UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
POSGRADO EN CIENCIAS BIOLÓGICAS
INSTITUTO DE BIOLOGÍA

SISTEMÁTICA Y EVOLUCIÓN DEL GÉNERO *CERATOZAMIA* (ZAMIACEAE, CYCADALES)

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS

PRESENTA:

LILÍ MARTÍNEZ DOMÍNGUEZ

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CIUDAD UNIVERSITARIA, CD. MX., OCTUBRE, 2023.

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Presente

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **19 de junio de 2023** se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la estudiante **MARTÍNEZ DOMÍNGUEZ LILÍ** con número de cuenta **520010868** con la tesis titulada "**SISTEMÁTICA Y EVOLUCIÓN DEL GÉNERO *CERATOZAMIA* (ZAMIACEAE, CYCADALES)",** realizada bajo la dirección del **DR. FRANCISCO ROBERTO VERGARA SILVA,** quedando integrado de la siguiente manera:

Presidente:	DR. ALFONSO OCTAVIO DELGADO SALINAS
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Sin otro particular, me es grato enviarle un cordial saludo.

ATENTAMENTE
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Ciudad Universitaria, Cd. Mx., a 20 de septiembre de 2023

COORDINADOR DEL PROGRAMA

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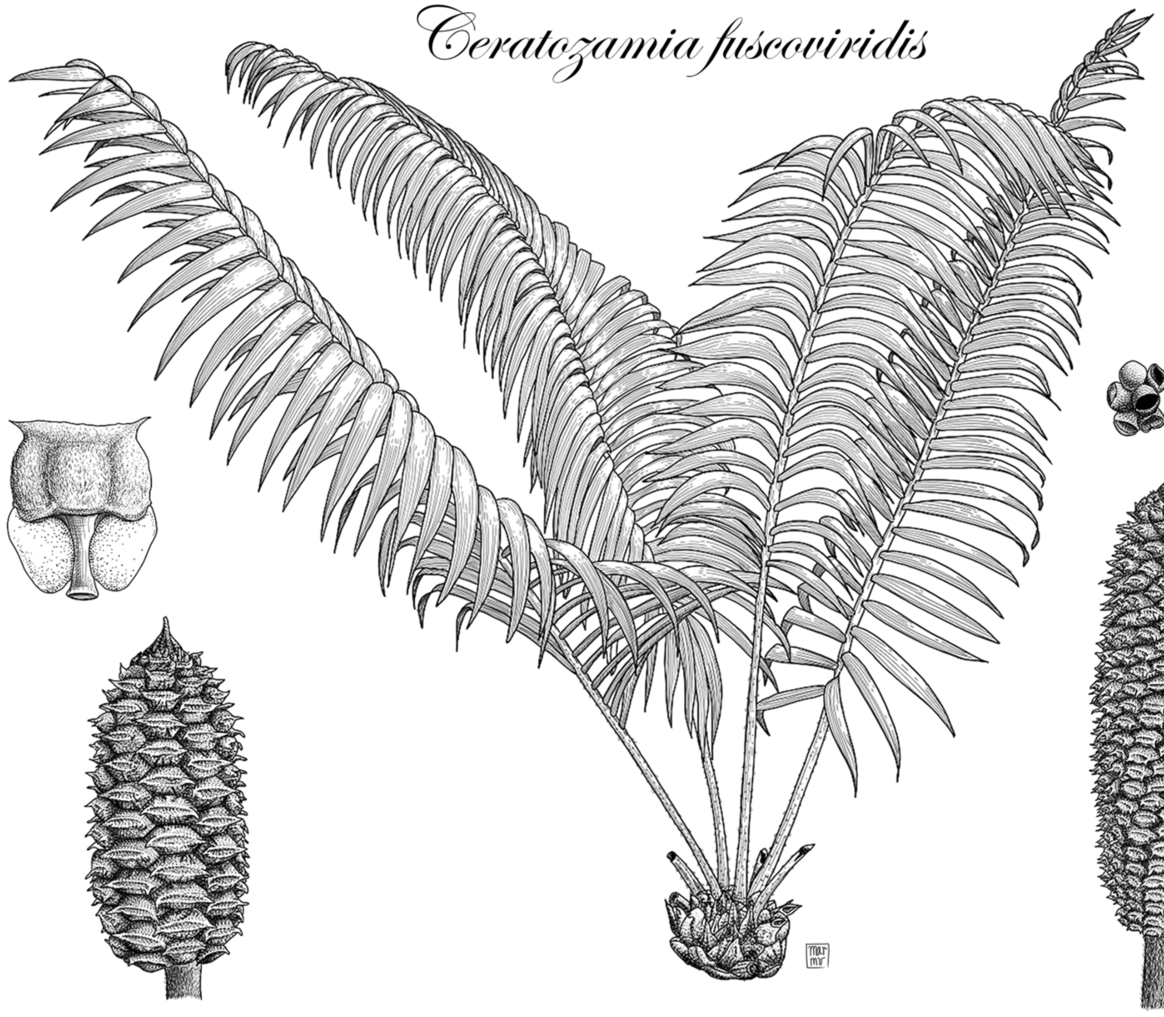
Ceratozamia fuscoviridis



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RESUMEN

Esta tesis se centra en el estudio del género *Ceratozamia* como organismo modelo para abordar cuestiones biológicas claves, que van desde el conocimiento de su diversidad hasta responder preguntas evolutivas. *Ceratozamia* es un linaje de gimnospermas dioicas restringido al Neotrópico, que comprende aproximadamente el 10% de la diversidad del orden Cycadales. Las características morfológicas de las estructuras accesorias de los estróbilos en las gimnospermas han recibido particular atención por su importancia para comprender el origen y evolución de las plantas con semillas. Sin embargo, en el caso de Cycadales el estudio de la variación de los estróbilos ovulíferos y poliníferos, así como la inferencia de los factores intrínsecos y extrínsecos que han dado origen a su diversidad morfológica han sido escasamente abordados. Esto se debe a que los ciclos reproductivos de estas plantas dioicas son largos, al desconocimiento de los patrones fenológicos y la necesidad de sistematización de este tipo de datos en colecciones botánicas. Para responder a estas preguntas se generó un marco taxonómico robusto debido a que *Ceratozamia* ejemplifica y reúne dentro de su diversidad de especies diferentes escenarios que caracterizan, al menos en parte, a los complejos de especies ampliamente distribuidos en la flora Neotropical. Algunos de los casos comunes en estos grupos son la reciente especiación, la similitud morfológica y genética, la alta variabilidad morfológica y la diversidad críptica, los cuales dificultan la tarea de delimitación de especies. Aquí se utilizaron datos morfológicos cualitativos y cuantitativos, moleculares, fenológicos, geográficos y ecológicos para la circunscripción a través de un marco integrado con diversas fuentes de evidencia bajo taxonomía integrativa, que culminó con el planteamiento de una monografía botánica. Se descubrieron cuatro especies nuevas, tres de las cuales habitan en México y una de ellas en Belice. La diversidad de *Ceratozamia* se circunscribió a 36 especies distribuidas desde Tamaulipas en México como límite norte por el oriente y Guerrero en el extremo occidental cuyo patrón de distribución continúa en las zonas montañosas de México, Guatemala y Belice con la región central de Honduras como límite sur. También se clarificaron los límites morfológicos y geográficos entre especies del género, se revisó la nomenclatura, se designaron tipos nomenclaturales, se abordó la distribución de cada especie, incluyendo ampliación del rango de distribución para algunas especies, y se elaboró una clave dicotómica. En relación al marco explícitamente evolutivo, la fenología se evaluó mediante un monitoreo intensivo en poblaciones silvestres de tres especies que comparten afinidades filogenéticas, morfológicas y geográficas. Se

