparasitica* clade. Recent molecular phylogenetic data support the cladistic work of Caputo *et al.* (1996), establishing *Z. roezlii* as one of the most derived taxa of the 24 analysed (Caputo *et al.*, 2004). The weight of Norstog's (1980) argument for the primacy of chromosome fusion in the karyotypic evolution of *Zamia* has been turned on its phylogenetic head.

When chromosome numbers and karyotype data are superimposed on the molecular phylogenetic tree (Fig. 2), most early diverging cycad taxa have $2n = 16$ or 18, with mostly metacentric and submetacentric chromosomes. These data strongly suggest that highly symmetrical karyotypes with low chromosome numbers are plesiomorphic in the genus, with more derived congeners possessing greater karyotypic asymmetry and higher chromosome numbers, as hypothesized by Khoshoo (1969) for cycads specifically and by Stebbins (1971) for plants in general. Chromosomal fission was supported as the mechanism increasing chromosome numbers by the noted structural instability of centromeres in taxa with higher chromosome numbers, which were easily broken during squashes (including *Z. roezlii*; Moretti, 1990; Caputo *et al.*, 1996).

CHROMOSOMAL REARRANGEMENTS OTHER THAN FISSION IN *ZAMIA*

Chromosomal fission is not the only proposed mechanism of karyotypic evolution from low to high chromosome numbers in *Zamia*. Fission is not expected to occur in isolation, and different mechanisms of karyotypic change are not mutually exclusive (Jones, 1998). Polysomy following pericentric inversions (Vovides & Olivares, 1996) or, to a lesser extent, unequal translocations may explain ascending interspecific and intraspecific chromosome series. Hybridization of individuals showing or not showing chromosome fission may also explain ascending interspecific and

intraspecific chromosome series (Caputo *et al.*, 1996; Napolitano *et al.*, 2004). As suggested by Matthey (1952) and Tobias (1953), counting the number of chromosome arms (i.e. the *nombre fondamentale*) can distinguish between fission events and other karyotypic changes. Chromosomal fission maintains the total number of chromosome arms, whereas pericentric inversion and/or hybridization of different karyotypes may either add or subtract from the total arm count (Stebbins, 1971). If chromosomal fission alone is responsible for karyotype evolution in *Zamia*, there should be no change in the number of chromosome arms as chromosome numbers increase. For example, if $2n = 16$ is taken as the ancestral chromosome number in the genus *Zamia* (with 32 chromosome arms), those species with $2n = 18$ would also have 32 arms. Pericentric inversions of the different combinations of telocentric chromosomes and/or hybridization were almost certainly involved in generating the chromosome arm numbers observed, a conclusion based on the size and number of chromosomes. Chromosome arm counts for all taxa in this study (refer to Appendix 2) suggest that chromosomal fission, pericentric inversion, hybridization of different karyotypes and a combination of these mechanisms occurred in the evolutionary history of this genus, although chromosomal fission must have generated the first diploid individual with an odd (instead of even) number of chromosomes (Caputo *et al.*, 1996).

POTENTIAL TESTS FOR CHROMOSOMAL REARRANGEMENTS

Molecular analysis has generated limited evidence for any chromosomal rearrangements, including chromosomal fission and pericentric inversion, as the main mechanism driving karyotypic evolution in *Zamia*. Interspecific karyotype comparison of telomere sequences (Kondo & Tagashira, 1998), fluorochrome staining (Tagashira & Kondo, 1999) and ribosomal DNA (rDNA) signals (Tagashira & Kondo, 2001) have all produced ambiguous results. Notably, strong telomere sequences of the *Arabidopsis* (DC.) Heynh. type were frequently found in centromeric and interstitial regions on *Zamia* chromosomes, whereas terminal signals were weak. These signals completely contrast with those in *Vicia faba* L., in which ancestral metacentric chromosomes showed the telomere sequence at the terminal ends of each arm (i.e. not in the centromeric position), whereas each derived telocentric chromosome showed signals at both ends (Schubert *et al.*, 1992). This finding caused Kondo & Tagashira (1998) to question whether *Arabidopsis*-type telomere sequences exist in cycads or whether they should be applied to interspecific chromosome differences. These molecular techniques may be more

applicable to the mechanisms underlying intraspecific chromosome series (Tagashira & Kondo, 2001).

Observations of meiotic behaviour would help to distinguish between various mechanisms of karyotype evolution (Moretti, 1990). Products of chromosome fission pair with derived homologues at meiosis (Todd, 1970; Caputo *et al.*, 1996), but pericentric inversion often inhibits complete synapsis (Sturtevant, 1917; Kolnicki, 2000). Recently, Cafasso *et al.* (2009) have shown that patterns of repetitive DNA sequences dispersed across the genomes of *Zamia* spp. correlate with many phylogenetic groups previously established in the literature, although different banding patterns are found among some closely related taxa. For example, the nine species in the *Zamia loddigesii* Miq. complex in Mexico (Vovides & Olivares, 1996; Stevenson, Moretti & Gaudio, 1998) show similar DNA sequence patterns [note that *Z. loddigesii* is now recognized as *Z. prasina*, for example, by Vovides & Olivares (1996)], whereas the banding pattern in *Z. neurophyllidia* D.W.Stev. differs from that of the other closely related basal Central American taxa (Cafasso *et al.*, 2009). Repeated sequences create multiple sequence homologies on chromosomes, which may cause alternative pairing arrangements and may, in turn, promote chromosomal fission. Unfortunately, meiotic behaviour is elusive in cycads (Moretti, 1990; Moretti *et al.*, 1991; Vovides & Olivares, 1996). *C*-values would provide great inferential distinctions between chromosomal fission and polysomy-related mechanisms. Duplicated centromeres and added telomere sequences would increase the *C*-value much less than would the duplication of entire chromosomes. There are only six cycad records in the *C*-value database (Ohri & Khoshoo, 1986; Leitch *et al.*, 2001; Murray, Leitch & Bennett, 2004). However, Cafasso *et al.* (2009) reported that all *Zamia* spp. analysed with both flow cytometry and quantitative dot blot analysis showed similar genomic DNA content to the standard *Z. angustifolia* Jacq., $1C = 12.05$ pg (D. Cafasso, Università degli Studi di Napoli Federico II, Naples, personal communication). Although Cafasso *et al.* (2009) have yet to publish the *C*-values, their results provide strong evidence for chromosomal fission in these taxa.

