

Article

Ceratozamia schiblii (Zamiaceae): A New Cycad Species from the Eastern Mountains of Oaxaca, Mexico

Miguel Angel Pérez-Farrera ^{1,*} , José Said Gutiérrez-Ortega ^{2,*} , Timothy J. Gregory ³, Jeffrey Chemnick ⁴, Silvia Salas-Morales ⁵, Michael Calonje ⁶ and Pedro Díaz-Jiménez ⁷

- ¹ Herbario Eizi Matuda, Laboratorio de Ecología Evolutiva, Instituto de Ciencias Biológicas, Universidad de Ciencias y Artes de Chiapas, Tuxtla Gutiérrez 29039, Mexico
- ² RIKEN Interdisciplinary Theoretical and Mathematical Sciences Program, Wako, Saitama 351-0198, Japan
- ³ University of California Botanical Garden, Berkeley, CA 94720, USA; cycadtim@gmail.com
- ⁴ Ganna Walska Lotusland, Santa Barbara, CA 93108, USA; jeffchemnick@cox.net
- ⁵ Sociedad para el Estudio de los Recursos Bióticos de Oaxaca, San Sebastián Tutla, Oaxaca 71246, Mexico; sshibli@hotmail.com
- ⁶ Montgomery Botanical Center, Coral Gables, FL 33156, USA; michaelc@montgomerybotanical.org
- ⁷ Jardín Botánico Araceario Thomas B. Croat, Comalcalco 86650, Mexico; aroid764@hotmail.com
- * Correspondence: perezfarreram@gmail.com (M.A.P.-F.); josesgo@gmail.com (J.S.G.-O.)

Abstract: *Ceratozamia schiblii* (Zamiaceae), a new species endemic to the highlands of eastern Oaxaca, Mexico, is described. The new species is part of the *C. robusta* species complex, which is a group of closely related species with very similar morphology. Among them, the new species can be distinguished by its brown leaflets in emerging leaves, a very long peduncle in seed cones, and the green color of mature pollen cones. Compared with the other species in the *C. robusta* species complex, *C. schiblii* has one of the longest rachides, and the highest number of leaflet pairs. The recognition of *C. schiblii* apparently resolves the total number of species within the *C. robusta* species complex. The region where *C. schiblii* populations occur coincides with the Neogene refugia areas of the Neotropical flora, which adds evidence to the importance of the mountain systems from Oaxaca and Chiapas, Mexico, in the diversification history of cycads.

Keywords: cycads; floristic refugia; Mesoamerica; morphological analyses; species complex



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1. Introduction

In recent years, knowledge about the systematics of Mexican cycads has advanced due to the increasing usage of morphological, anatomical, and genetic data to evaluate novel, as well as previously known, discoveries. This multidisciplinary approach has resulted in a significant increase in the number of newly described species across the three cycad genera native to Mexico: *Dioon* Lindl., *Zamia* L., and *Ceratozamia* Brongn. Indeed, some 26 cycad species in Mexico have been described in the last 20 years. Most Mexican species are endemic to the southern states of Oaxaca, Chiapas, and Veracruz, a region recognized as one of the main hotspots of cycad diversity in the New World [1–3]. The majority of cycad species are considered threatened [4], so an increased understanding of the species richness and diversity within this region will contribute to their conservation.

Ceratozamia (Zamiaceae) is a Neotropical cycad genus with 36 recognized species, 33 of which occur exclusively in Mexico [5]. The genus is associated with tropical montane cloud forests, oak forests, and evergreen tropical forests, spanning a wide range of elevations from near sea level to approximately 1950 m above sea level (m a.s.l.) [6]. All species are defined according to qualitative and quantitative morphological variation of leaves, leaflets, and reproductive structures [2,6]. Generally, there is a correspondence between geographic isolation and species' range delimitation; and it is generally expected that isolated populations occurring in distinct mountain ranges are likely to show qualitative variation, which is an indicator that they might belong to separate species. However, the

taxonomic delimitation of species based solely on diagnostic morphological characters is not always possible. Some species closely resemble each other morphologically, and in many cases, populations previously thought to belong to a single species were subsequently separated into two or more taxa when statistical approaches to test quantitative diagnostic trait differentiation are applied [2,6,7].

Ceratozamia species can be classified into different “species complexes” [2,7]. A species complex refers to a group of species that share morphological features and are expected to be phylogenetically closely related. This morphological similarity among members of a species complex often makes it difficult to distinguish one species from the others within the group. One of the largest complexes in the genus is the “*C. robusta* species complex” [2], which has a wide distribution through Chiapas, Tabasco, Veracruz, Guerrero, and Oaxaca in Mexico, extending to Belize and Guatemala (Figure 1). The species in this complex are distinguished from other species in the genus by having larger trunks, leaves, and cones. The habitats for the species in the complex are usually associated with karstic forests and can be found in a wide range of elevations and associated vegetation [6,8,9]. In recent years, the identity and status of many populations that were previously circumscribed as *C. robusta* has been clarified with the help of morphological analyses [6,8–11]. Subsequently, it has been determined that the *C. robusta* species complex now consists of the species *C. robusta* Miq., *C. subroseophylla* Mar.-Domínguez & Nic.-Mor., *C. aurantiaca* Pérez-Farr., Gut.Ortega, J.L.Haynes & Vovides, *C. dominguezii* Pérez-Farr. & Gut.Ortega, *C. oliversacksii* D.W.Stev., Mart.-Domínguez & Nic.-Mor., and *C. leptoceras* Mart.-Domínguez, Nic.-Mor., D.W.Stev. & Lorea-Hern. [8–11]. However, there are still several populations within the *C. robusta* species complex in Oaxaca that need further study to determine their taxonomic circumscription.