encontró que existe un marcado sobrelape entre las fenofases críticas para el éxito reproductivo (la receptividad y la liberación de polen), lo que permitió inferir que potencialmente podría existir flujo de genes entre poblaciones y que la dinámica de los insectos polinizadores con las plantas conduce, en cierta medida, la especiación. Se elaboró una filogenia utilizando información molecular y morfológica como base para explorar la evolución morfológica de *Ceratozamia* con énfasis en las estructuras reproductivas. En este contexto, los estróbilos ovulíferos fueron más filogenéticamente estructurados y los resultados sugieren: (1) un modelo de selección estabilizadora, y (2) que el fenotipo reproductivo podría ser influenciado por variables ambientales donde las especies habitan. Finalmente, se combinan las perspectivas histórica y contemporánea sobre los conceptos taxonómicos de especies y los métodos de delimitación de especies aplicados en los diferentes géneros del orden Cycadales para discutir los cambios a través del tiempo y los enfoques utilizados. Aquí se argumenta que este conocimiento aunado a los actuales enfoques aplicados en los grupos biológicos, pueden influir en las direcciones futuras de estudio. Para concluir, se señala que la discusión de trabajos taxonómicos y los métodos de delimitación de especies de esta tesis resaltan que la delimitación de especies es un problema desafiante abierto a debate. En general, este estudio mostró como los herbarios contribuyen significativamente al trabajo taxonómico y, por ende, a hacer frente a la crisis de la biodiversidad por la pérdida acelerada de especies. El monitoreo en campo de cícadas debería expandirse de manera sistemática para registrar patrones fenológicos, los cuales podrían proveer datos relevantes para someter a prueba nuevas hipótesis evolutivas.

ABSTRACT

This dissertation focuses on the study of *Ceratozamia* as a model organism to address key biological issues ranging from knowledge of its diversity to answering evolutionary questions. *Ceratozamia* is a lineage of dioecious gymnosperms restricted to the Neotropics that comprises approximately 10% of the diversity in the order Cycadales. The morphological characteristics of the structures of the strobili in gymnosperms have received special attention because of their importance for understanding the origin and evolution of seed plants. However, in the case of Cycadales, the study of pollen and ovulate strobili variation as well as the inferences of the intrinsic and extrinsic factors involved in the origin of morphological diversity in cycads have been scarcely addressed. This is due to the long-life cycles of these dioecious plants, the lack of knowledge of their phenological patterns, and the need to systematize this type of data in botanical collections. To answer these questions, a robust taxonomic framework was generated because *Ceratozamia* exemplifies and brings together within its diversity different species circumscriptions that characterize, at least in part, the species complexes widely distributed in the Mesoamerican Flora. Some of the common issues within these groups are morphological and genetic similarity, high morphological variability, and cryptic diversity that make the task of species delimitation difficult. Qualitative and quantitative morphological, molecular, phenological, geographical, and ecological data were used for species delimitation via an integrated framework with several sources of evidence under an integrative taxonomy approach that culminated in a botanical monograph. Four new species were discovered, three found Mexico and one in Belize. The diversity of *Ceratozamia* was circumscribed to comprise 36 species distributed along the mountainous regions of Mexico, Guatemala, Belize, and Honduras, with the northern limits at the east and west in the Mexican states of Tamaulipas and Guerrero, respectively, and Atlántida Department in Honduras as the southern limit. The morphological and geographic limits among species of this genus were clarified, nomenclature was reviewed, nomenclatural types were designated, the distribution of each species was addressed including range extension for some species, and a dichotomous key was provided. In relation to the explicit evolutionary framework, phenology was evaluated through intensive monitoring in wild populations of three species that share phylogenetic, morphological, and geographic affinities. A marked overlap between critical phenophases for the reproductive success (receptivity and open pollination) was found, which suggests that gene flow among populations along with the dynamic between plant and insect pollinator may play a role in speciation. A phylogeny based on molecular and morphological data was produced to explore the morphological evolution of *Ceratozamia* with an emphasis on reproductive structures. In this context, ovulate strobili were more phylogenetically structured, and the results suggested (1) a stabilizing selection model, and (2) that the reproductive phenotype could be influenced by environmental variables where the species occur. Finally, historical and contemporary perspectives were combined to analyzed taxonomic concepts and species delimitation methods as applied in the different genera within the Cycadales with the goal to discuss the changes through time and the approaches that were used. This knowledge was integrated in the current approaches applied to biological groups and should influence future directions of study. In conclusion, the discussion of taxonomic treatments and species delimitation methods included in this thesis assume that species delimitation is a challenging problem open for debate. Overall, this study showed how herbaria contribute significantly to taxonomic work as we face the biodiversity crisis for accelerated species loss. Field monitoring of cycads should be systematically expanded to record phenological patterns, which could provide relevant data to test new evolutionary hypotheses.

I. INTRODUCCIÓN GENERAL

La especie es la unidad fundamental de trabajo en prácticamente todos los campos de la biología, pero la definición y la delimitación de especies han sido controversiales (Sites & Marshall, 2003, 2004; Rieppel, 2009; Zachos, 2016). A pesar de las diferencias en las definiciones de especies que van desde considerar la monofilia o la parafilia dentro de sus criterios para el reconocimiento de las hipótesis de especies (Sites & Marshall, 2003; Templeton, 1989), la mayoría de los conceptos propuestos coinciden en el rechazo de la polifilia (Wheeler & Meier, 2000). Debido a lo anterior y a la naturaleza intrínseca del proceso evolutivo que puede derivar en discordancia entre los diferentes caracteres que expresan las entidades evolutivas, la delimitación de especies es una tarea cargada de complejidad para la cual se han implementado diversas estrategias (de Queiroz, 2007; Carstens et al., 2013). Aunque existen varias estrategias de delimitación, el enfoque de taxonomía integrativa, que se basa en el uso de diferentes caracteres (fuentes de evidencia) como criterios de delimitación y métodos de análisis, ha resultado prometedor para abordar la problemática relacionada con la discordancia de caracteres (Dayrat, 2005; DeSalle et al., 2005; Padial et al., 2010).

La taxonomía integrativa ha sido de particular interés para el análisis de los complejos de especies, los cuales son grupos de organismos con un número de especies incierto por la dificultad para reconocer los límites entre ellas (Prata et al., 2018). Los complejos de especies se deben a múltiples factores como la alta variación morfológica intra e interespecífica, la alta similitud morfológica entre especies afines y la introgresión (Leliart et al., 2009; Prata et al., 2018). Estos grupos están ampliamente representados en la flora Neotropical y su errónea identificación, así como la ausencia de estudios sobre estos grupos tienen implicaciones directas en la sobre y/o subestimación de la diversidad, y por ende en los métodos que se implementan tanto para la evaluación de las categorías de riesgo como para realizar estrategias eficientes de conservación (Wheeler et al., 2012; Halme et al., 2015). En este trabajo, se sometieron a prueba las hipótesis de especies en el género *Ceratozamia* Brongn., un grupo de cícadas en peligro de extinción con una historia taxonómica caracterizada por la carencia de revisiones taxonómicas inclusivas y una historia evolutiva reciente que está marcada por una alta similitud morfológica vegetativa (Condamine et al., 2015; Martínez-Domínguez et al., 2018a; IUCN, 2022).

La historia de *Ceratozamia* ejemplifica diversos escenarios que están comúnmente presentes en la sistemática de los complejos de especies desde cuestiones directamente ligadas con su evolución hasta aspectos prácticos propios de la taxonomía. Dentro del primer grupo están las especies con numerosos polimorfismos que dificultan el reconocimiento de patrones, tal es el caso de las especies afines a *Ceratozamia miqueliana* H.Wendl., y *C. mexicana* (c.f. Pérez-Farrera et al., 2009; Martínez-Domínguez et al., 2017, 2018a; Medina-Villarreal & González-Astorga, 2016; Vovides et al., 2016). Otras causas son la convergencia morfológica, la cual se ha descrito para formas de folíolos en el género (Stevenson et al., 1986; Medina-Villarreal et al., 2019), la similitud morfológica, misma que está presente en la mayoría de las especies tal como muestra la propuesta de 7 complejos de especies dentro del género y el reciente descubrimiento de diversidad críptica (Vovides et al., 2004; Martínez-Domínguez et al., 2017). En relación a los aspectos *sensu stricto* taxonómicos se encuentran el tipo de caracteres comúnmente analizados y el método para el descubrimiento y delimitación de especies. Contrario a los requerimientos en el género, los taxónomos han centrado los esfuerzos en la descripción de especies nuevas bajo el enfoque tradicional y con escaso énfasis en documentar y aplicar métodos para analizar la variación morfológica, lo cual en conjunto ha limitado el conocimiento sobre el rango de distribución de las especies (e.g. Pérez-Farrera et al., 2007; Haynes et al., 2008; Vovides et al., 2008a,b). Además, en los grupos biológicos con ciclos de vida y reproductivos largos se dificulta el uso de caracteres reproductivos, tal como ha ocurrido en *Ceratozamia*, el cual es un grupo con un sistema dioico en el que los datos de análisis han sido principalmente los vegetativos, mientras que los caracteres reproductivos y su fenología permanecen en el desconocimiento (Norstog & Nicholls, 1997; Stevenson 1981; Vovides et al., 2004; Whitelock, 2004).

