

## *Zamia grijalvensis* sp. nov. (Zamiaceae, Cycadales) from Chiapas, Mexico with notes on hybridization and karyology

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*Zamia grijalvensis*, a new cycad species from Chiapas, México, is described and illustrated. This species is similar to *Z. lacandona* with respect to leaves, but differs in habit, morphology and colour of the female and male cones. The female cone of *Z. grijalvensis* is reddish–brown to orange–brown, whereas that of *Z. lacandona* is dark brown with lighter beige tomentum. The male cone of *Z. grijalvensis* is erect, whereas that of *Z. lacandona* is decumbent. The new species may be of natural hybrid origin and is discussed in the light of other findings among cycads. Preliminary diploid chromosome counts for *Z. grijalvensis*  $2n = 19, 20$  and karyotypes appear variable, as previously found in its congeners with wide distribution, such as *Z. paucijuga* and *Z. prasina*, both of which are characterised by high diploid numbers with a high number of telocentric and few metacentric chromosomes. These changes in chromosome number are probably the result of stressful environmental factors.

The concept of Meso-America used here is that of Flora Mesoamericana (Missouri Botanical Garden) that covers the south easternmost states of Mexico including the Yucatan Peninsula and all of the Central American republics. *Zamia* is a neotropical cycad genus with the greatest distribution of all American cycads ranging from southeastern USA, the Greater Antilles, Mexico and Central America to Bolivia. *Zamia* is the most ecologically and morphologically diverse cycad genus (Norstog and Nicholls 1997) with about 69 known species (Osborne et al. 2012). In Meso-America alone there are about 37 described species, 16 of which are known from Mexico. Mexico is considered a centre of diversity for neotropical cycads (ca 54 spp.), harbouring about 24% of all cycad species worldwide and over 90% are endemic. Mexico has the second highest cycad diversity worldwide after Australia (Vovides et al. 2007).

During botanical explorations and ecological impact studies for a hydroelectric project, we collected an unknown *Zamia* in the middle part of the Grijalva river basin. Living material was collected and held at the National Cycad Collection of the Francisco Javier Clavijero Botanic Garden (JBC) in Xalapa, and at the Univ. de Ciencias y Artes de Chiapas (UNICACH). After observations in nature, and on specimens in cultivation at the JBC for over three years, no morphological changes due to phenotypic plasticity were noted. This permitted us to consider it new to science.

The coriaceous leaflet texture and subfalcate to falcate, bright green leaflet morphology presented by the new species prompted us to include it within the *Zamia splendens*-group as defined by Schutzman and Vovides (1998). This includes *Z. splendens* Schutzman (now *Z. katzeriana* (Regel) Rettig, referred to here as the *Z. katzeriana*-group), *Z. cremnophila* Vovides, Schutzman & Dehgan, *Z. standleyi* Schutzman, *Z. lacandona* Schutzman & Vovides and *Z. purpurea* Vovides, Rees & Vázquez-Torres. All these species bear stiff, coriaceous, dark to light green toothed leaflets that range in shape from linear, lanceolate, oblanceolate, elliptical, long elliptical, to falcate.

### Chromosome studies

Root tip mitosis was obtained according to the technique of Vovides (1983) but using a 0.2% aqueous solution of colchicine and a pretreatment of 8 h at room temperature. Two individuals from the type locality held in the National Cycad Collection of the JBC under accession numbers 2009-040A and 2009-018A were examined and three good metaphase cells from each were used for chromosome counts. In these, counts of  $2n = 19$  and  $20$  were obtained respectively (Fig. 3). Photomicrographs were taken by a Canon digital camera fitted to a Zeiss Photomicroscope (Fomi III) fitted with  $\times 63$  planapochromatic objective and phase contrast optics.

***Zamia grijalvensis* Pérez-Farrera, Vovides & Martínez-Camilo sp. nov. (Fig. 1, 2)**

*Zamia lacandonae* affinis, a qua foliis magis numerosis (14–29 paribus vs 7–12 paribus), strobilis microsporangiatibus erectiusculis (vs. decumbentibus), strobilis megasporangiatibus atrorubello-brunneis (vs. spadiceis), foliolis primaevis

*xerampelinis* (vs *rubellis* vel *porphyreo-castaneis*), nec non *dentibus foliolorum longioribus* (0.76–3.14 mm vs 1.0–1.25 mm) differt.