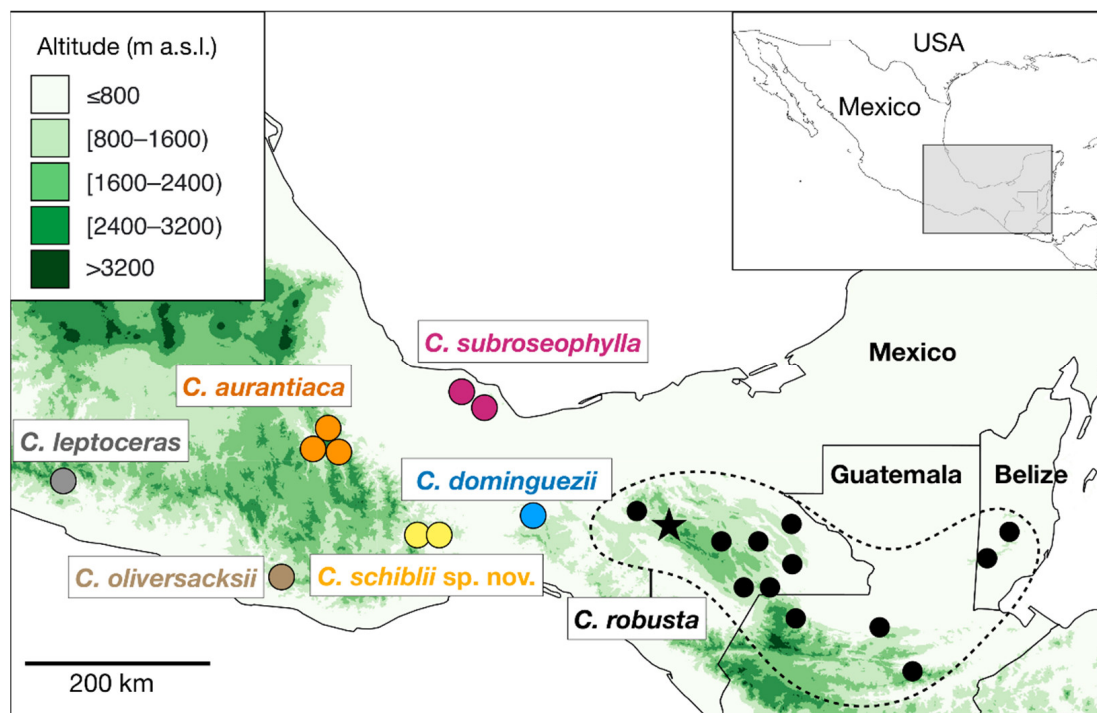


Figure 1. Distribution map of the known populations of the seven species in the *Ceratozamia robusta* species complex. The figure is updated from our previous studies [8,9]. The putative new species from eastern Oaxaca (*C. schiblii* sp. nov.) occurs near the center of the distribution range and is represented by yellow circles. Black circles surrounded by a dotted line indicate the occurrence of populations of *C. robusta*, the species with the widest distribution in the complex. The star symbol indicates the neotype locality of *C. robusta* (Parque Nacional Cañón del Sumidero, Chiapas, Mexico). The species *C. aurantiaca*, *C. subroseophylla*, *C. dominguezii*, *C. oliversacksii*, and *C. leptoceras* are also part of the species complex. Green color scale indicates the elevation in meters above sea level (m a.s.l.).

In the course of evaluating various populations of the *Ceratozamia robusta* complex, we found that the populations from the eastern highlands of Oaxaca, Mexico, in the municipalities of Santiago Lachiguiri and Santa María Guienagati, have several morphological characteristics that distinguish them from other members in the species complex. By analyzing qualitative and quantitative trait variations of these populations compared to the other species within the complex, we have determined that these populations are distinct enough to represent a new species, which we here describe and illustrate as *C. schiblii* sp. nov.

2. Material and Methods

A total of 87 adult plants representing four populations from Chiapas, Oaxaca, and Veracruz belonging to all species (except *C. leptoceras*) in the *C. robusta* complex were analyzed in this study. We were unable to include *C. leptoceras* due to fieldwork limitations, but we consider the qualitative traits of this species according to its original description [10]. The geographic distribution of the species was mapped using QGIS [12] and the elevation raster layers of 30 s resolution of the NASA Shuttle Radar Topographic Mission (SRTM) 90 m Digital Elevation Database v4.1 deposited in DIVA-GIS.org [13]. We utilized morphometric data collected in previous studies [8,9] for the species *C. aurantiaca*, *C. robusta*, *C. subroseophylla*, and *C. dominguezii*, and herein we have added data for nine individuals of *C. oliversacksii*, as well as for 16 individuals of the putative new species, *C. schiblii* (Table 1, Figure 1).

Table 1. Species compared in this study. Name of sampling locality, elevation in m above sea level (a.s.l.), and number of samples analyzed (*n*) in morphometric analyses are provided.

Species	Key	Elevation (m a.s.l.)	Locality	<i>n</i>
<i>C. aurantiaca</i>	aur	480	El Faro, Sierra Norte, Oaxaca (type population)	16
<i>C. robusta</i>	rob	1200	Cañón del Sumidero, Chiapas (neotype population)	20
<i>C. subroseophylla</i>	sub	500	Santiago Tuxtla, Veracruz (type population)	11
<i>C. dominguezii</i>	dom	130	Uxpanapa, Veracruz (type population)	15
<i>C. oliversacksii</i>	oli	840	San Gabriel Mixtepec, Oaxaca	9
<i>C. schiblii</i>	sch	1290	Santiago Lachiguiri, Oaxaca (type population)	16

Following a similar methodology used in our previous studies [8,9], we observed the qualitative trait variation among the examined species (Table 2) and compared the measurements of seven leaf and leaflet traits commonly used as diagnostic characters in *Ceratozamia* [6,8–11] (Table 3). For the quantitative data, we performed the following tests implemented in PAST v.3.4 [14] to determine whether the morphometric variation can be used to distinguish the various species. First, Welch’s ANOVA was used to test whether the means of the trait variation across species were significantly different (Table 3). Moreover, for each trait, Tukey’s Q of pairwise differentiation between the putative new species *C. schiblii* sp. nov. and the other five species was calculated to distinguish the traits that significantly differ with respect to the others. By combining all the trait data, a linear discriminant analysis (LDA) was conducted to test whether the overall variation among species can distinguish the six species considered a priori. Finally, the pairwise Mahalanobis distances were calculated to test whether the six species are significantly separated, which would indicate that the overall morphological differentiation is well sorted among the examined species. Herbaria specimens collected from the examined populations were deposited in HEM, XAL, and MEXU (acronyms according to [15]).

Table 2. Comparison of morphological variation among species of the *Ceratozamia robusta* species complex.

Trait	<i>C. aurantiaca</i>	<i>C. robusta</i>	<i>C. subroseophylla</i>	<i>C. dominguezii</i>	<i>C. oliversacksii</i>	<i>C. leptoceras</i>	<i>C. schiblii</i> sp. nov.
Trunk	Erect to decumbent	Erect	Erect to decumbent	Erect to decumbent	Erect	Erect to decumbent	Erect to decumbent
Color of emerging leaves	Orange-brown to orange	Reddish-brown	Yellowish-brown	Caramel color	Green	Green to copper-green	Brown
Crown of leaves	Open	Closed	Open	Open	Open	Open	Open
Texture of leaflets	Coriaceous	Papyraceous	Papyraceous	Papyraceous	Papyraceous	Membranaceous	Coriaceous
Veins on abaxial surface of leaflets	Not visible	Not visible	Visible	Not visible	Not visible	Visible	Not visible
Apex of seed cone	Mucronate	Acuminate	Mucronate	Acuminate	Acuminate	Acute	Mucronate
Habit of mature seed cones	Erect	Erect	Erect	Erect	Erect	Erect	Pendulous
Peduncle of seed cone	Short	Short	Short	Short	Short	Short	Long

Table 3. Morphometric differentiation of seven traits among the six *Ceratozamia* species examined. Overall differentiation estimated as Welch's *F* in ANOVA and *p*-values is indicated. Number of samples (adult individuals) per population used in the analyses is indicated in Table 1.