EVOLUTIONARY IMPLICATIONS OF CHROMOSOMAL FISSION IN *ZAMIA* AND CYCADALES AS A WHOLE

What does chromosomal fission mean in terms of evolutionary patterns in *Zamia*? The large size of chromosomes in all cycad taxa excluding Zamiaceae (Marchant, 1968) indicates that chromosomal fission has been rare or absent in these taxa. Even if chromosomal fission is common in *Zamia* and the lineage

including *Microcycas*, it is unlikely that this would result in substantial adaptive radiation. Effective population size is so small in these taxa that genetic drift should be much more important than selection (Gorelick, 2009; Gorelick & Olson, 2011) and, without selection, there can be no adaptation, let alone adaptive radiations. Lack of diversity in the cycad fossil record (Taylor, Taylor & Krings, 2009) is consistent with lack of adaptive radiations in cycads. From the perspective of explaining large-scale patterns of diversity for all cycads, chromosomal fission is therefore relatively unimportant compared with mechanisms in other taxa, e.g. whole-genome duplication (polyploidy) in flowering plants (Gorelick, 2001; Gorelick & Olson, 2011).

However, patterns at the microevolutionary scale in *Zamia* seem to be most parsimoniously explained by chromosomal fission. Taxa with inherently weak centromeres may have greater adaptive potential than their relatives with more stable centromeres. Chromosomal fission decreases genetic hitchhiking by severing transcentromeric linkages, allowing for direct selection on newly unlinked genes (Todd, 1970; Levin, 2000). High chromosome numbers in *Zamia* have been correlated with morphological variability and stressful or widely variable habitats (Moretti & Sabato, 1984; Moretti *et al.*, 1991; Caputo *et al.*, 1996; Vovides & Olivares, 1996; Napolitano *et al.*, 2004). Moretti & Sabato (1984) speculated that stressful habitats might cause chromosomal fission, allowing species to invade and survive in a wider habitat range than their relatives with lower chromosome numbers.

Ancient lineages, such as cycads, may not show correlation between derived karyotype and morphology in all taxa (Ehrendorfer, 1976). The discrepancies between stable habitat, primitive morphology and high chromosome number in *Z. roezlii* that drove Norstog's (1980) treatment still persist. Schutzman, Vovides & Dehgan (1988) suggested that these observable discrepancies may have their origins in palaeoclimatic conditions. Shifts in habitat stability over geological time may have affected the ancestors of extant *Zamia*. Continued research into historical climate shifts within the habitat range of *Zamia* may explain the origins of seemingly confounding taxa such as *Z. roezlii*.

Karyotype evolution can change course over evolutionary time (Jones, 1977, 1998). However, evidence from karyotypes, phylogenetics and phytogeography suggests that chromosomal fission is the main mechanism in *Zamia*, consistent with the predictions of the minimum interaction theory of karyotypic evolution (Imai *et al.*, 1986; Imai, Satta & Takahata, 2001). By reducing the effects of linkage disequilibrium, chromosomal fission is likely to account for the small-scale radiations in this group.