**Holotype:** Mexico, Chiapas, Montañas del Norte. Jul 2010 M. A. Pérez-Farrera 2620a (HEM). Isotypes: MEXU, MO, XAL.



Figure 1. (A) Conical male cone of *Zamia grijalvensis*. Note thick tomentose peduncle, (B) cylindrical female cone of *Z. grijalvensis*, (C) base of petiole, (D) habit of leaf.

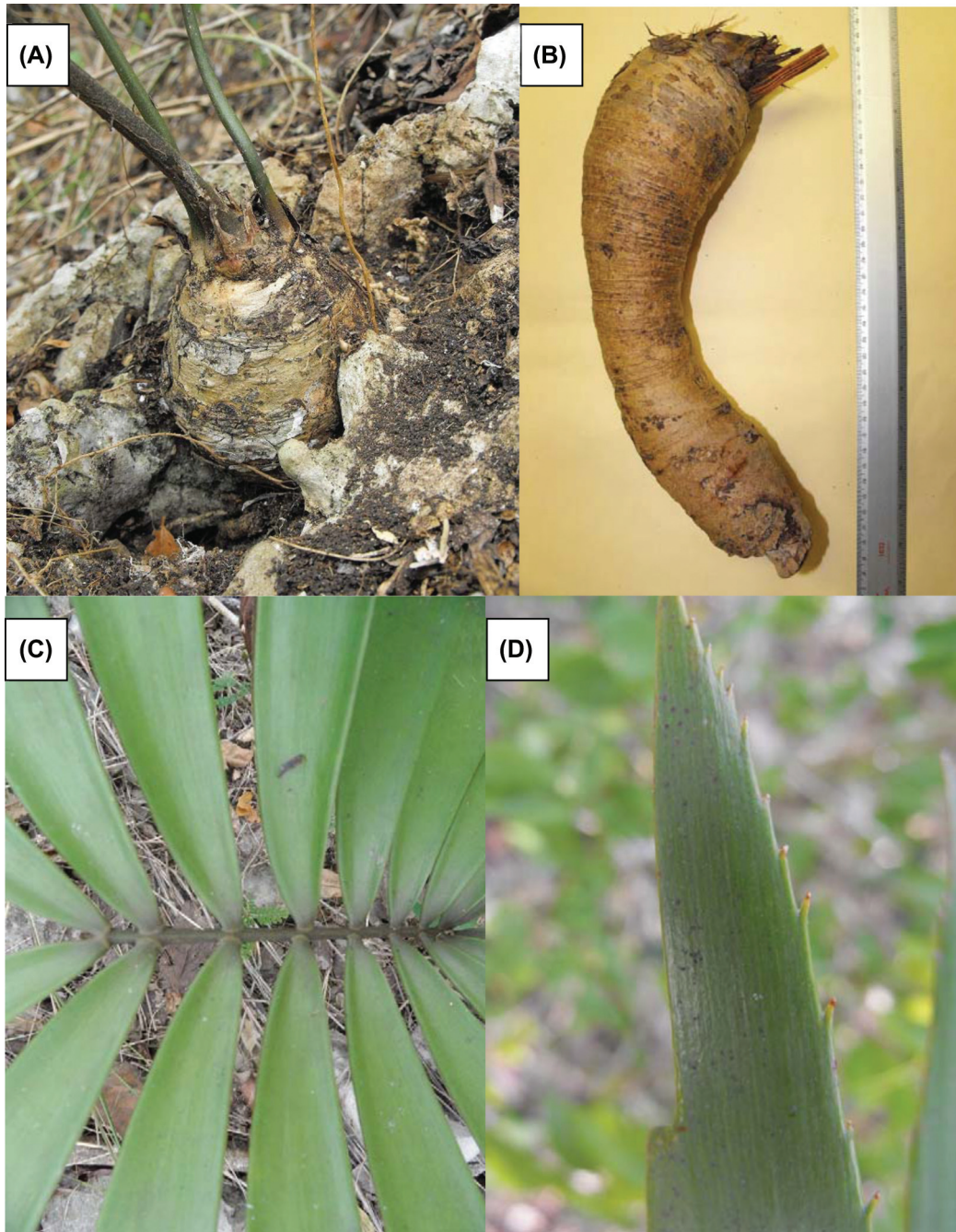


Figure 2. (A) Detail of trunk, leaf cataphyll and base of petioles, (B) trunk detail, note the constricted middle portion, (C) leaflet articulations, (D) details of leaflet marginal teeth.