Key	Trait	<i>F</i>	<i>p</i>
A	Length of petiole	9.12	<0.0001
B	Length of rachis	10.58	<0.0001
C	Number of leaflet pairs	17.43	<0.0001
D	Length of median leaflets	2.052	0.08
E	Distance between median leaflets	15.13	<0.0001
F	Width of median leaflets	14.05	<0.0001
G	Width of basal leaflets	14.39	<0.0001

3. Results

Our analyses of qualitative (Table 2) and quantitative traits (Figure 2) show that the populations from the eastern highlands of Oaxaca, Mexico, are distinct from others within the *Ceratozamia robusta* species complex and merit recognition as a new species. The plants of this new species, which we herein name *C. schiblii* sp. nov., differ from the other species in the complex by having coriaceous leaflets, a feature only shared with *C. aurantiaca* (Table 2). However, the two species can be clearly distinguished by the color of their emerging leaves, which are orange-brown to orange in *C. aurantiaca* and brown in *C. schiblii*. The second qualitative feature that readily distinguishes the new species from its congeners is the extremely long peduncles that make the seed cones pendulous at maturity, whereas they remain erect in all the other species within the complex (Table 2).

The multiple statistical tests based on morphometric data supported the differentiation among species. All traits except the length of median leaflets showed significant overall differentiation among species (Table 3). In pairwise comparisons between *C. schiblii* and the other members of the complex (Table 4), it is notable that *C. schiblii* has petioles that are significantly longer than *C. aurantiaca* and *C. oliversacksii* and much shorter than *C. subroseophylla* (Figure 2A). The rachides in *C. schiblii* are significantly longer than in *C. oliversacksii*, but shorter than *C. dominguezii*, which is the species with the longest leaves and rachides [9] (Figure 2B). Most notable is that the number of leaflet pairs in *C. schiblii* is the highest among the species in the complex, but not significantly higher than in *C. oliversacksii*, which also has a high number of leaflet pairs (Figure 2C). The median leaflets were also found to be significantly longer than *C. robusta* and *C. oliversacksii*, but not different from the remaining species (Figure 2D). The distance between leaflets was similar to that observed in *C. aurantiaca* and *C. robusta*, but longer than in *C. subroseophylla* and *C. oliversacksii*, and shorter than *C. dominguezii* (Figure 2E). The width of the median leaflets of *C. schiblii* was among the narrowest in the complex (Figure 2F), with *C. oliversacksii* being the species with the narrowest leaflets. *C. schiblii* has wider basal leaflets than *C. robusta* and *C. oliversacksii* but does not differ significantly from the other species.

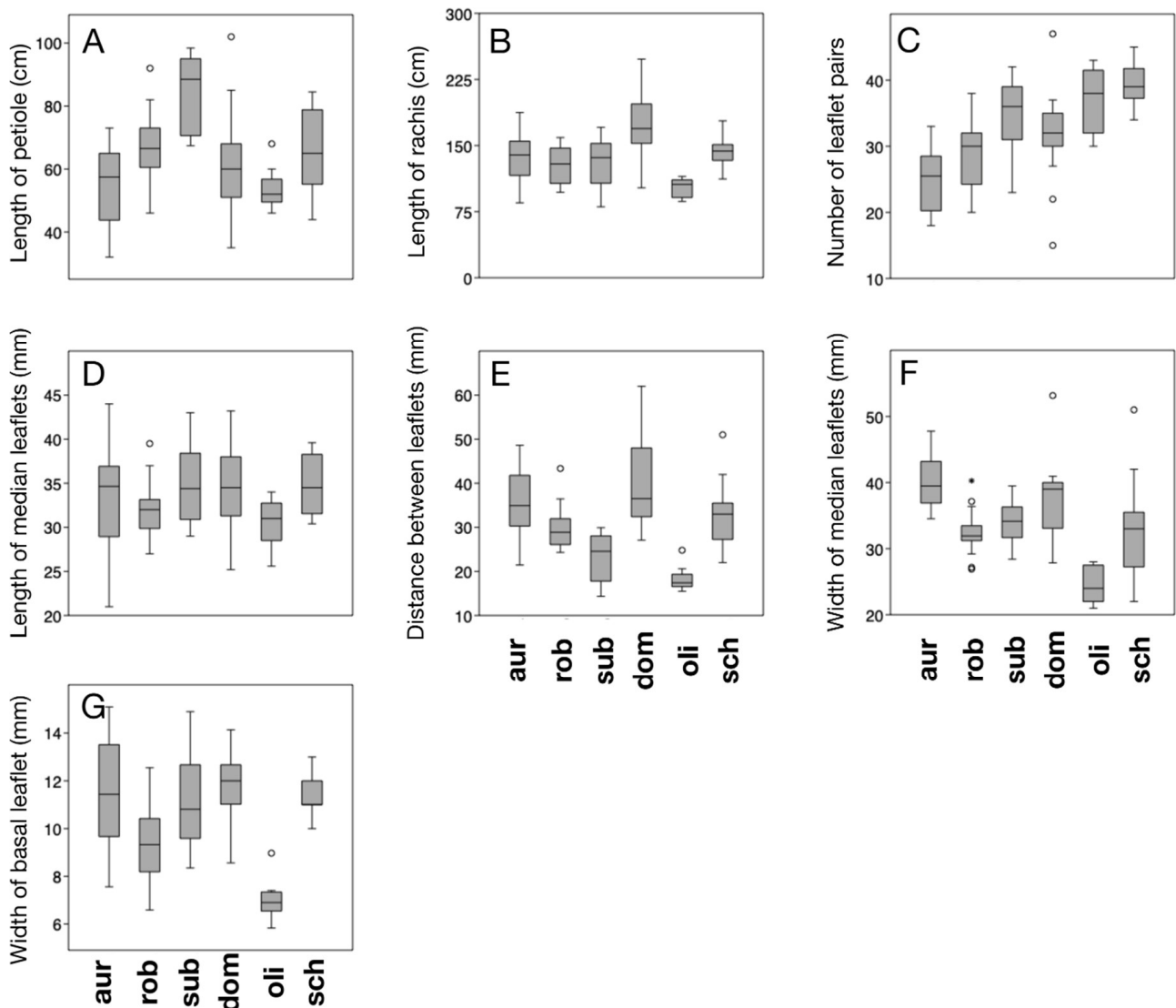


Figure 2. Morphometric variation among the six species in the *Ceratozamia robusta* species complex analyzed in this study: *Ceratozamia aurantiaca* (aur), *C. robusta* (rob), *C. subroseophylla* (sub), *C. dominguezii* (dom), *C. oliversacksii* (oli), and *C. schiblii* (sch). The order of plots corresponds to the traits (A–G) listed in Table 3. Whiskers indicate standard error and circles indicate outliers.