Además de las cuestiones relacionadas con la delimitación de especies, *Ceratozamia* pertenece a un linaje antiguo de los denominados comúnmente como “fósiles vivientes” (Renner, 2011), por lo que la evolución de las especies y los caracteres representan desafíos adicionales. Este género de distribución exclusivamente Neotropical con aproximadamente 30 especies está conformado por algunas especies con disyunción geográfica que poseen caracteres morfológicos vegetativos prácticamente idénticos, tal es el caso de *C. hondurensis* J.L.Haynes, Whitelock, Schutzman & R.S.Adams y *C. euryphyllidia* Vázq.Torres, Sabato & D.W.Stev., endémicas a Honduras y a la región de Uxpanapa-Chimalapas en México, respectivamente (Stevenson et al., 1986; Martínez-Domínguez et al., 2018a). También posee especies con escaza diferenciación molecular lo que podría estar relacionado con la reciente especiación del género hace aproximadamente 12 millones de años o con potenciales eventos de hibridación (Pérez-Farrera et al., 2014; Condamine et al., 2015).

Ceratozamia es uno de los géneros más diversos de cícadas cuyo centro de diversidad es México (Nicolalde-Morejón et al., 2014). Las especies son más abundantes en bosque mesófilo de montaña y selva alta perennifolia (Vovides et al., 2004). Se distingue del resto del orden Cycadales por sus esporófilas con dos cuernos en el extremo distal, las cuales, al igual que el resto de las cícadas, están arregladas en estróbilos simples (Norstog & Nicholls, 1997). Aunque se han descrito numerosas especies nuevas y se han realizado varios esfuerzos para abordar las relaciones filogenéticas, la delimitación de especies y las relaciones filogenéticas dentro del género continúan siendo discutibles (González & Vovides, 2002, 2012; Medina-Villarreal et al., 2019). Su taxonomía se basa en la combinación de un conjunto de caracteres morfológicos mayormente homoplásicos y no en sinapomorfías que caractericen a las especies (Martínez-Domínguez et al., 2018a). Esta situación ha conllevado a confusión taxonómica principalmente en las especies del género que se describieron con pocos caracteres morfológicos y que poseen amplia distribución como es el caso de *C. mexicana* y *C. robusta* Miq. (c.f. Medina-Villarreal & González-Astorga, 2016; Vovides et al., 2016). En general, en C*eratozamia* los realineamientos taxonómicos continúan y las revisiones taxonómicas son ausentes cuya última monografía data de casi un siglo cuando la diversidad estaba prácticamente inexplorada (Vovides et al., 2004; Martínez-Domínguez et al., 2018a). En este trabajo se emplean diferentes tipos de caracteres (morfológicos tanto vegetativos como reproductivos, moleculares, ecológicos y biología reproductiva) para delimitación de especies bajo la aproximación de taxonomía integrativa. Bajo estas consideraciones, se presenta la monografía de *Ceratozamia*, la cual es la primera para un género de cícadas Neotropicales. El énfasis de análisis a nivel evolutivo se centra en el uso de caracteres reproductivos y en los patrones fenológicos, ambos poco explorados. Por tanto, utilizamos el registro fósil, una filogenia con una combinación de diferentes tipos de caracteres, la reconstrucción de caracteres ancestrales y las variables ambientales donde las especies habitan para explorar la evolución morfológica en este grupo de gimnospermas.

I.I. *Objetivo general*

Realizar la monografía del género *Ceratozamia,* inferir las relaciones filogenéticas y explorar las potenciales causas de la diversificación de las especies y evolución de los caracteres morfológicos.

I.II. *Objetivos particulares*

- Evaluar el aporte de los caracteres morfológicos cuantitativos y cualitativos con énfasis en los caracteres reproductivos a nivel genérico, en un contexto taxonómico.
- Circunscribir las especies de *Ceratozamia* bajo los supuestos de la taxonomía integrativa, en los casos donde se requiera.
- Realizar el tratamiento taxonómico (monográfico) para el género *Ceratozamia.*
- Evaluar el aporte de los patrones fenológicos reproductivos en la evolución de *Ceratozamia.*
- Reconstruir la filogenia de *Ceratozamia* utilizando evidencia morfológica (cualitativa y cuantitativa) y molecular, empleando matrices combinadas.
- Analizar los patrones de evolución de tamaños de estróbilos ovulíferos y poliníferos en *Ceratozamia*.

I.III. *Metodología general*

I.III.I. *Modelo de estudio*

El género *Ceratozamia* pertenece a la familia Zamiaceae del orden Cycadales, un grupo de plantas comúnmente conocidas como cícadas (Nicolalde-Morejón et al., 2014). Las cícadas son las plantas con semillas más antiguas cuyo origen se remonta a 300 m.a. aproximadamente, las cuales fueron abundantes durante el Mesozoico alcanzando una distribución mundial (Norstog & Nicholls, 1997). Su declive inició en el Cretácico tardío y continúo hasta la actualidad. La diversidad actual es de aproximadamente 350 especies agrupadas en 10 géneros, distribuidas únicamente en las zonas tropicales y subtropicales del mundo (Norstog & Nicholls, 1997; Erdei et al., 2012; Calonje et al., 2013-2023). La distribución actual es restringida y en algunos casos discontinua ocupando las zonas de latitudes alrededor del Ecuador con picos de diversidad en 27° S y 18° N de latitud (Fragniere et al., 2015).