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APPENDIX 1

SUMMARY OF SPECIES INCLUDED IN THE ANALYSIS, INCLUDING LOCATION OF ORIGIN, ACCESSION NUMBERS AND CHROMOSOME NUMBERS

Species	Accession number†	Origin of plant	2n	Reference
<i>Z. acuminata</i> Oersted ex Dyer*	FTG76-612	Veraguas, Panama	24	a,b
	FTG89-163 or FTG 87-385	Panama or Costa Rica		
<i>Z. angustifolia</i> Jacq.	FTG75-261	Bahamas	16	a,c
	n/a	Eleuthera Island, Cuba		
<i>Z. chigua</i> Seemann*	FTG72-80	Rio Colima, Colombia	16	a1
<i>Z. cremnophila</i> Vovides, Schutzman & Dehgan*	FTG87-339A; FTG87-339B; FTGS87-33A; FTGS87-339B; FTG339C S-619, 622, 625 (XAL, FLAS)	Tabasco, Mexico Tabasco, Mexico	16	d,e
<i>Z. cunaria</i> Dressler & D.W.Stev.	NYDWS1635-A8; NYDWS1635-A114	Panama	23	b
<i>Z. cunaria</i> Dressler & D.W.Stev.	NYDWS1635-A9	Panama	24	b
<i>Z. erosa</i> O.F.Cook & G.N.Collins	NAP1038-0; NAP1044-0; NAP1083-0	Puerto Rico	16	d1
<i>Z. fischeri</i> Miq.*	n/a NAP537-2; NAP537-4; NY1601A; NY1601B; NY3940A; NY1603; NY1693C; NY1558	Mexico San Luis Potosi, Mexico	16	f,g
<i>Z. floridana</i> A.DC	n/a	?	16	f
<i>Z. furfuracea</i> L.f.*	FTG66-341A,B NAP501-2 n/a	Veracruz, Mexico Veracruz, Mexico Veracruz, Mexico	18	a,c,d
<i>Z. furfuracea</i> × <i>Z. spartea</i>	FTG80-164	Cultivated	18	h
<i>Z. herrerae</i> S.Calderón & Standl.			23	i
			24	i
<i>Z. imperialis</i> A.S.Taylor, J.L.Haynes & Holzman	FTG76-607	Santa Fe, Panama	22	a2
<i>Z. inermis</i> Vovides, J.D.Rees & Vásquez-Torres*	XAL79-049	Veracruz, Mexico	16	j
<i>Z. integrifolia</i> L.f.*	NAP499-3 n/a	Florida, USA ?	16	c,d
<i>Z. ipetiensis</i> D.W.Stev.*	NYDWS1159-A75; NYDWS1159-A76; NYDWS1159-A77; NYDWS1159; A78; NYDWS-A79; NYDWS1159-A80	Panama	23	b
<i>Z. lacandona</i> Schutzman & Vovides			16	k
			17	k
			18	k
<i>Z. lindenii</i> Regel ex André	FTG77-931	Rio Palenque, Ecuador	16	a
<i>Z. loddigesii</i> Miq.*	FTG69-565 NAP539-0; FTG60-465 n/a	Mexico Veracruz, Mexico ?	18	a,c,d
<i>Z. manicata</i> Linden ex Regel*	NAP186M; NAP199M; NAP204M; FTG84-272; FTG87-122	Colombia	18	b
<i>Z. muricata</i> Willd.*	n/a FTG77-1003	?	23	c
	FTG77-1024	Puerto Cabello, Venezuela	24	a
<i>Z. neurophyllidia</i> D.W.Stev.*	FTG77-1024	Rio Siquirres, Costa Rica	18	a3
<i>Z. obliqua</i> A.Braun	FTG76-608	Cabo Corriente, Colombia	18	a

APPENDIX 1 *Continued*

Species	Accession number†	Origin of plant	2n	Reference	
<i>Z. paucijuga</i> Wieland*	NAP928-74	Oaxaca, Mexico	19	h	
	NAP928/63	Oaxaca, Mexico	23	h,l	
	n/a	Punta Minizo, Oaxaca			
	NAP1014-0	Pinotepa Nacional, Oaxaca; Pochutla, Oaxaca	24	d	
	n/a	Punta Minizo, Oaxaca	25	h,l	
	NAP928-24	Oaxaca, Mexico			
	n/a	San Gabriel Mixtepec, Oaxaca	26	l	
	NAP928-M11	Nayarit, Mexico	27	h,l	
	n/a	Maria Cleofa Island, Nayarit			
	NAP928-M5	Nayarit, Mexico	28	h,l	
<i>Z. poeppigiana</i> Mart. & Eichler*	n/a	Miramar, Nayarit, Mexico			
	n/a	Ecuador, Peru	16	m	
<i>Z. portoricensis</i> Urb.*	FTG58-59	Cuba	16	a,d	
	NAP1098-0; NY 1621-87A	Puerto Rico			
<i>Z. prasina</i> W.Bull.	XAL84-265	Campeche, Mexico	17	n	
	FTG13-84A; FTG13-84B	Belize	22	g1,h1	
	NY1-89-11	Northern Belize			
	NY101-91	Northern Belize	23	h1	
	NAP1606-9	Quintana Roo, Mexico	24	h1,n	
	XAL75-005; XAL81.009.02; XAL84-262; XAL85-008.03	Tabasco, Mexico			
	NAP1606-10	Northern Belize	25	h1,n	
	XAL84-222; XAL80-178; XAL85-008.02	Campeche, Mexico			
	NYDW1138A; NYDWS1138B	Quintana Roo, Mexico	26	g1,h1,n	
	NAP2282-0	Campeche, Mexico			
	XAL84-263; XAL85-008.01	Yucatan, Mexico			
	FTG60-466	Yucatan, Mexico	27	a4,h1,n	
	NAP1606-M292	Central Belize			
	XAL84-264	Campeche, Mexico			
	NAP1606-17	Central Belize	28	h1	
	<i>Z. pseudoparasitica</i> Yates	FTG77-403	Veraguas, Panama	16	a
		<i>Z. pumila</i> L.*	NAP530-1; NAP530-4; NAP530-5;	Dominican Republic	16
NAP531-1; NAP1021-0;	Cuba				
NAP1023-0; NAP1045-0					
n/a					
<i>Z. purpurea</i> Vovides, J.D.Rees & M.Vázquez-Torres*	XAL78-639	Veracruz, Mexico	16	j	
	<i>Z. pygmaea</i> Sims	FTG58-869	Cuba	16	a,c,d
NAP534-0		Cuba			
n/a		Cuba			
<i>Z. roezlii</i> Regel*	FTG71-535M	Rio San Juan, Colombia	22	o	
	FTG73-118	Rio Anchicaya, Colombia	24	a5	
	FTG71-535	Rio San Juan, Colombia	25	a5,o	
	FTG71-535	Rio San Juan, Colombia			
	FTG76-610	Rio San Juan, Colombia	26	a5,o	
	FTG76-610	Rio San Juan, Colombia			
<i>Z. skinneri</i> Warsz.*	n/a	n/a	18	c	
<i>Z. soconuscensis</i> Schutzman, Vovides & Dehgan*	S-676, 678, 685, 694 (XAL, FLAS)	Chiapas, Mexico	16	e	