Table 4. Pairwise morphological trait differentiation between the new species *Ceratozamia schiblii* and the other examined species in the *C. robusta* species complex. Values indicate *F*-values calculated in ANOVA. Abbreviations: sch = *C. schiblii*; aur = *C. aurantiaca*; rob = *C. robusta*; sub = *C. subroseophylla*; dom = *C. dominguezii*; oli = *C. oliversacksii*; ns = not significant; *: $p < 0.05$; **: $p < 0.005$; ***: $p < 0.001$.

	Trait	sch vs. aur	sch vs. rob	sch vs. sub	sch vs. dom	sch vs. oli
A	Length of petiole	5.49 *	0.27 ns	14.83 ***	0.76 ns	6.46 *
B	Length of rachis	0.88 ns	6.42 *	1.55 ns	9.77 **	49.16 ***
C	Number of leaflet pairs	112.9 ***	58.84 ***	8.61 *	17.1 ***	2.32 ns
D	Length of median leaflets	0.45 ns	6.55 *	0.00 ns	0.01 ns	11.27 *
E	Distance between median leaflets	1.16 ns	2.47 ns	14.49 ***	4.77 *	32.33 ***
F	Width of median leaflets	11.78 **	0.09 ns	0.24 ns	3.20 ns	10.82 **
G	Width of basal leaflets	0.09 ns	23.51 ***	0 ns	1.13 ns	140.4 ***

The multivariate analyses were clearer at distinguishing the overall morphometric variation among species. The linear discriminant analysis (Figure 3) separated the convex-

hulls of each species with little overlap among them. The two most important discriminant axes summarized the total variation as 59.1% and 23.3%, respectively. According to the loading scores represented as biplots in Figure 3, the traits with the highest contribution were the length of petioles (trait A), length of the rachides (trait B), and the number of leaflet pairs (trait C) (Figure 3), which is denoted by the longest biplots. The confusion matrix resulting from the LDA showed that most of the samples were correctly assigned to their species, with very few exceptions (Table 5). Likewise, the squared Mahalanobis distances among the six evaluated species were significantly high (Table 6), indicating that the overall morphometric variation is sufficient to distinguish the species in the *C. robusta* species complex.

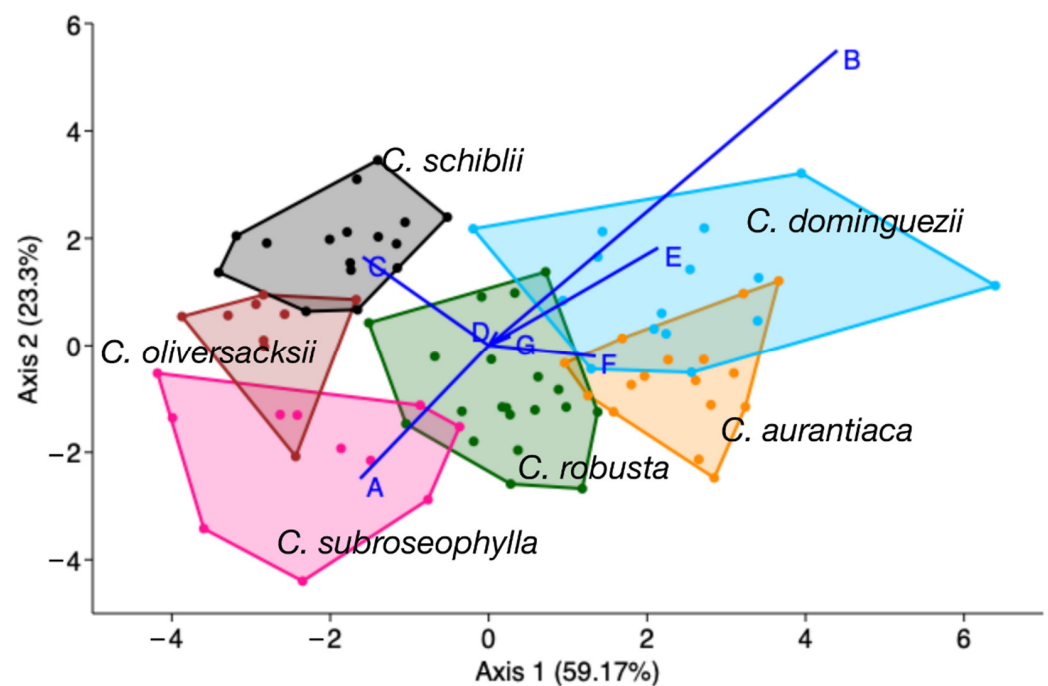


Figure 3. Linear discriminant analysis plot showing the dispersion of the samples of six compared species along the two main axes. Biplots A–F indicate the direction and relative loading score of each trait variation along the two axes. Traits A–G correspond to those listed in Table 3: A, length of petiole; B, length of rachis; C, number of leaflet pairs; D, length of median leaflets; E, distance between median leaflets; F, width of median leaflets; G, width of basal leaflets.

Table 5. Confusion matrix obtained from the Linear Discriminant Analysis (LDA). Rows indicate given groups, and columns indicate groups predicted by the LDA. Bold values indicate correctly assigned individuals to their own species, supporting their taxonomic identity. Abbreviations: aur = *C. aurantiaca*; rob = *C. robusta*; sub = *C. subroseophylla*; dom = *C. dominguezii*; oli = *C. oliversacksii*; sch = *C. schiblii*.

	aur	rob	sub	dom	oli	sch	Total
aur	13	1	0	2	0	0	16
rob	1	18	0	1	0	0	20
sub	0	0	11	0	0	0	11
dom	2	1	0	11	0	1	15
oli	0	0	0	0	9	0	9
sch	0	0	0	0	0	16	16
Total	16	20	11	14	9	17	87

Table 6. Squared Mahalanobis distances (below the diagonal) and *p*-values (above the diagonal) among the six examined species. All values are significant, suggesting complete morphological sorting among species. Abbreviations: aur = *C. aurantiaca*; rob = *C. robusta*; sub = *C. subroseophylla*; dom = *C. dominguezii*; oli = *C. oliversacksii*; sch = *C. schiblii*.

	Aur	rob	sub	dom	oli	sch
aur		<0.001	<0.001	<0.001	<0.001	<0.001
rob	7.8582		<0.001	<0.001	<0.001	<0.001
sub	26.485	11.609		<0.001	0.001	<0.001
dom	6.2325	9.7395	32.998		<0.001	<0.001
oli	32.383	12.87	16.229	33.386		<0.001
sch	25.085	14.141	16.102	20.021	10.575	

With the evidence presented, we propose the recognition of a new cycad species in the genus *Ceratozamia* that we name *C. schiblii* sp. nov.