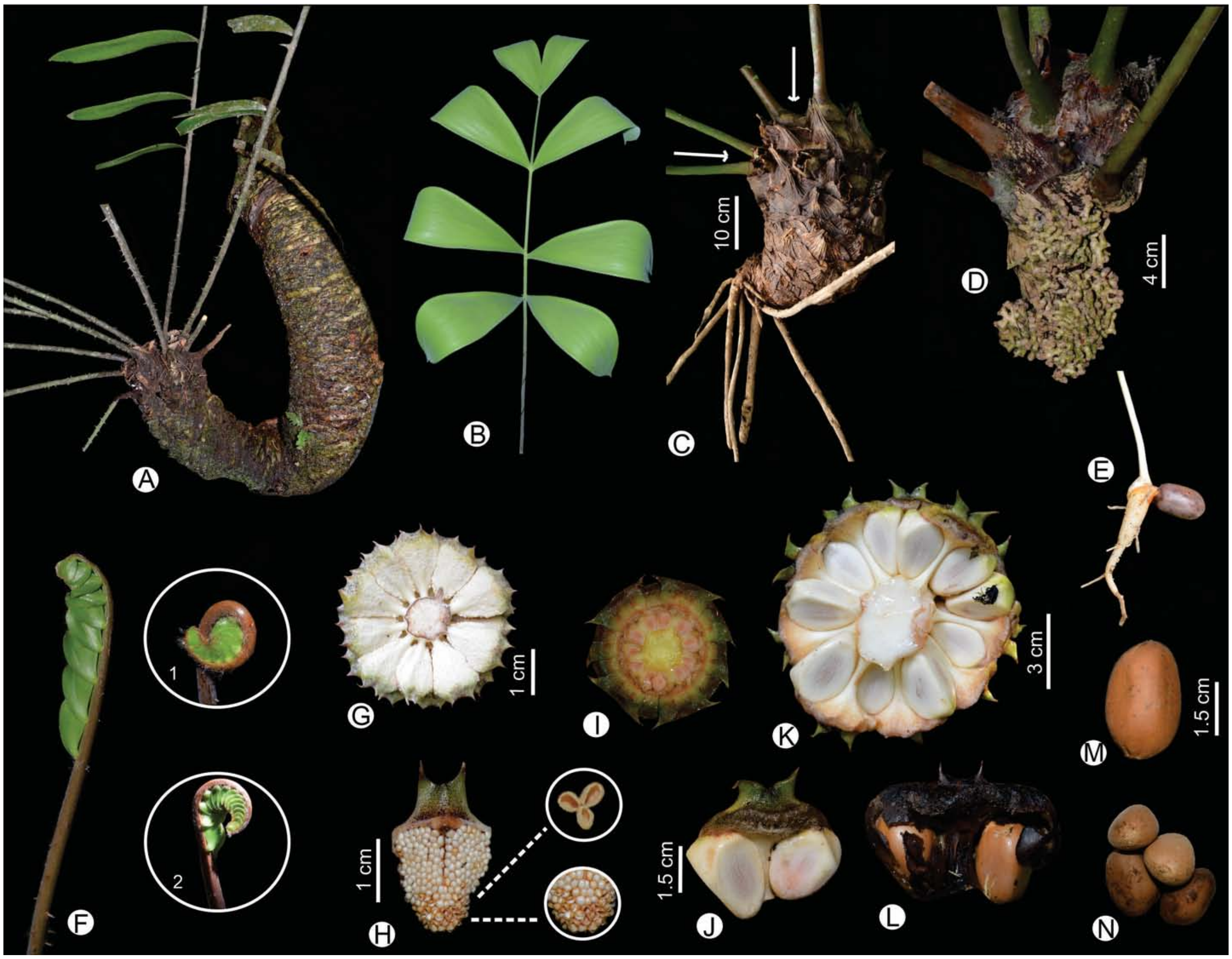

Figura 1. Morfología de una cícada: el género *Ceratozamia*. A. Tallo decumbente. B. Hoja. C. Crecimiento del tallo, las flechas indican el crecimiento simpodial. D. Raíces coraloides. E. Sistema radicular. F. Hoja emergente, 1 vernación, 2 inicio del desarrollo de la hoja. G. Corte transversal del estróbilo polinífero. H. Microsporófila, el círculo superior muestra un sinangio y el círculo inferior muestra detalle del polen en los soros. I. Corte transversal de estróbilo ovulífero inmaduro. J. Megasporófila con la sarcotesta carnosa de la semilla. K. Corte transversal de estróbilo ovulífero maduro. L. Degradación de la sarcotesta de las semillas. M. Semilla madura (se observa esclerotesta). N. Grupo de semillas (se observa la chalaza).

Morfológicamente, las cícadas se caracterizan por sus tallos hipogeos o epigeos cubiertos por las bases de las hojas y los restos de catáfilas (Norstog & Nicholls, 1997; Figura 1). Son de tipo paquicaule por su forma columnar con casi el mismo tamaño desde la base hacia el ápice siguiendo un crecimiento radial, no posee capa de cámbium vascular, y la corteza externa y la médula interna son parenquimatosas (Stevenson, 1980). En el caso de *Ceratozamia* son semihipogeos a epigeos y presentan bifurcación apical (Martínez-Domínguez et al., 2018a; Figura 1). Esta bifurcación sigue una arquitectura con ramificación anisotómica que se da a partir de una pseudo-dicotomía donde una es un brote vegetativo y la otra una reproductiva, lo cual se conoce como modelo de Norstog (Stevenson, 1988, 2020). *Ceratozamia* presenta tallos bifurcados que pueden tener hasta 5 ápices, cada uno produciendo estructuras tanto vegetativas como reproductivas.

En general, las cícadas poseen tres tipos de hojas que surgen desde el mismo meristemo apical donde crece el tallo (Figura 1): 1) las catáfilas que son estructuras de protección producidas en grupos; 2) los nomófilos (hojas fotosintéticas) pueden tener diferentes colores y formas; y 3) las esporófilas que contienen los gametos masculinos (polen) o femeninos (óvulos) (Norstog & Nicholls, 1997; Jones, 2002). En *Ceratozamia,* los tres tipos de estructuras poseen tricomas en mayor o menor abundancia (Stevenson, 1981). Las catáfilas son triangulares completamente o parcialmente cubiertas por tricomas (Stevenson, 1981). Los nomófilos son compuestos por un raquis y pecíolo cilíndrico a subcilíndrico donde el primero posee dos canales paralelos, y folíolos dispuestos de manera opuesta a subopuesta; los folíolos poseen margen entero, la base carece de callosidades o anillos en la inserción con el raquis (Brongniart, 1846; Vovides et al., 2004; Martínez-Domínguez et al., 2018a).

Las esporófilas tanto poliníferas como ovulíferas están arregladas de manera helicoidal a lo largo de un eje central con excepción del género *Cycas* L., donde las esporófilas ovulíferas están colocadas una sobre la otra sin una estructura determinada (Norstog & Nicholls, 1997). El arreglo de las esporófilas se denomina estróbilo, el cual es una estructura simple (Norstog & Nicholls, 1997). Los estróbilos poseen una parte infértil que recibe el nombre de pedúnculo, generalmente cubierto por tricomas, y una porción fértil donde se insertan las esporófilas sobre un eje infértil. En *Ceratozamia* las esporófilas poseen un extremo distal con 2 cuernos y en el extremo apical del estróbilo (tanto ovulífero como polinífero) se presenta una estructura infértil que es una prolongación independiente (Norstog & Nicholls, 1997; Nicolalde-Morejón et al., 2014; Figura 1).

El polen de las cícadas es longitudinalmente monosulcado y bilateralmente simétrico (Dehgan & Dehgan, 1988). Está contenido en los sacos polínicos o esporangios que se abren longitudinalmente, los cuales están agrupados formando sinangios y dispuestos sobre toda la parte interna de la microsporófila (Dehgan & Dehgan, 1988; Vovides et al., 2020; Figura 1). Los granos son elípticos a ligeramente circulares con exina conspicua en *Ceratozamia* (Chamberlain, 1912). Cada esporófila ovulífera posee dos óvulos dispuestos en ambos lados de la megasporófila que están separados por un eje (Norstog & Nicholls, 1997). La excepción se presenta en *Cycas* donde pueden existir más de 2 óvulos por cada una de estas estructuras que son más similares a una hoja (Norstog & Nicholls, 1997). Las semillas poseen una endotesta interior fina, una esclerotesta que es rígida de color café a café claro y una sarcotesta exterior carnosa (Chamberlain, 1912). Esta última posee colores muy brillantes en la mayoría de los géneros; sin embargo, en *Ceratozamia* es de color generalmente pálido que va de un tono rosa pálido a ligeramente blanco amarillento (Martínez-Domínguez et al., 2018a; Figura 1).

Las cícadas poseen dos tipos de raíces que se ramifican formando un sistema complejo (Norstog & Nicholls, 1997; Figura 1). La raíz principal es fuertemente contráctil y desarrolla crecimientos secundarios. El segundo tipo de raíz son las coraloides que son asociaciones entre las raíces de la planta con cianobacterias; éstas presentan crecimiento lateral y se desarrollan sobre la superficie del suelo formadas a partir de raíces jóvenes papilosas denominadas precoraloides (Ahern & Staff, 1994).

I.III.II. *Materiales y métodos*

Esta tesis posee cuatro ejes básicos de análisis, los cuales están estrechamente interrelacionados (Figura 2): 1) revisión de bibliografía científica, 2) revisión de colecciones científicas, 3) trabajo de campo y 4) trabajo de laboratorio. En general, el flujo de trabajo para este proyecto de tesis fue dinámico tanto dentro de cada eje como entre ellos.

La revisión de bibliografía científica fue el punto de partida, en particular para recopilar los nombres con las correspondientes descripciones originales (protólogos) de las especies descritas de *Ceratozamia*. Además, permitió reunir información histórica sobre el uso de los nombres para conocer los diferentes conceptos taxonómicos de las especies. Esto implica sintetizar información de revisiones taxonómicas parciales, Floras regionales (como Flora mesoamericana, floras estatales, entre otras) y monografías. Se generó una síntesis integral de información sobre el género que permitió trazar la historia de los conceptos taxonómicos históricos, los conceptos de especie y detectar los puntos focales, es decir, grupos de especies para un análisis más detallado a nivel de delimitación de especies.