**Paratypes:** Mexico, Chiapas, Montañas del Norte. Jul 2009. N. Martínez-Meléndez 2647 female (HEM); Chiapas, Montañas del Norte. May 2009. N. Martínez-Meléndez 2635 male (HEM).

**Etymology**

The species is named in honour of the Grijalva River (Río Grijalva) that crosses half the territory of Chiapas through the physiographic region of the Central Depression and Northern Mountain Range. Throughout its course, this river generates over 50% of the hydroelectric energy produced in Mexico.

**Description**

Perennial pachycaulous, dioecious plants. Stem subterranean, 24–42 × 6.3–8.0 cm. Leaves usually one, occasionally two (depending upon condition and age of the plant), ascending to arching, 122.5–171.5 × 48–86 cm; leaflets in 14–29 pairs per leaf, opposite to subopposite in the apical region, subalternate to alternate in the mid and basal part of the leaf, linear to sub falcate in the mid portion to slightly lanceolate in apical leaflets, 24.5–43.0 × 2.2–3.2 cm, acuminate at apex, equal to unequal; margin subrevolute with marginal teeth in upper half of leaf; spines terete 0.76–3.14 mm long, attenuate at base, reddish–brown at



Figure 3. Mitotic metaphase cells of *Zamia grijalvensis*: (A)  $2n = 19$ , (B)  $2n = 20$ . Scale bars = 10  $\mu\text{m}$ .

emergence, dark green when mature, articulation ring-like, pale brown, 6.2–10.9 mm wide rachis; eophyll leaflets four. Petiole terete, orange at base, dark green to brown, covered with brown tomentum, armed with short stout prickles for approximately 75% of its length from the base,  $41\text{--}92 \times 9.4\text{--}17.1$  mm. Leaf cataphylls triangular, chartaceous,  $2.5\text{--}3.0 \times 2.0\text{--}2.4$  cm. Cone cataphylls narrowly triangular, papyraceous,  $3.5\text{--}4.0 \times 1.0\text{--}1.2$  cm. Pollen cones one or two per stem, erect, cylindrical, ochre to brown, apiculate, tomentulose,  $11.3\text{--}23.7 \times 1.02\text{--}1.4$  cm; peduncle puberulent,  $7.6\text{--}19.10 \times 0.38\text{--}0.63$  cm; microsporophylls cuneiform, puberulent, with the distal ends truncate-hexagonal with a horizontal groove,  $0.56\text{--}0.61 \times 0.26\text{--}0.38$  cm; microsporangia spheroidal, (8–)12 per sporophyll, four or six proximal to each margin of fertile area, aggregated into sori of two microsporangia each, dehiscing by longitudinal sutures. Ovulate cone cylindrical, erect, barrel-shaped, apiculate at apex, dark reddish-brown to orange-brown, tomentulose,  $14.2\text{--}22.5 \times 5.0\text{--}6.6$  cm; peduncle puberulent,  $5.4\text{--}11.6 \times 1.6\text{--}1.9$  cm; megasporophylls cuneiform-peltate, the distal ends scutiform with discernable hexagonal-truncate distal face with a horizontal groove,  $2.1\text{--}2.7 \times 1.08\text{--}1.61$  cm, with two ovules per megasporophyll. Seed ovoid to angular, with the fleshy sarcotesta turning bright orange at maturity,  $1.65\text{--}1.98 \times 0.94\text{--}1.24$  cm; sclerotesta smooth, light brown with 5–6 visible lines radiating from the micropyle. Chromosome number  $2n = 19, 20$  (Fig. 3).