APPENDIX 1 *Continued*

Species	Accession number†	Origin of plant	2n	Reference
<i>Z. spartea</i> A.DC*	NAP986-0; FTG59-1063 XAL80-474	Oaxaca, Mexico Oaxaca, Mexico	18	d,j1
<i>Z. splendens</i> Schutzman*	FTG83-310A	Chiapas, Mexico	16	d
<i>Z. standleyi</i> Schutzman*	n/a FTG76-977	Honduras Olanchita, Honduras	16	a6,m
<i>Z. sylvatica</i> Chamberlain*	NAP997-0; NAP1060-0; FTG83-317	Oaxaca, Mexico	18	d
<i>Z. variegata</i> Warsz.	FTG87-96A; FTG87-96C; FTG87-96D	Chiapas, Mexico	21	g2
	NY685A; NY685B; FTG87-96B	Chiapas, Mexico	22	g2
<i>Z. vazquezii</i> D.W.Stev., Sabato & De Luca*	n/a NYVT3990; FTG87-111	Veracruz, Mexico Veracruz, Mexico	18	c,g3
<i>Z. wallisii</i> A.Braun*	n/a	Colombia	16	m
<i>Chigua restrepoi</i> D.W.Stev.		Colombia	18	p
<i>Microcycas calocoma</i> (Miq.) A.DC	NAP428-3	Cuba	26	d

^aNorstog (1980); ^{a1}as *Z. sp.* <helecho>; ^{a2}as *Z. skinneri* A.S.Taylor, J.L.Haynes & Holzman; ^{a3}as *Z. skinneri* Warsz. ex Dietrich.; ^{a4}first as *Z. loddigesii* Miq., then as *Z. polymorpha* D.W.Stev., Moretti & Vázquez-Torres; ^{a5}as *Z. chigua* Seem.; ^{a6}as *Z. tuerckheimii* Donn.Sm.; ^bCaputo *et al.* (1996); ^cTagashira & Kondo (1999); ^dMoretti (1990); ^{d1}as *Z. amblyphyllidia* D.W.Stev.; ^eSchutzman *et al.* (1988); ^fMarchant (1968); ^eMoretti *et al.* (1991); ^{e1}first as *Z. sp. nov.*, then as *Z. polymorpha* D.W.Stev., Moretti & Vázquez-Torres; ^{e2}as *Z. picta* Dyer; ^{e3}as *Z. fischeri* Miq.; ^hNapolitano *et al.* (2004); as *Z. polymorpha* D.W.Stev., Moretti & Vázquez-Torres; ⁱNicolalde-Morejón, Vovides & Stevenson (2009); ^jVovides (1983); ^{j1}as *Z. loddigesii* var. *angustifolia* J.Schust.; ^kAndrew Vovides, Instituto de Ecología, A.C., Xalapa, personal communication; ^lMoretti & Sabato (1984); ^mA. Moretti, unpublished data (in ^b); ⁿVovides & Olivares (1996); first as *Z. loddigesii* Miq., then as *Z. polymorpha* D.W.Stev., Moretti & Vázquez-Torres; ^oNorstog (1981) as *Z. chigua* Seem.; ^pMoretti *et al.* (1993); references consistent for Appendices 2 and 3.

*Genome content analysed in Cafasso *et al.* (2009).

†Place of cultivation abbreviations: FTG, Fairchild Tropical Garden, FL, USA; NAP, Botanical Garden of Naples, Italy; NY, New York Botanical Garden, New York, NY, USA; XAL, INIREB Herbarium, Xalapa, Mexico.