El segundo eje corresponde a la revisión de colecciones científicas, lo cual es fundamental considerando que los especímenes son una fuente central de información para taxonomía y evolución. En total se revisaron, ya sea de manera física o electrónica, aproximadamente 1450 ejemplares botánicos depositados tanto en herbarios nacionales como extranjeros. También se consultó el portal Global Plants web portal (http://plants.jstor.org/). Con esta información se generó una base de datos geográfica con coordenadas, elevación y tipos de vegetación, y una base de datos morfológica asociada al ejemplar revisado con datos del colector y fecha de colecta. Estos dos puntos permitieron detectar áreas donde era necesario realizar exploración botánica debido al bajo muestreo, seleccionar casos potenciales de análisis o que requerían realizar correcciones de identificación y estimar las fechas óptimas para realizar el trabajo de campo en función de la probabilidad de encontrar estructuras reproductivas y/o de su grado de maduración.

El trabajo de campo se dividió en dos actividades, la recolecta de ejemplares botánicos y monitoreo en campo. La primera parte en este género fue vital debido a que la mayoría de los ejemplares botánicos depositados en los herbarios son infértiles. Además, uno de los objetivos fue documentar la variación intra e interpoblacional, por lo que la recolecta a nivel poblacional fue necesaria para caracterizar la variación al interior de las poblaciones. En total se realizaron recolectas en 94 poblaciones que representan al total de especies de *Ceratozamia*, de las cuales 45 corresponden a recolectas realizadas explícitamente durante el transcurso de este proyecto de investigación y se realizaron principalmente en la zona centro y sur de México, y en Honduras. Este muestreo consideró cubrir el rango de distribución para cada una de las especies. En cada población se recolectaron y/o midieron aproximadamente 20 individuos para documentar la variación morfológica. Por otra parte, el monitoreo de poblaciones en algunas especies fue para obtener datos fenológicos (medir los estróbilos, registrar cambios de coloración, entre otros) para lo cual se realizaron visitas periódicas a las poblaciones. Esto permitió detectar patrones con fines evolutivos y establecer fechas de colecta, en particular en aquellas especies donde se desconocía por completo la fenología.

El eje final fue el trabajo de laboratorio mismo que consistió en realizar extracción de ADN, amplificación y secuenciación de un conjunto de marcadores moleculares. Las secuencias fueron empleadas a diferente nivel para circunscripción y para los análisis filogenéticos. En el primer caso se utilizó un enfoque poblacional para las especies considerando cinco (5) individuos para cada población de las especies, mientras que en el segundo caso un (1) individuo por especie.

I.IV. ***Estructura de la tesis***

Los análisis realizados para cubrir cada uno de los objetivos de la tesis, así como los resultados obtenidos se presentan a manera de artículos científicos. La tesis está estructurada en 5 capítulos que contienen en total 8 artículos científicos. Algunos de los resultados permitieron la preparación de 3 artículos de divulgación científica los cuales están publicados (ANEXO I, II, III). Además, las descripciones de especies distribuidas en la región mesoamericana se prepararon para el proyecto Flora Mesoamericana, mismo que se encuentra aceptado para publicación.

El capítulo I consiste en la delimitación de especies, por lo que, incluye la circunscripción del complejo de especies distribuido en la región del Soconusco y la descripción de 3 especies nuevas. El abordaje de estos grupos de especies surgió de la revisión de literatura y revisión de colecciones científicas (Figura 2), que mostraron los siguientes escenarios: 1) poblaciones de *Ceratozamia* en Guerrero no previamente reportadas y que representaron una ampliación del rango de distribución del género; 2) *Ceratozamia* en la Sierra Norte de Oaxaca; 3) el complejo de especies *C. robusta* y 4) las especies con distribución en el Soconusco cuyos límites morfológicos y geográficos eran poco definidos.

El capítulo II presenta la monografía botánica de *Ceratozamia,* el cual fue seleccionado como artículo de requisito. En este apartado se someten a prueba directamente las hipótesis de especies para que sean reproducibles y comprobables como entidades biológicas. Se incluyen mapas de distribución para cada una de las especies, descripciones detalladas, caracterización del hábitat, ejemplares revisados y notas taxonómicas puntuales para cada especie. También se incluyó una clave de identificación para el género, descripción de los caracteres utilizados, sinonimias y designación de tipos nomenclaturales. Además, en este apartado, como parte del tratamiento monográfico, se describió una especie nueva.

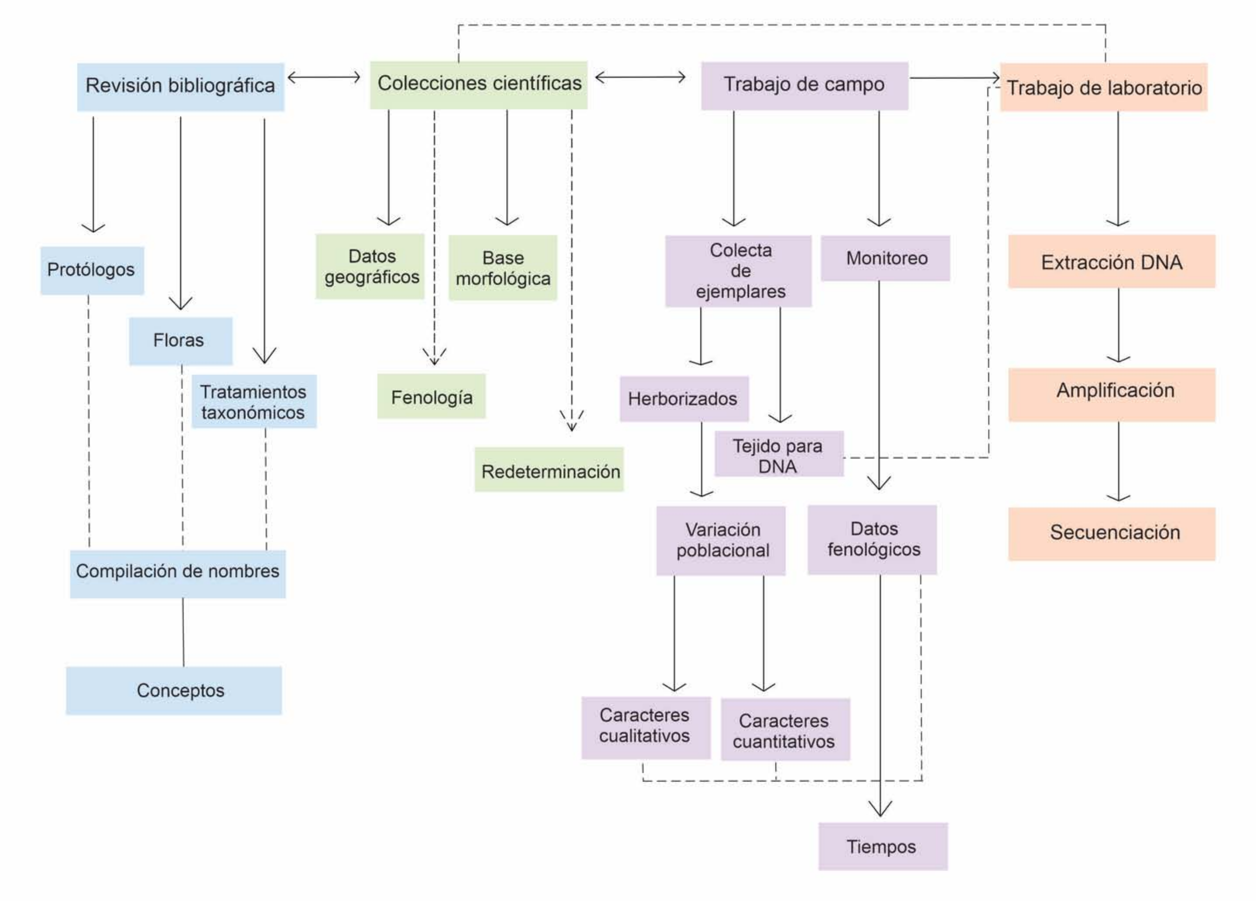

Figura 2. Flujo de trabajo desarrollado para el proyecto sistemática y evolución del género *Ceratozamia*.