New Species Description

Ceratozamia schiblii Pérez-Farr. & Gut.Ortega sp. Nov. (Figures 4–8).

Holotype:—MEXICO. Oaxaca: Lachidola, 1290 m, 30 November 2021, Pérez-Farrera M. A., 4074 ♂ with Díaz Jiménez P., García González J., Pérez F. J. & Moreno Méndez G. (HEM). Isotypes: (XAL, MEXU).

Ceratozamia schiblii is distinguished from other species in the genus by its brown leaflets in emerging leaves, pendulous seed cone with a long peduncle, and the green color of mature pollen cones.

Plant rupicolous, unbranching. **Stem** cylindrical, erect, decumbent in older plants, covered with persistent leaf bases, 20–220 cm tall, 20–24 cm in diameter. **Cataphylls** persistent, brown and densely tomentose at emergence, triangular apex acuminate. **Leaves** pinnate 15–24 forming an open crown, erect, ascending, 161.2–262.5 cm long, 60.8–79.2 cm wide, brown at emergence, turning olive green at maturity. **Petiole** terete, 43.9–84.5 cm long, armed, dense with thick prickles. **Rachis** terete, 112–178 cm long, erect, with spaced prickles diminishing into apical part of rachis, green. **Leaflets** 34–45 pairs, coriaceous, opposite to subopposite in the medium part of the leaf, subopposite to alternate in the basal part, linear, subfalcate to falcate, margin entire, apex acute to acuminate, asymmetric; base broad attenuate, articulation green; veins 21–36, parallel, inconspicuous, not readily visible; median leaflets 30.4–39.6 cm long, 3–4.5 cm wide, 2.2–5.1 cm between leaflets, articulation 1–1.3 cm wide. **Microstrobilus** (pollen cone) solitary, conical, erect, olive green when mature, 22–33 cm long, 6.09–7.02 cm in diameter; peduncle densely tomentose, 4–8 cm long, 1.5–2.8 cm in diameter, brown. **Microsporophyll** cuneiform, distal face bicornate, 16–20 mm long, 10–14 mm wide, sporangia zone on abaxial surface 10–13 mm long, microsporangia grouped in sori of 3–4. **Megastrobilus** (seed cone) solitary, cylindrical, erect when emerging, descendent when mature, 25–28 cm long, 10.7–12 cm in diameter, brown at maturity; apex mucronate; peduncle tomentose, 20–21.5 cm long, 1.5–1.8 cm in diameter, brown. **Megasporophylls** peltate, bicornate, distal face 3.4–3.9 cm wide, 1.2–1.8 cm tall, pubescent, brown (including horns) at maturity, base light brown; peduncle tomentose, light-yellow to light-orange at maturity. **Seed** ovoid, sarcotesta cream when immature, orange-brown when mature, sclerotesta beige, with micropylar ridges 1.4–1.7 cm in diameter, 2.6–3.1 cm long.

Specimens examined: MEXICO. Oaxaca: Lachidola, 12 September 2021, Pérez-Farrera M. A. 4065 with García González J. & José Perez F. (HEM); 1260 m, 9 May 2019, Pérez-Farrera M.A. & Calonje M. 3764 ♀(HEM); Santa María Guienagati, 9 March 2022, Pérez-Farrera M.A. with Díaz Jiménez P. & Villar Morales D. 4093 ♀(HEM). 1200 m, 25 January 1996, Cerón C. M. 249 with Salas Morales S.M. & Morales H. (SERO, MEXU, XAL); Guevea de Humboldt, 1300 m, 30 March 1991, Campos V. A. & Torres C. R. 3614 (CAS; MEXU); La Chiviza, 1170 m, 24 Aug 1984, Torres R. & Martínez C. 5920 (CAS, MEXU).