APPENDIX 2

COUNTS OF TOTAL CHROMOSOMES, INDIVIDUAL CHROMOSOME TYPES AND TOTAL CHROMOSOME ARMS OF
ZAMIA SPP. ANALYSED

Species	2n	Karyotype						Total arms	Reference
		m	sm	st	t	T	M : T		
<i>Z. angustifolia</i>	16	12	2	2			14 : 2	32	a
<i>Z. angustifolia</i>	16	13	2	1			15 : 1	32	c
<i>Z. chigua</i>	16	9	5	1		1	14 : 2	31	a1
<i>Z. cremnophila</i>	16	12	2	1	1		14 : 2	32	e
<i>Z. cremnophila</i>	16	12	3	1			15 : 1	32	d
<i>Z. erosa</i>	16	9	5	2			14 : 2	32	d1
<i>Z. fischeri</i>	16	12	4				16 : 0	32	f
<i>Z. fischeri</i>	16	12	3	1			15 : 1	32	g
<i>Z. floridana</i> (a)	16	12	4				16 : 0	32	f
<i>Z. floridana</i> (b)	16	12	4				16 : 0	32	f
<i>Z. inermis</i>	16	12	1	3			13 : 3	32	j
<i>Z. integrifolia</i>	16	11	3	2			14 : 2	32	c
<i>Z. integrifolia</i>	16	12	2	2			14 : 2	32	d
<i>Z. lacandona</i>	16								k
<i>Z. lindenii</i>	16	11	3			2	14 : 2	30	a
<i>Z. poeppigiana</i>	16								m
<i>Z. portoricensis</i>	16	12	3	1			15 : 1	32	d
<i>Z. portoricensis</i>	16	13	2	1			15 : 1	32	a
<i>Z. pseudoparasitica</i>	16	9	5			2	14 : 2	30	a
<i>Z. pumila</i>	16	13	1	2			14 : 2	32	d
<i>Z. pumila</i>	16	12	2	2			14 : 2	32	c
<i>Z. purpurea</i>	16	12	2		2		14 : 2	32	j
<i>Z. pygmaea</i>	16	10	5			1	15 : 1	31	a
<i>Z. pygmaea</i>	16	12	3	1			15 : 1	32	c
<i>Z. pygmaea</i>	16	13	1	2			14 : 2	32	d
<i>Z. soconuscensis</i>	16	12	2	2			14 : 2	32	e
<i>Z. splendens</i>	16	8	6	2			14 : 2	32	d
<i>Z. standleyi</i>	16	12	2			2	14 : 2	30	a6
<i>Z. standleyi</i>	16								m
<i>Z. wallisii</i>	16								m
<i>Z. lacandona</i>	17								k
<i>Z. prasina</i>	17	12	1	1	1	2	13 : 4	32	n
<i>Z. furfuracea</i>	18	11	1			6	12 : 6	30	a
<i>Z. furfuracea</i>	18	10	2	3		3	12 : 6	33	d
<i>Z. furfuracea</i>	18	10	2	1		5	12 : 6	31	c
<i>Z. furfuracea</i> × <i>Z. spartea</i>	18	10	2	4		2	12 : 6	36	h
<i>Z. lacandona</i>	18								k
<i>Z. loddigesii</i>	18	10	2			6	12 : 6	30	d
<i>Z. loddigesii</i>	18	9	3		1	5	12 : 6	30	a
<i>Z. loddigesii</i>	18	9	3	2	2	2	12 : 6	34	c
<i>Z. manicata</i>	18	11	1	2		4	12 : 6	32	b
<i>Z. neurophyllidia</i>	18	6	6	1		5	12 : 6	31	a3
<i>Z. obliqua</i>	18	9	3	2		4	12 : 6	32	a
<i>Z. skinneri</i>	18	11	1	3		3	12 : 6	33	c
<i>Z. spartea</i>	18	10		5	3		10 : 8	36	j1
<i>Z. spartea</i>	18	11	1	3	1	2	12 : 6	34	d
<i>Z. sylvatica</i>	18	10	2	5	1		12 : 6	36	d
<i>Z. vazquezii</i>	18	10	1	1		6	11 : 7	30	c
<i>Z. vazquezii</i>	18	10	2			6	12 : 6	30	g3

APPENDIX 2 *Continued*

Species	2n	Karyotype						Total arms	Reference
		m	sm	st	t	T	M : T		
<i>Z. paucijuga</i>	19	3	6	4	1	5	9 : 10	33	h
<i>Z. variegata</i>	21	9	3	4		5	12 : 9	37	g2
<i>Z. imperialis</i>	22	6	3	3		10	9 : 13	34	a2
<i>Z. prasina</i>	22	6	2	1	2	11	8 : 14	33	h1
<i>Z. prasina</i>	22	6	3	3	2	8	9 : 13	36	g1
<i>Z. roezlii</i>	22	7	2	3	1	9	9 : 13	35	o
<i>Z. variegata</i>	22	6	2	4	4	6	8 : 14	38	g2
<i>Z. cunaria</i>	23	4	3	1	2	13	7 : 16	33	b
<i>Z. herrerae</i>	23								i
<i>Z. ipetiensis</i>	23	3	4	1	1	14	7 : 16	32	b
<i>Z. muricata</i>	23	7	1	3	3	9	8 : 15	37	c
<i>Z. paucijuga</i>	23	5	5	5		8	10 : 13	38	l
<i>Z. paucijuga</i>	23	5	2	5	1	10	7 : 16	36	h
<i>Z. prasina</i>	23	5	2	3	3	10	7 : 16	36	h1
<i>Z. acuminata</i>	24	4	4	1		15	8 : 16	33	a
<i>Z. acuminata</i>	24	3	4	9		8	7 : 17	40	b
<i>Z. cunaria</i>	24	1	5	4		14	6 : 18	34	b
<i>Z. herrerae</i>	24								i
<i>Z. muricata</i>	24	5		6		13	5 : 19	35	a
<i>Z. paucijuga</i>	24	4	2	3		15	6 : 18	33	d
<i>Z. prasina</i>	24	4	2	2		16	6 : 18	32	h1
<i>Z. prasina</i>	24	2	4	2		16	6 : 18	32	n
<i>Z. roezlii</i>	24	3	5			16	8 : 16	32	a5
<i>Z. paucijuga</i>	25	4	2	4	2	13	6 : 19	37	l
<i>Z. paucijuga</i>	25	3	2	6	2	12	5 : 20	38	h
<i>Z. prasina</i>	25	3	2	4		16	5 : 20	34	h1
<i>Z. roezlii</i>	25	1	5	2	2	15	6 : 19	35	a5
<i>Z. roezlii</i>	25	3	2	1	2	17	5 : 20	33	o
<i>Z. paucijuga</i>	26	2	1	7		16	3 : 23	36	l
<i>Z. prasina</i>	26	2	2	4	2	16	4 : 22	36	h1
<i>Z. prasina</i>	26	2	2	1	1	20	4 : 22	32	n
<i>Z. prasina</i>	26	2	2	8	5	9	4 : 22	43	g1
<i>Z. roezlii</i>	26	3	2	4		17	5 : 21	35	a5
<i>Z. roezlii</i>	26	3	3	1		19	6 : 20	33	o
<i>Z. paucijuga</i>	27	1	2	6		18	3 : 24	36	l
<i>Z. paucijuga</i>	27	1	2	4	2	18	3 : 24	36	h
<i>Z. prasina</i>	27	1	2	4		20	3 : 24	34	h1
<i>Z. prasina</i>	27	1	1	6		19	2 : 25	35	a4
<i>Z. prasina</i>	27		1	5		21	1 : 26	33	n
<i>Z. paucijuga</i>	28		2	4	4	18	2 : 26	38	l
<i>Z. paucijuga</i>	28		3	7		18	3 : 25	38	h
<i>Z. prasina</i>	28		4	3		21	4 : 24	35	h1
<i>Chigua restrepoi</i>	18						12 : 6		p
<i>Microcyas calocoma</i>	26	2	3	7	4	10	5 : 21	42	d