El capítulo III se centra en la fenología. Esta área es una de las menos estudiadas no sólo en el género *Ceratozamia* sino en el orden Cycadales. Aquí se muestran los resultados de un monitoreo intensivo realizado en la región central de Veracruz con tres especies como modelo. Se discuten las implicaciones generales de los patrones fenológicos en el contexto evolutivo y los retos para conocer los patrones fenológicos.

En el capítulo IV se incluyen dos ejes: 1) la reconstrucción filogenética de *Ceratozamia* utilizando diferentes fuentes de evidencia y 2) la evolución de caracteres. El énfasis está en las estructuras reproductivas, particularmente sobre los patrones de evolución de los tamaños de los estróbilos ovulíferos y poliníferos que permiten generar hipótesis sobre los modelos evolutivos de estas estructuras. Además, se evalúa el potencial aporte de las variables ambientales como conductores de la especiación en este género. Esta integración de evidencia junto con los escenarios paleogeográficos y paleoecológicos que han tenido lugar en las zonas donde se distribuyen las especies extintas y extantes del género, permiten la discusión de potenciales escenarios evolutivos.

Finalmente, el capítulo V es producto del proceso de candidatura. En esta sección se analizan y describen las tendencias de publicación de especies nuevas, los conceptos taxonómicos históricos de las especies y los conceptos de especies utilizados en el orden Cycadales. Además, se discuten perspectivas evolutivas con base en los diferentes estudios realizados para abordar escenarios de especiación y diversificación desde diferentes áreas como la ecología, la biología del desarrollo, entre otras.

II. RESULTADOS

II.I. Capítulo I.

Describiendo la diversidad en *Ceratozamia*: delimitación de especies a través de diferentes escalas

II.I.I. A novelty in *Ceratozamia* (Zamiaceae, Cycadales) from the Sierra Madre del Sur, Mexico: biogeographic and morphological patterns, DNA barcoding and phenology. 2020. PhytoKeys, 156: 1–25. DOI: 10.3897/phytokeys.156.53502

II.I.II. A new Mexican *Ceratozamia* (Zamiaceae) species with notes on reproductive phenology. 2022. Nordic Journal of Botany, 2022(1): 1–11. DOI: 10.1111/njb.03348

II.I.III. *Ceratozamia oliversacksii* (Zamiaceae), a new species of gymnosperm from western Oaxaca, Mexico. 2022. Kew Bulletin, 77: 211–219. DOI: 10.1007/S12225-021-09992-X

II.I.IV. Species delimitation in *Ceratozamia* (Zamiaceae) from Southwestern Mexico, in light of reproductive and climatic diversification. 2023. Organisms Diversity & Evolution, 23: 275–293. DOI: 10.1007/s13127-022-00598-0

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A novelty in *Ceratozamia* (Zamiaceae, Cycadales) from the Sierra Madre del Sur, Mexico: biogeographic and morphological patterns, DNA barcoding and phenology

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Abstract

Ceratozamia is a genus of cycads occurring in eastern Mexico and Central America. In this study, we describe a new species from the Pacific region of Mexico in Guerrero state. This locality represents the most northwestern Mexico distribution for the genus. We focus the comparison of this species with the most geographically proximate and phenotypically relevant lineages for this taxon. We followed an integrative taxonomy approach to evaluate the classification of these species, including geographic location, morphology, DNA barcoding and phenology as primary sources of systematic data. Within the morphological dataset, reproductive structures are described in detail and new characters are proposed for microsporophylls. The comparative morphology of these structures facilitated the elucidation of differences in forms and species for identification. The two chosen DNA barcoding markers – namely, the chloroplast genome coding region *matK* and the nuclear ribosomal internal transcribed spacer (ITS) region – had low divergence, allowing only 61% of species identification, suggesting slow molecular evolutionary rates. Besides employing these three basic sources of evidence, we introduced phenology as additional information for species circumscription. In addition, this work includes a brief review of the genus at the species-level. This is therefore the most recent review for *Ceratozamia* across its full geographic range (latitudinal and elevational). Overall, this work further contributes to a comprehensive framework for systematic studies in Mexican cycads.

Keywords
cloud forest, cycads, Guerrero, integrative taxonomy

Introduction

The Sierra Madre del Sur (SMS) is a biogeographic province assigned to the Mexican Transition Zone, which includes neartic and neotropical ecosystems (Marshall and Liebherr 2000). This Mexican province runs northwest-southwest parallel to the Pacific Ocean coast throughout 1,200 km. It has great diversity of species, due to its high climatic and topographical heterogeneity (Morrone 2005; Santiago-Alvarado et al. 2016). Its rugged orography has been influenced by ancient geological events. In parallel, regional climatic cycles are relevant to explain its ecological heterogeneity, including overlaps between the Neotropical and Nearctic biotas, both of which house high species richness and endemics (Morrone 2010). The SMS has been divided into three subprovinces, in which the eastern Sierra Madre del Sur subprovince is the most heterogeneous in structural composition. This subprovince includes two districts: the Guerrero and Oaxaca highlands (Morrone 2017).

One of the least represented habitats in the SMS, and perhaps the least studied, is the SMS cloud forest. This type of vegetation is distributed unequally between 600 and 1,800 meters of elevation, and has a very disjointed and fragmented range caused by different climatic cycles (Luna-Vega et al. 1999). Moreover, the cloud forest is the most diverse habitat in Mexico per unit area and is found in small fragmented zones mainly in the Guerrero and Oaxaca highland districts (Rzedowski 1996; Challenger 1998).

The distribution of *Ceratozamia* Brongn. (Cycadales) is restricted to areas with high humidity in the main mountain systems of Mexico and Central America. The genus occurs in a spectrum of habitats: evergreen tropical forest, oak-pine forest, and cloud forest in the Sierra Madre Oriental (SMO), Sierra Madre de Chiapas (SMCh), SMS proper, and mountains of eastern Central America. The greatest diversity of this genus is found in the SMO. In the SMS, *Ceratozamia* has only been reported in Oaxaca (Vovides et al. 2004; Contreras-Medina 2016), but the north central portion of the SMS also may have suitable habitats for the genus. This is particularly so in Guerrero, which has small patches of cloud forest and is part of this biogeographic province (Luna-Vega et al. 1999).

Ceratozamia is easily diagnosed by the presence of two horns at the distal end of the sporophylls (Norstog and Nicholls 1997). This genus is one of the most complex morphological assemblages within the family Zamiaceae, because some of its species show considerable intrapopulation variation and most characters commonly used to diagnose species in other cycad genera are polymorphic (Martínez-Domínguez et al. 2017). Taxonomic and molecular systematic studies have contributed to clarify species identification and to provide taxonomic revisions with keys at the regional level (e.g. Pérez-Farrera et al. 2009; Martínez-Domínguez et al. 2016, 2017, 2018a). Generally, quantitative morphological characters provide limited information among closely related species (Martínez-Domínguez et al. 2017). However, reproductive characters have been poorly studied at the species level, both morphologically and in terms of phenological cycles. Detailed studies and descriptions of organs in the reproductive structures – mainly micro- and megasporophylls – could yield potentially useful diagnostic characters in the genus (Martínez-Domínguez et al. 2018a,b).