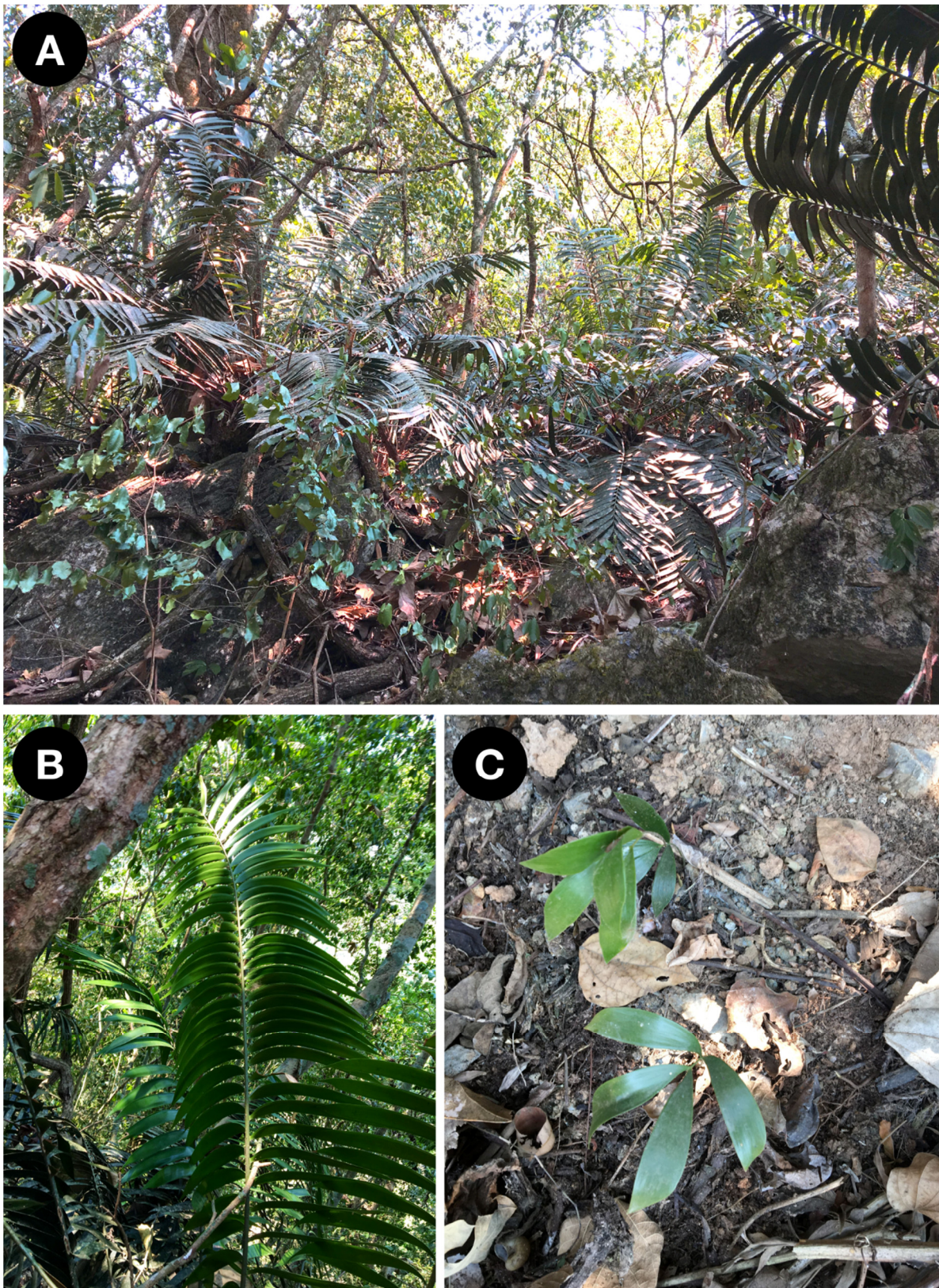


Figure 4. *Ceratozamia schiblii* sp. nov. in habitat. (A) Adult plants. (B) Erect leaves and details of leaflets. (C) Seedlings.



Figure 5. Some of the distinctive features of *Ceratozamia schiblii*. (A) Leaves are one of the longest and with the highest number of leaflet pairs among the species of the *C. robusta* species complex (left: M.C.; right, M.A.P.-F.). (B) Megastrobili (seed cones) are pendulous and peduncles long. (C) Emerging leaves are of brown color. Photo A by Cesar Daniel Coutiño Ovando. Photos B and C by Miguel Angel Pérez Farrera.

Habitat description: *Ceratozamia schiblii* grows between 1100 and 1300 m a.s.l., in karstic tropical montane cloud forest and *Quercus* forest, according to the vegetation classification of Rzedowski [16]. The associated flora includes *Pinus chiapensis* (Martínez) Andresen, *P. oocarpa* Schiede ex Schltdl., *Cupressus lusitanica* Mill., *Quercus laeta* Liebm., *Q. castanea* Née, *Clethra macrophylla* M.Martens & Galeotti, *Saurauia serrata* DC., *Podocarpus matudae* Lundell, *Chamaedorea pinnatifrons* H. Wendl., *Chamaedorea oblongata* Mart., *Chamaedorea tepejilote* Liebm., *Begonia nelumbonifolia* Schltdl. & Cham., *Anthurium cerrobaulense* Matuda, *A. scandens* (Aubl.) Engl., *Monstera deliciosa* var. *sierrana* G.S. Bunting, *Philodendron sagittifolium* Liebm., *Syngonium chiapense* Matuda, *Litsea glaucescens* Kunth, *Smilax purpusii* Brandege, *Monnina xalapensis* Kunth, *Oreopanax xalapensis* Kunth Decne. & Planch., *Cleyera theaeoides* (Sw.) Choisy, *Styrax ramirezii* Greenm., *Ternstroemia tepezapote* Schltdl. & Cham., *Hedyosmum mexicanum* C. Cordem., *Palicourea elata* (Sw.) Borhidi, *Arachnothryx laniflora* (Benth.) Planch., *Cyathea divergens* Kunze, *Oreomunnea mexicana* (Standl.) J.-F. Leroy, among others.

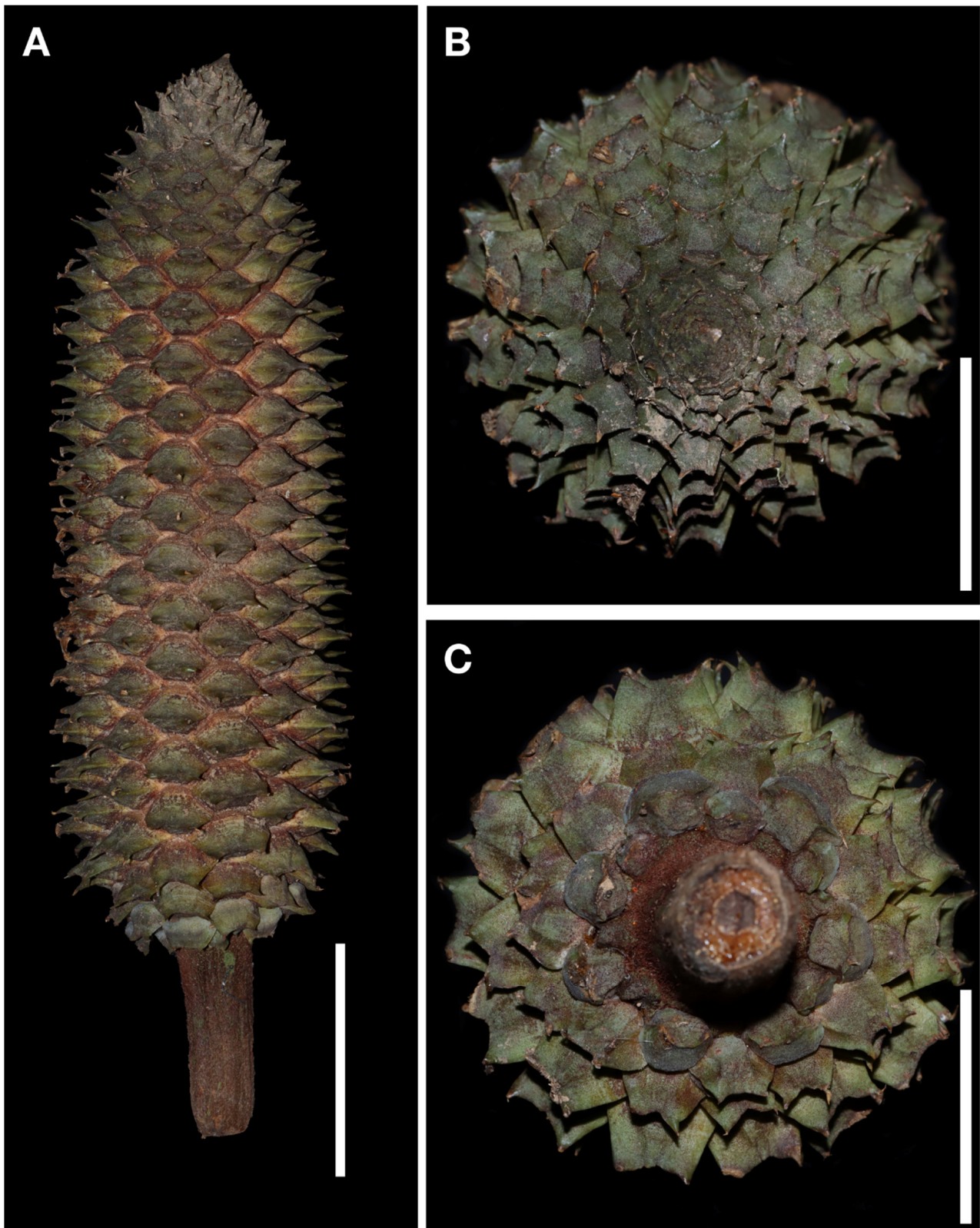


Figure 6. Megastrobilus (seed cone) of *Ceratozamia schiblii*. (A) Lateral view (scale = 10 cm); (B) view from above (scale = 5 cm), (C) view from below (scale = 5 cm). Photos by José García González.