See Appendix 1 for references.

APPENDIX 3

MEAN CHROMOSOME LENGTHS (μm) FOR INDIVIDUAL CHROMOSOME AND CONSOLIDATED TYPES

Species	2n	Mean Chromosome Size (μm)						Reference
		m	sm	st	t	T	M : T	
<i>Z. angustifolia</i>	16	10.937	7.960	6.976			9.449 : 6.976	a
<i>Z. angustifolia</i>	16	12.991	8.386	8.153			10.689 : 8.153	c
<i>Z. chigua</i>	16	10.646	10.671	8.408		6.923	10.659 : 7.666	a1
<i>Z. cremnophila</i>	16	15.050	10.774	8.921	7.415		12.912 : 8.168	d
<i>Z. cremnophila</i>	16	12.113	8.575	6.912			10.344 : 6.912	e
<i>Z. erosa</i>	16	15.391	13.774	10.569			14.583 : 10.569	d1
<i>Z. fischeri</i>	16							f
<i>Z. fischeri</i>	16	28.668	22.030	18.574			25.349 : 18.574	g
<i>Z. floridana</i> (a)	16							f
<i>Z. floridana</i> (b)	16							f
<i>Z. inermis</i>	16	14.333	12.115	9.515			13.224 : 9.515	i
<i>Z. integrifolia</i>	16	14.941	11.177	9.471			13.059 : 9.471	c
<i>Z. integrifolia</i>	16	17.675	13.049	10.658			15.362 : 10.658	d
<i>Z. lacandona</i>	16							k
<i>Z. lindenii</i>	16	10.426	8.987			4.185	9.706 : 4.185	a
<i>Z. poeppigiana</i>	16							m
<i>Z. portoricensis</i>	16	15.554	9.807	10.413			12.680 : 10.413	d
<i>Z. portoricensis</i>	16	14.562	9.153	11.190			11.858 : 11.190	a
<i>Z. pseudoparasitica</i>	16	10.717	8.789			6.164	9.753 : 6.164	a
<i>Z. pumila</i>	16	15.427	8.906	10.205			12.166 : 10.205	d
<i>Z. pumila</i>	16	13.624	7.374	8.781			10.499 : 8.781	c
<i>Z. purpurea</i>	16	17.369	11.854		11.423		14.611 : 11.423	j
<i>Z. pygmaea</i>	16	14.409	10.657			10.708	12.533 : 10.708	a
<i>Z. pygmaea</i>	16	19.163	11.349	11.617			15.256 : 11.617	c
<i>Z. pygmaea</i>	16	13.944	9.686	9.491			11.815 : 9.491	d
<i>Z. soconuscensis</i>	16	13.223	12.994	8.556			13.108 : 8.556	e
<i>Z. splendens</i>	16	16.104	15.062	13.029			15.583 : 13.029	d
<i>Z. standleyi</i>	16	10.855	9.530			6.609	10.192 : 6.609	a4
<i>Z. standleyi</i>	16							m
<i>Z. wallisii</i>	16							m
<i>Z. lacandona</i>	17							k
<i>Z. prasina</i>	17	11.042	7.872	5.455	5.732	5.579	9.457 : 5.589	n
<i>Z. furfuracea</i>	18	11.413	8.925			7.718	10.169 : 7.718	a
<i>Z. furfuracea</i>	18	17.630	14.199	12.702		12.422	15.914 : 12.562	d
<i>Z. furfuracea</i>	18	14.920	10.908	11.150		9.011	12.914 : 10.080	c
<i>Z. furfuracea</i> x <i>Z. spartea</i>	18	7.641	5.989	4.997		4.728	6.815 : 4.863	h
<i>Z. lacandona</i>	18							k
<i>Z. loddigesii</i>	18	13.840	10.336			9.225	12.088 : 9.225	d
<i>Z. loddigesii</i>	18	11.282	9.917		6.912	7.835	10.600 : 7.374	a
<i>Z. loddigesii</i>	18	13.576	12.322	8.327	8.719	9.385	12.949 : 8.810	c
<i>Z. manicata</i>	18	7.143	5.661	5.197		4.441	6.402 : 4.819	b
<i>Z. neurophyllidia</i>	18	11.785	11.949	11.877		8.242	11.867 : 10.060	a3
<i>Z. obliqua</i>	18	11.338	10.496	7.424		7.237	10.917 : 7.330	a
<i>Z. skinneri</i>	18	17.663	15.181	9.661		10.671	16.422 : 10.166	d
<i>Z. spartea</i>	18	16.416		11.81	11.389		16.416 : 11.600	j1
<i>Z. spartea</i>	18	29.944	25.479	20.349	19.670	16.851	27.712 : 18.957	d
<i>Z. sylvatica</i>	18	29.721	21.096	19.353	18.784		25.408 : 19.068	d
<i>Z. vazquezii</i>	18	17.104	14.158	11.431		10.845	15.631 : 11.138	c
<i>Z. vazquezii</i>	18	24.938	20.042			15.040	22.490 : 15.040	g3
<i>Z. paucijuga</i>	19	13.441	10.652	11.981	6.066	8.266	12.047 : 8.771	h