Recently, during a review of collections of *Ceratozamia* deposited at the FCME herbarium, our research group found a specimen collected in 1984 from the state of Guerrero. However, this material lacked reproductive structures indispensable for unequivocal identification. Because the previously known biogeographic pattern of this genus was restricted to southeastern and central Mexico, this discovery was a novelty, as the corresponding coordinates would represent the northernmost locality of *Ceratozamia* for the Mexican Pacific. Given that similar specimens from the same geographic point had not been collected again, we explored the corresponding area in search of this underdescribed population. After conducting extensive fieldwork in Guerrero to collect fresh material and monitor the attendant reproductive process, we compared the new specimens with all known species in the genus with an initial focus on similar species using geographic and morphological criteria. Formally, we adopted an integrative taxonomic approach (*sensu* De Salle et al. 2005; Goldstein and DeSalle 2010) that involves a set of inferential rules for corroboration or refutation of species hypotheses based on multiple sources of evidence ('taxonomic circle' *sensu* DeSalle et al. 2005). Elsewhere, we have successfully used this approach for species-level identification in *Ceratozamia*, explicitly considering intraspecific morphological variation (Martínez-Domínguez et al. 2017). To identify this unidentified herbarium sheet and additional specimens from two Guerrero populations, which ultimately turned out to match the new taxon herein described, we use geographic location information, both qualitative and quantitative morphological characters, character-based DNA barcoding and phenological data. Finally, we have explored how this taxonomic discovery might alter our understanding of biogeographic and evolutionary patterns in *Ceratozamia*.

Materials and methods

Specimen collection and morphological character coding

Twenty-one specimens were collected for the newly described taxon from two localities in Guerrero, Mexico. Leaf tissue was collected from all individuals for DNA sequencing and preserved in silica-gel. In total, we sampled 8 to 10 specimens approximately per population for *Ceratozamia robusta* Miq., and *C. subroseophylla* Mart.-Domínguez & Nic.-Mor., three and two populations, respectively (Appendix I). These specimens were collected during 2014 and 2017 by our research group, which have been deposited at the CIB and MEXU. In these collections, we have included the type locality and associated populations that correspond to the distribution range. Species selection was further based upon morphological similarities with the initial *Ceratozamia* specimen from Guerrero. For this species, a set of 40 continuous, quantitative characters were coded along with another series of 39 discrete, qualitative characters. Additionally, we examined all herbarium specimens for the 30 species recognized in the genus, which are deposited at CIB, ENCB, FCME, MEXU, US, NY, and XAL. From the herbarium specimens for all species, we evaluated a set of 25 continuous and 23 discrete characters.

Biogeographic information for specimens

Herbarium specimens of *Ceratozamia* were reviewed and their geographic coordinates were used to a compile a database. This information was verified in the geographic information system ArcMap GIS v.10.2. Ambiguous and/or doubtful geographic data were omitted; in cases where precise locality data were available, we georeferenced each locality with Google Earth Pro (2020) (http://earth.google.es/). To determine elevation for all registered samples, points of occurrence were superimposed on the 'digital elevation model' available from CONABIO (Guevara and Arroyo-Cruz 2016). The vegetation type for the populations of Guerrero taxon was characterized and classified following the biogeographical provinces of cloud forest according to CONABIO (2008).

DNA extraction, PCR amplification and DNA sequencing

Genomic DNA was extracted from five individuals for each population collected in Guerrero using the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). Additionally, DNA was extracted from a single individual for *Ceratozamia chamberlainii* Mart.-Domínguez, Nic.-Mor. & D.W. Stev., *C. delucana* Vázq.Torres, A. Moretti & Carvajal-Hern., *C. mexicana* Brongn., and *C. totonacorum* Mart.-Domínguez & Nic.-Mor. Samples from these taxa were not included in previous works (cf. Nicolalde-Morejón et al. 2011; Martínez-Domínguez et al. 2017; Medina-Villarreal et al. 2019). For amplification of the nuclear ribosomal internal transcribed spacer (ITS) region, primers 5a fwd (5'CCTTATCATTTAGAGGAAGGAG3') and 4 rev (5'TCCTCCGCTTATTGATATGC3') were used, whereas primers fwd (5'ATACCCCATTTTATTCATCC3') and rev (5'GTACTTTTATGTTTACGAGC3') were used for the maturase K (*matK*) chloroplast genome locus. The latter region was amplified in *Ceratozamia subroseophylla* (an ITS region sequence was previously available) and included in the molecular matrix. Choice of markers followed previous publications where they performed best in terms of number of diagnostic sites (Martínez-Domínguez et al. 2016, 2017). Amplification products were visualized in 1% ethidium bromide-stained, agarose gels. Bands of the expected molecular weight were purified with a QIAquick PCR Purification Kit (QIAGEN) and sent to Laboratorio de Secuenciación Genómica de la Biodiversidad y la Salud (LANABIO; Instituto de Biología, UNAM) for automated DNA sequencing. All DNA sequence data were deposited in GenBank.

Character-based DNA barcoding

Electropherograms were edited and assembled using the program Sequencher v.4.8 (Gene Codes Corp., Ann Arbor, MI, USA). Sequences were aligned in BioEdit v.7.0.9 using the 'multiple alignment' option in Clustal X (Thompson et al. 1997; Hall 1999). We manually checked and edited these alignments with MacClade v.4.03 (Maddison and Maddison 2001). A matrix of these sequences, plus a subset of sequences for *Ceratozamia* including previously published ITS and *matK* sequences currently available in GenBank, was then saved in Nexus format for further use (Suppl. material 1: File S1). All markers were assembled with SequenceMatrix v.1.7.8 (Vaidya et al. 2011). The resulting dataset corresponding to all species currently and correctly ascribed to the genus in Mexico (Nicolalde-Morejón et al. 2011; Martínez-Domínguez et al. 2017) was subject to character-based DNA barcoding in CAOS (Character Attribute Organization System; Sarkar et al. 2008). Following the recommendations in the CAOS manual, a guide tree was prepared using the molecular matrix of *Ceratozamia* DNA sequences. The total length of the concatenated ITS and *matK* data matrix was 1813 nucleotide sites, including gaps. Cladistic analyses on this matrix in TNT v.1.5 (Goloboff et al. 2008) generated nine shortest, equally parsimonious trees. Because relationships in the corresponding strict consensus tree were not completely resolved (Suppl. material 2: Fig. S1), this tree was manually edited to avoid polytomies, preserving the arrangement of resolved clades in all trees. The topology of the resulting guide tree was saved in the Nexus format for further CAOS analyses. Identification of DNA diagnostics was carried out with the *P-gnome* program. Only simple pure ('sPu' *sensu* Sarkar et al. 2008) characters (i.e. attributes) with confidence value of 1.00 were selected.

Reproductive phenology data

The populations registered for the Guerrero taxon, as well as two populations for both *Ceratozamia robusta* and *C. subroseophylla*, were monitored for phenological observations of ovulate strobili (Appendix I). In addition, herbarium specimens of known wild collections in CIB, MEXU, and XAL were examined for complementary information in this regard (Suppl. material 3: File S2). All observations were categorized into one of the following three states, which were determined according to Martínez-Domínguez et al. (2018b): (i) receptivity (R); (ii) late ovulate (LO); and (iii) degraded (D). Determining phenological patterns for other species in the genus was not possible due to the lack of reproductive structures in herbaria.

Results

Biogeographic patterns

The genus *Ceratozamia* has a continuous but restricted distribution along two of the major mountain ranges in Mexico – namely, the Sierra Madre Oriental (SMO) and the Sierra Madre del Sur (SMS), Belize, and some lowlands in Los Tuxtlas (Veracruz, Mexico) and Honduras (Fig. 1). Elevational ranges for most species are broad, usually occurring between 800 to 1,400 meters. However, some species are distributed at the extremes of this general pattern. *Ceratozamia miqueliana* H.Wendl., can occur at elevations as low as 19 meters, and *C. zaragozae* Medellín-Leal at elevations up to 2,030 meters (Fig. 2). The species with the greatest elevation range are *C. fuscoviridis* W. Bull, *C. miqueliana*, *C. robusta*, and *C. zoquorum* Pérez-Farr., Vovides & Iglesias. In turn, the two species with the narrowest range of elevational variation are *C. hildae* G.P.Landry & M.C.Wilson and *C. euryphyllidia* Vázq.Torres, Sabato & D.W.Stev. The new populations registered from Guerrero are the northernmost for the genus on the western ranges of the coastal Pacific Ocean region; this location corresponds to the northern end of the SMS province, from 1,100 to 1,400 meters of elevation (Fig. 1).