#### Habitat

*Zamia grijalvensis* grows in deciduous tropical forest (nomenclature according to Rzedowski 1978) that is structured into three strata. The upper canopy is dominated mainly by *Pseudobombax ellipticum* (Kunth) Dugand, *Cochlospermum vitifolium* Spreng., *Zuelania guidonia*

Britton & Millsp., *Bursera simaruba* Sarg., *Chrysophyllum mexicanum* Brandege, *Ficus pertusa* L.f., *Licaria* aff. *coriacea* Kosterm., *Neea* aff. *tenuis* Standl., *Eugenia capuli* Schltdl., *E. gaumeri* Standl., *Coccoloba cozumelensis* Hemsl., *Neopringlea integrifolia* S. Watson, *Inga laurina* Willd. The middle stratum is dominated by *Caesalpinia pulcherrima* (L.) Sw., *Polygonum* sp., *Cnidioscolus tubulosus* I. M. Johnst., *Chusquea* sp., *Piper pseudolindenii* C. DC., *Croton guatemalensis* Lott, *Psychotria erythrocarpa* Schltdl., *Guettarda combsii* Urb. subsp. *seleriana* (Loes.) Borhidi, *Bauhinia* sp., *Guadua longifolia* (E. Fourn.) R. W. Pohl, *Chamaedorea glaucifolia* H. Wendl. The lower herbaceous/shrubby layer consists of *Agave grijalvensis* B. Ullrich, *Russelia purpusii* T. & S. Brandege, *Selenicereus grandiflorus* (L.) Britton & Rose, *Anthurium faustomirandae* Pérez-Farrera & Croat, *A. schlechtendalii* Kunth, *A. huixtlense* Matuda, *Euphorbia* spp. and *Salvia* spp. The habitat presents a regular topography with slopes of up to  $45^\circ$  and cliff faces. The geology of this region corresponds to early Tertiary continental marine strata from the Cenozoic, upper Cretaceous (Ferrusquía-Villafranca 1998). The rock type is limestone forming a karstic topography with a shallow tropical rendzina soil (Ferrusquía-Villafranca 1998) where *Z. grijalvensis* is found growing at an altitudinal range between 650 and 700 m a.s.l.

#### Conservation status

The precise locality for *Z. grijalvensis* is here deliberately omitted in order to avoid decimation of its population by illegal collecting. We have found only one population of this species in a small geographic area between limits of the Central Depression and mountains of northern Chiapas. *Zamia grijalvensis* is under severe threat due to habitat transformation by subsistence agriculture and cattle

grazing. The species is also at risk because it grows in deciduous tropical forest subject to uncontrolled annual forest fires. The population has no more than around 300 individuals with poor regeneration. Because of its small population size and apparently highly restricted distribution we recommend the IUCN red list category of CR B.1. (IUCN 2005) until further explorations uncover more populations and perhaps a wider distribution.

### Similar species

*Zamia grijalvensis* is similar to *Z. lacandona* in that both species present only one, rarely two, ascending to arched leaves per crown. Emergent leaves of *Z. lacandona* are reddish to purplish brown with 7–12 pairs of leaflets, whereas *Z. grijalvensis* emergent leaves are reddish to purplish brown with 14–29 pairs of leaflets; the ovulate cone is brown to orange–brown when mature and both ovulate and pollen cones are erect, whereas the ovulate cone of *Z. lacandona* is dark brown with lighter beige megasporophyll facets when mature and only the pollen cone is decumbent. Also *Z. grijalvensis* differs in stem morphology and in leaflet articulation colour: in *Z. grijalvensis* the stems are consistently constricted or compressed in their middle part and the leaflet articulation with the rachis is pale brown, whereas in *Z. lacandona* the stem is conical and the leaflet articulation with rachis is dark brown. *Zamia lacandona* grows in tropical evergreen rain forest in the Lacandon forest, whereas *Z. grijalvensis* grows in seasonally dry deciduous tropical forest in the Central Depression of Chiapas. The phenology and pollinators are not known in detail for *Z. grijalvensis* but through cladistic inference (Vovides et al. 1997) we can confidently say that the pollinators are most probably snout weevils in the families Cucurionidae or Belidae. The reproductive phenology is similar to most *Zamia* spp., where cone initiation starts at beginning of the rainy season (mid to late spring) and female cone maturity during August–October (autumn).