APPENDIX 3 *Continued*

Species	2n	Mean Chromosome Size (μm)						Reference
		m	sm	st	t	T	M : T	
<i>Z. variegata</i>	21	10.066	7.650	6.522		6.399	8.858 : 6.460	g2
<i>Z. imperialis</i>	22	14.593	12.032	7.761		7.501	13.312 : 7.631	a2
<i>Z. prasina</i>	22	9.392	7.221	6.033	7.269	6.062	8.307 : 6.455	h1
<i>Z. prasina</i>	22	18.794	14.291	13.418	12.400	12.548	16.543 : 12.789	g1
<i>Z. roezlii</i>	22	12.219	11.383	8.487	8.138	7.973	11.801 : 8.199	o
<i>Z. variegata</i>	22	9.975	6.370	4.832	5.441	5.386	8.173 : 5.220	g2
<i>Z. cunaria</i>	23	12.287	10.710	8.770	6.553	6.458	11.499 : 7.260	b
<i>Z. herrerae</i>	23							i
<i>Z. ipetiensis</i>	23	15.457	11.495	7.793	7.503	7.569	13.476 : 7.621	b
<i>Z. muricata</i>	23	13.798	10.088	8.702	9.616	10.371	11.943 : 9.563	c
<i>Z. paucijuga</i>	23							l
<i>Z. paucijuga</i>	23	13.639	9.123	6.751	7.604	7.133	11.381 : 7.163	h
<i>Z. prasina</i>	23	8.992	6.061	4.879	4.189	5.199	7.527 : 4.756	h1
<i>Z. acuminata</i>	24	13.990	8.700	7.648		8.439	11.345 : 8.043	a
<i>Z. acuminata</i>	24	14.187	11.201	8.420		9.226	12.694 : 8.823	b
<i>Z. cunaria</i>	24	20.186	17.281	11.608		10.290	18.734 : 10.949	b
<i>Z. herrerae</i>	24							j
<i>Z. muricata</i>	24	11.400		8.888		9.139	11.400 : 9.013	a
<i>Z. paucijuga</i>	24	27.319	17.934	13.737		15.686	22.626 : 14.712	d
<i>Z. prasina</i>	24	10.301	7.498	5.745		5.914	8.900 : 5.829	h1
<i>Z. prasina</i>	24	12.647	10.800	9.023		6.585	11.724 : 7.804	n
<i>Z. roezlii</i>	24	9.865	8.704			6.542	9.284 : 6.542	a5
<i>Z. paucijuga</i>	25							l
<i>Z. paucijuga</i>	25	10.500	8.537	5.968	7.025	6.136	9.518 : 6.376	h
<i>Z. prasina</i>	25	12.640	8.298	6.989		7.160	10.469 : 7.074	h1
<i>Z. roezlii</i>	25	7.864	8.249	7.620	5.900	5.766	8.056 : 6.429	a5
<i>Z. roezlii</i>	25	11.548	11.588	8.240	7.651	7.088	11.568 : 7.660	o
<i>Z. paucijuga</i>	26							l
<i>Z. prasina</i>	26	12.361	8.923	6.164	7.504	7.625	10.642 : 7.098	h1
<i>Z. prasina</i>	26	9.548	6.388	4.774	4.241	5.183	7.968 : 4.733	n
<i>Z. prasina</i>	26	17.884	12.941	10.708	9.878	10.560	15.413 : 10.382	g1
<i>Z. roezlii</i>	26	9.247	9.847	6.935		7.087	9.547 : 7.011	a5
<i>Z. roezlii</i>	26	9.451	9.303	7.580		6.870	9.377 : 7.225	o
<i>Z. paucijuga</i>	27							l
<i>Z. paucijuga</i>	27	15.717	12.778	7.646	8.521	8.574	14.248 : 8.247	h
<i>Z. prasina</i>	27	7.776	8.663	6.844		7.374	8.220 : 7.109	h1
<i>Z. prasina</i>	27	13.501	8.471	5.926		6.857	10.986 : 6.391	a4
<i>Z. prasina</i>	27		10.698	7.879		7.951	10.698 : 7.915	n
<i>Z. paucijuga</i>	28							l
<i>Z. paucijuga</i>	28		10.212	8.118		8.660	10.212 : 8.389	h
<i>Z. prasina</i>	28		6.145	5.782		6.140	6.145 : 5.961	h1
<i>Chigua restrepoi</i>	18							p
<i>Microcycas calocoma</i>	26	21.159	14.435	12.199	11.931	11.839	17.797 : 11.990	d

See Appendix 1 for references.