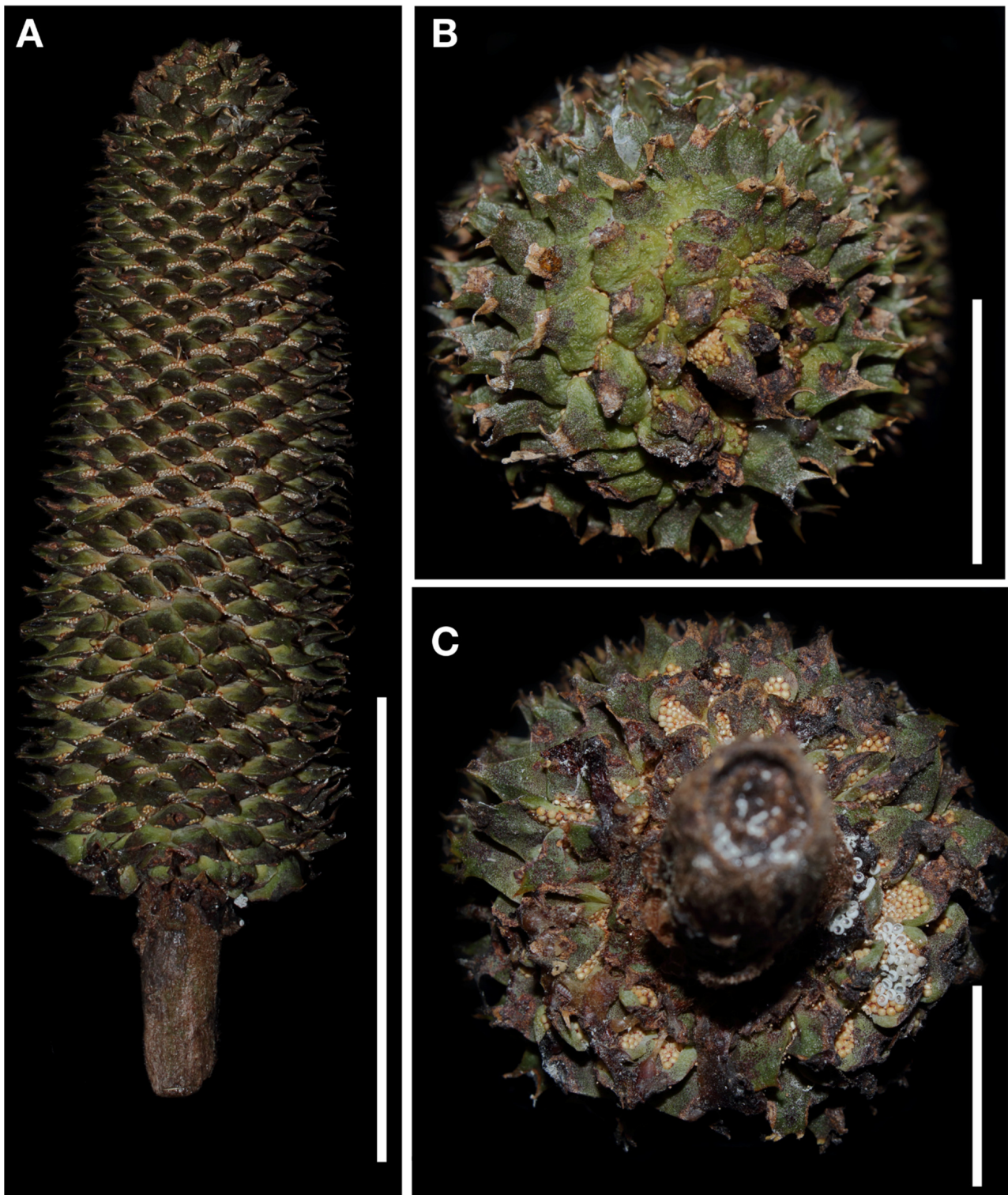


Figure 7. Microstrobilus of *Ceratozamia schiblii* at near pollen dehiscence phase. (A) Lateral view (scale = 10 cm); (B) view from above (scale = 2 cm), (C) view from below (scale = 2 cm). Photos by José García González.

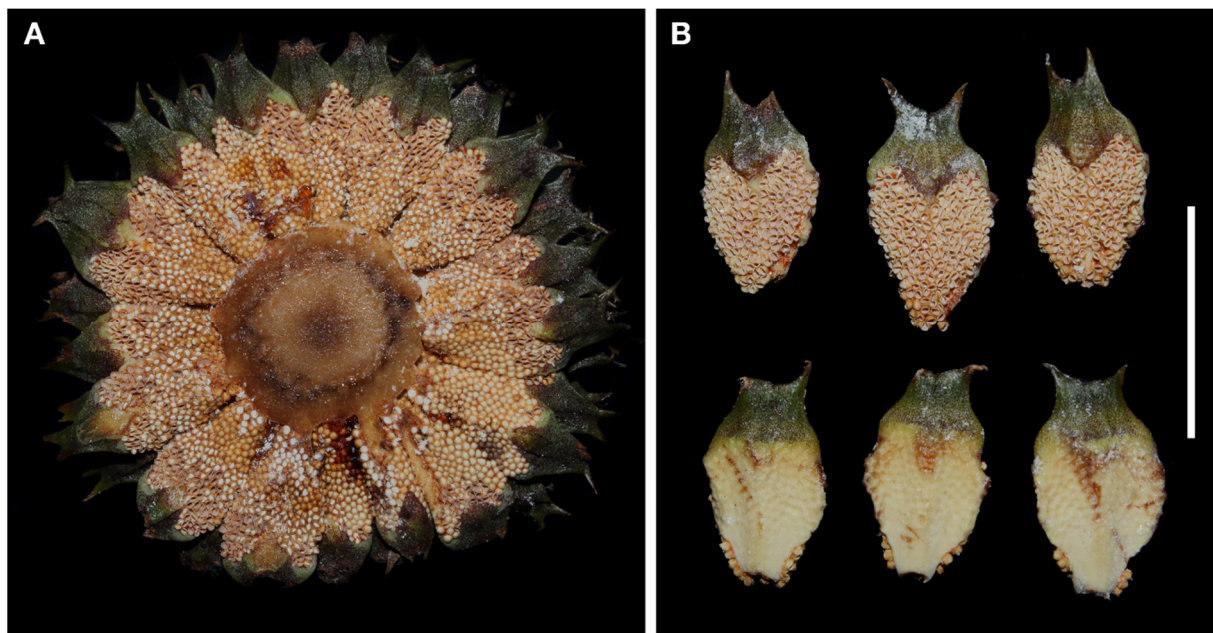


Figure 8. Microsporophylls of *Ceratozamia schiblii*. (A) Transversal sectioned microstrobilus (pollen cone) with exposed microsporophylls. (B) Microsporophylls abaxial side (top) and adaxial side (bottom). Scale = 2 cm. Photos by José García González.