### A hybridization process?

We believe that the new species may be of natural hybrid origin. One parent may be *Zamia loddigesii* Miq., or species in the *Z. katzeriana* species complex that includes *Z. lacandona* (Schutzman and Vovides 1998) whose populations have since become fragmented. Natural hybrids in cycads have been documented in the past, but no detailed analysis has been made. Johnson (1963) reported hybrid swarms in New South Wales, Australia involving *Macrozamia communis* L. Johnson, *M. secunda* C. Moore and *M. pauli-guilielmi* W. Hill & F. Muell. Norstog (1987) performed artificial crosses between a male *Zamia furfuracea* L.f. (considered an introgressive hybrid with a coastal dune form by Schutzman 2004) and a female *Z. spartea* thus obtaining vigorous fertile F1 hybrids true to the female parent. Later the F2 generation showed leaf and leaflet morphology ranging between that of *Z. spartea* (leaflets linear) and *Z. furfuracea* (leaflets obovate to oblanceolate) and encompassing the intermediate leaflet morphology found in both *Z. loddigesii* and *Z. paucijuga* Wieland

(linear-lanceolate to lanceolate). In a morphometric study using Anderson pictorialized scatter diagram (Stace 1980) involving a suspected introgressive hybrid (*Z. furfuracea* inland form) between the putative parents *Z. loddigesii* and *Z. furfuracea* (coastal dune form) Vovides (1988) found that the putative hybrid was intermediate between the two parents for six morphological and 11 anatomical characters. This gave us reason to speculate that *Z. loddigesii* from the Gulf of Mexico seaboard and isthmus of Tehuantepec area could be of ancient hybrid origin and *Z. paucijuga* derived from the isthmian stock occupying the more stressed habitats of the Pacific seaboard from the isthmus to Nayarit (Moretti and Sabato 1984, Vovides and Olivares 1996). Introgressive hybridization tendencies have been observed in other Meso-American zamias such as *Z. neurophyllidial/Z. skinneri* Warz. ex A. Dietrich, *Z. prasina* (= *Z. polymorpha*) and *Z. variegata* Warz. (Schutzman 2004). The issue of hybridization in cycads needs to be studied by future anatomical and molecular techniques.

### Chromosome numbers and karyology

*Zamia* species show the highest variation in chromosome number and karyotype among extant Cycadales (Marchant 1968, Moretti and Sabato 1984, Vovides and Olivares 1996, Norstog and Nicholls 1997). With the exception of *Z. lacandona* ( $2n = 16, 17, 18$ ) and *Z. grijalvensis* ( $2n = 19, 20$ ), all known chromosome numbers of the *Z. katzeriana* species group are  $2n = 16$ . The cytology of the new species, with up to six telocentric chromosomes, appears to present centric fissions and/or Robertsonian changes as found in *Z. paucijuga* Wieland ( $2n = 23, 25, 26, 27, 28$ ; Moretti and Sabato 1984) and *Z. prasina* Bull. (formerly *Z. loddigesii* Miq. of the Yucatan peninsula ( $2n = 17, 24, 25, 26, 27$ ; Vovides and Olivares 1996). It has been observed that these *Zamia* species with varying chromosome numbers, asymmetric karyotype and a high number of telocentric chromosomes occupy a greater geographic distribution and generally seasonally dry habitats. Stressful habitats might cause chromosomal fission, allowing species to adapt and to occupy and survive in a wider habitat range of increasing dryness (Moretti and Sabato 1984, Vovides and Olivares 1996). However, causal factors for karyotype variation may be multiple and are yet to be determined (Khoshoo 1969, Jones 1998). Recently Olson and Gorelick (2011) suggested that there may be an evolutionary trend towards karyotype asymmetry in *Zamia*. However, since only two individuals of *Z. grijalvensis* were investigated, it is clearly too early to draw any meaningful conclusions.

### Diagnostic key to *Zamia* species in Chiapas

1. Leaflets without marginal teeth ..... *Z. soconuscensis*  
– Leaflets with marginal teeth ..... 2
2. Leaflets papyraceous ..... *Z. herrerae*  
– Leaflets coriaceous ..... 3
3. Leaflets long elliptical to elliptical ..... *Z. katzeriana*  
– Leaflets not long elliptical to elliptical, but lanceolate or linear ..... 4

4. Leaflets with yellow spots ..... *Z. variegata*  
 – Leaflets without yellow spots ..... 5
5. Leaflets linear in the mid portion of leaves ... *Z. loddigesii*  
 – Leaflets falcate in the mid portion of leaves ..... 6
6. Leaflets 7–12 pairs per leaf; pollen cones decumbent ...  
 ..... *Z. lacandona*  
 – Leaflets 14–29 pairs per leaf; pollen cones erect ...  
 ..... *Z. grijalvensis*

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