APPENDIX 4

RECOMMENDATIONS FOR IDIOGRAMS IN VOVIDES & OLIVARES (1996)

After reviewing the karyotypes and idiograms of *Z. prasina* and *Z. polymorpha* (first as *Z. loddigesii*, then as *Z. polymorpha*) presented by Vovides & Olivares (1996), we recommend that only the karyotypes be used in future analyses. The chromosome counts and the mean chromosome sizes are quite different between the two chromosome presentations (refer to Appendices 4a and 4b). On discussion with Vovides, we agree that the small size of both the median region (m) and submedian region (sm) chromosomes in the idiograms is uncharacteristic of *Zamia* chromosome forms presented in both this and all other publications. The mean chromosome sizes in the idiograms are consistently smaller than those in the karyotypes. It is possible that the small chromosomes presented in the idiograms resulted from pericentric inversion or unequal translocation but, at this point, we prefer to focus on the chromosomes in karyotypes.

APPENDIX 4A. COUNTS OF CHROMOSOME TYPES REPORTED IN KARYOTYPES AND IDIOGRAMS FOR *ZAMIA PRASINA* IN VOVIDES & OLIVARES (1996)

Locality	Type	2n	Chromosome count								Total arms
			M	m	sm	st	t	T	M:T		
Champoton, Campeche	Karyotype	17		12	1	1	1	2	13:4	32	
Champoton, Campeche	Idiogram	17		9	4	1	1	2	13:4	32	
n/a	Karyotype	24		2	4	2		16	6:18	32	
Balacan, Tabasco	Idiogram	24		4	4	7	2	7	8:16	41	
Oxcutzcab, Yucatan	Idiogram	24		6	1	3		14	7:17	34	
Xuilib, Yucatan	Idiogram	24		5	1	3		15	6:18	33	
Valladolid, Yucatan	Idiogram	24		7	3	3	1	10	10:14	38	
n/a	Karyotype*	25							n/a	n/a	
Campeche, Campeche	Idiogram	25		4		6		15	4:21	35	
Between Chetumal and Cancun, Quintana Roo	Idiogram	25	1	6	4	5		9	11:14	41	
Xuilib, Yucatan	Idiogram	25		3	1	1	6	14	4:21	36	
n/a	Karyotype	26		2	2	1	1	20	4:22	32	
Valladolid, Yucatan	Idiogram	26		9	1	3	2	11	10:16	41	
Xuilib, Yucatan	Idiogram	26		3		4	4	15	3:23	37	
Champoton, Campeche	Karyotype	27			1	5		21	1:26	33	
Champoton, Campeche	Idiogram	27		4	1	3		19	5:22	35	

*Karyotype of $2n = 25$ is too overlapped for measurements.

APPENDIX 4B. MEAN CHROMOSOME SIZES REPORTED IN KARYOTYPES AND IDIOGRAMS FOR *ZAMIA POLYMORPHA* IN VOVIDES & OLIVARES (1996)

Locality	Type	2n	Mean chromosome size (µm)						
			M	m	sm	st	t	T	M : T
Champoton, Campeche	Karyotype	17		11.38	10.30	5.46	5.73	5.79	10.84 : 5.66
Champoton, Campeche	Idiogram	17		9.76	7.44	10.01	6.19	4.91	8.60 : 7.04
n/a	Karyotype	24			11.54	9.02		6.59	11.54 : 7.80
Balacan, Tabasco	Idiogram	24			3.56	3.24	3.89	3.32	3.56 : 3.48
Oxcutzcab, Yucatan	Idiogram	24		5.77	5.88	2.52		3.50	5.83 : 3.01
Xuilib, Yucatan	Idiogram	24		3.31	2.42	2.31		1.98	2.87 : 2.15
Valladolid, Yucatan	Idiogram	24		6.92	4.61	4.44	3.99	3.85	5.77 : 4.09
n/a	Karyotype*	25		10.40	8.47			7.90	9.43 : 7.90
Campeche, Campeche	Idiogram	25		2.83	5.12	2.48		1.99	3.97 : 2.23
Between Chetumal and Cancun, Quintana Roo	Idiogram	25	1.65	4.59	3.42	1.75		2.69	3.22 : 2.22
Xuilib, Yucatan	Idiogram	25			5.54	2.09	3.75	3.80	5.54 : 3.21
n/a	Karyotype	26		9.43	7.48	4.77	4.24	5.18	8.46 : 4.73
Valladolid, Yucatan	Idiogram	26		4.92	4.84	5.51	4.25	4.04	4.88 : 4.60
Xuilib, Yucatan	Idiogram	26		4.41	8.01	4.16	4.75	4.10	6.21 : 4.34
Champoton, Campeche	Karyotype	27			10.70	7.88		7.95	10.70 : 7.92
Champoton, Campeche	Idiogram	27		2.90	3.36	2.48		1.79	3.13 : 2.14

*Mean values for clear chromosomes of karyotype $2n = 25$ (1m, 2sm and 3T) are included solely for comparison with idiogram values.