Etymology: The specific epithet was chosen to honor Leo Schibli (1958–2004), a founding member of Sociedad para el Estudio de los Recursos Bióticos de Oaxaca, Asociación Civil (SERBO, A.C.). His field explorations and specimen collections in Oaxaca resulted in the discovery of several new cycad species.

Key to the species of the *Ceratozamia robusta* complex: We provide a key to the species in the *C. robusta* species complex. Moreover, although *C. mixeorum* does not belong to the *C. robusta* species complex, we include it to make a distinction between it and the new species, given their geographic proximity. More traits that can distinguish the two species are discussed below.

- 1a. Plants with erect cone
 - 2a: Plants with emerging orange leaves.....*C. aurantiaca*
 - 2b: Plants with green, reddish, yellowish, brown, or caramel emerging leaves
 - 3a: Plants with visible veins on leaflets
 - 4a: Plants with emerging yellowish-brown leaves.....*C. subroseophylla*
 - 4b: Plants with emerging green leaves
 - 5a: Leaflets membranaceous.....*C. leptoceras*
 - 5b: Leaflets papyraceous.....*C. oliversacksii*
 - 3b: Plants with inconspicuous veins on leaflets
 - 6a: Plants with emerging reddish-brown leaves.....*C. robusta*
 - 6b: Plants with newly emerging caramel colored leaves.....*C. dominguezii*
- 1b. Plants with pendent cone
 - 7a: Plants with emerging leaves of pea green color.....*C. mixeorum*
 - 7b: Plants with emerging leaves of brown color.....*C. schiblii*

4. Discussion

Before this study, we examined all the *Ceratozamia* specimens deposited in the herbaria MEXU, HEM, and XAL, and each was attributable to one of the already known *Ceratozamia* species; the only exceptions were the plants from the municipalities of Lachidola and Santiago Lachiguiri, in the eastern highlands of Oaxaca, Mexico. The plants from these sites were long recognized as *C. robusta* given their large trunks, leaves, and cones. With this study, we now show that those populations represent a new species, *C. schiblii*. *Ceratozamia*

schiblii stands out for the length of the seed cone peduncle, which is extremely long (more than 20 cm). The seed cones emerge around May, are erect, and become pendulous with maturity as the weight increases. The species is also characterized by the pollen cone, which is green at maturity, unlike most other species within the *C. robusta* complex, which are typically yellowish or brown.

The LDA analysis shows a separation between the populations within the *C. robusta* complex on the Pacific and Gulf slopes. In general, the species of the Gulf slope are much more robust (leaves, rachis, and petiole) than those of the Pacific slope. Similar to the other species within the complex, *C. schiblii* populations are found on a mountain range identified as a refugium for tropical plants during the Neogene climatic fluctuations [17]. This pattern of the geographic distribution of closely related species in the complex supports the idea that mountains in southeastern Mexico have played an important role in the divergence and allopatric speciation in cycads [18].

Ceratozamia schiblii occurs in the southernmost spurs of Sierra Norte, Oaxaca, very close to Sierra Mixe, which is a region that is home to *C. mixeorum* Chemnick, T.J.Greg. & Salas-Mor. It is possible to confuse *C. schiblii* with *C. mixeorum* because both have seed cones with extremely long peduncles, a characteristic that is atypical in the genus [19]. However, whereas *C. schiblii* is part of the *C. robusta* species complex, *C. mixeorum* is part of a different clade in the phylogeny of the genus [20], being sister to *C. matudae* Lundell. The morphological characteristics that define the *C. robusta* species complex are the single, tall (maximum around 2 m), and thick (maximum around 40 cm in diameter) trunks, as observed in adult *C. robusta* plants, long leaves (usually longer than 2 m), long median leaflets (usually 30 to 40 cm [Figure 2D]), wide median leaflets (usually 3 to 4 cm [Figure 2E]), and a low number of leaflet pairs (from 15, as in *C. dominguezii*, to 47 as in *C. oliversacksii* [Figure 2C]). *Ceratozamia mixeorum*, as currently circumscribed [19], does not belong to the *C. robusta* species complex because it has bifurcated (or multi-furcated) trunks that are much shorter (up to 125 cm) and thinner (up to around 20 cm); leaves are shorter (the longest being less than 2 m), leaflets are narrower (2 to 3 cm), and the number of leaflet pairs is much higher (from around 50 to 77). However, *C. mixeorum* has some traits in common with the species in the *C. robusta* complex such as a similar length of median leaflets, or the large size of seed and pollen cones, which may contribute to their confusion. We suggest considering multiple traits when making any diagnosis. In the field, *C. schiblii* can be distinguished as having brown emerging leaves (bright pea-green in *C. mixeorum*), linear or subfalcate-to-falcate leaflets (linear-lanceolate or falcate in *C. mixeorum*), and wider median leaflets of 3–4.5 cm (2.1–2.9 cm in *C. mixeorum*). Furthermore, leaves can be longer in *C. schiblii* (161–262 cm) than in *C. mixeorum* (146–198 cm). In addition, *C. schiblii* adults are unbranched whereas *C. mixeorum* adults sometimes present two or a few branches of nearly equal length, and multiple crowns of leaves. Finally, it is interesting to mention that *C. schiblii* grows at lower elevations, at around 1170 to 1300 m a.s.l., but *C. mixeorum* occurs in cloud forests at high elevations, from 1440 to 1900 m a.s.l. [19], similarly to its closely related species *C. matudae*.

Based on the presented evidence, we conclude that the total number of species belonging to the *C. robusta* species complex is seven. However, the possibility of the discovery of novel wild populations remains open, which may result in an increase in the species of the complex. Further studies to test the distinction of more new species might also be facilitated with the inclusion of anatomical variation analyses, as they have been useful in the case of the *C. miqueliana* species complex [21]. Moreover, further phylogeographic studies in the complex and at the genus level may contribute to the resolution of the relationships among the species, which is an ongoing task that remains unresolved due to incongruencies presented in previous studies [20,22].

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