The *Ceratozamia* taxon herein described from Guerrero is found in relictual cloud forest and the transition zone between cloud forest and oak forest on rocky limestone slopes. In contrast, *C. robusta* is found in evergreen tropical forest and oak forest, whereas *C. subroseophylla* only inhabits evergreen tropical forest. These two related *Ceratozamia* species occur on clay soils with isolated rocks of volcanic origin, whereas the Guerrero taxon occurs on karstic rocks. In the context of classifications of biogeographical provinces of cloud forest, the Guerrero taxon occurs in southern coastal mountain range and the Putla subregion.

Comparisons of quantitative and qualitative morphological characters

In terms of vegetative morphology, three general groups of species within *Ceratozamia* can be distinguished. These groups include plants with (i) very wide leaflets, between (2.5) 4.5–17.6 cm, oblong to oblanceolate and obovate, (ii) wide leaflets of 2.3–4.6 cm, lanceolate to linear, and (iii) narrow leaflets, 0.8–2.2 cm wide, linear to lanceolate. The first group includes the species *C. miqueliana*, *C. zoquorum*, *C. latifolia* Miq., *C. huastecorum* Avendaño, Vovides & Cast.-Campos, *C. euryphyllidia*, *C. decumbens* Vovides, Avendaño, Pérez-Farr. & Gonz.-Astorga, *C. hildae*, *C. morettii* Vázq.Torres & Vovides, *C. santillanii* Pérez-Farr. & Vovides, *C. hondurensis* J.L.Haynes, Whitelock, Schutzman & R.S.Adams, *C. chamberlainii*, and *C. totonacorum*. Plants belonging to this group of species also have hypogeous stems, few leaves, and small ovulate strobili, with the exception of *C. miqueliana*, *C. chamberlainii*, and *C. totonacorum*, which have ovulate strobili up to 30 cm long and epigeous stems. The second group has epigeous stems, many leaves, and cylindrical and long ovulate strobili; this group contains

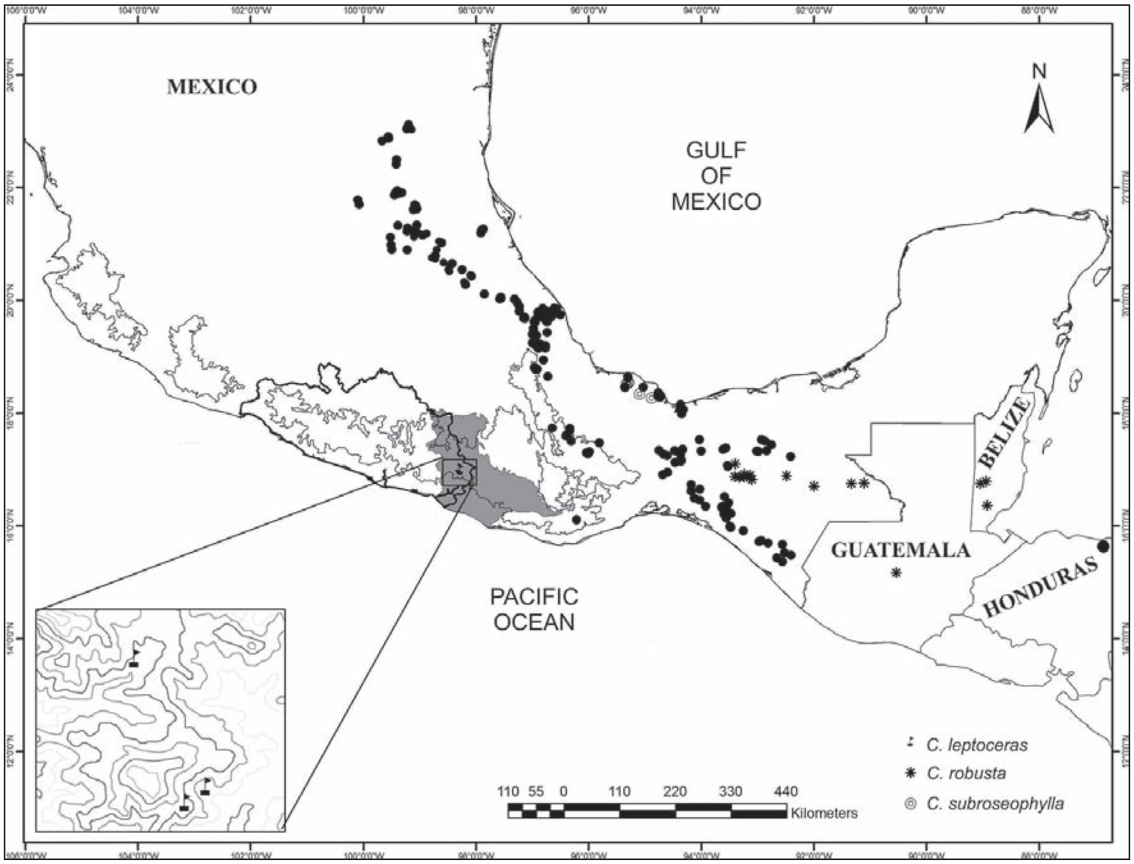

Figure 1. Distribution map of the genus *Ceratozamia* (black solid circles). Distributions for species morphologically similar to *Ceratozamia leptoceras* are represented with an asterisk and a double circle. Guerrero state and Putla subregion of cloud forest are shown with outline and shaded area, respectively. Inset: points corresponding to the Guerrero mountain range where *Ceratozamia* was collected.

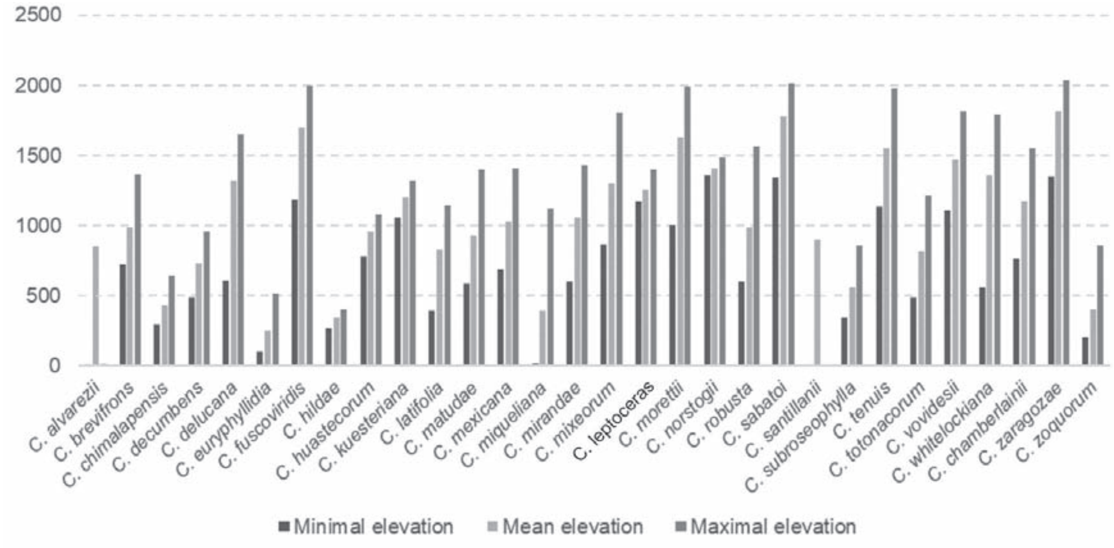

Figure 2. Elevational distribution of *Ceratozamia*. Intervals of altitudinal distribution of *Ceratozamia* species are shown.

C. mexicana, *C. subroseophylla*, *C. robusta*, *C. whitelockiana* Chemnick & T.J.Greg., *C. mixeorum* Chemnick, T.J.Greg. & Salas-Mor., *C. delucana*, and *C. brevifrons* Miq. The third group has epigeous and semi-epigeous stems and greater variation in relation to the size of ovulate strobili; it includes *C. zaragozae*, *C. norstogii* D.W.Stev., *C. alvarezii* Pérez-Farr. Vovides & Iglesias, *C. mirandae* Vovides, Pérez-Farr. & Iglesias, *C. vovidesii* Pérez-Farr. & Iglesias, *C. matudae* Lundell, *C. tenuis* (Dyer) D. W. Stev. & Vovides, *C. sabatoi* Vovides, Vázq.Torres, Schutzman & Iglesias, *C. chimalapensis* Pérez-Farr. & Vovides, *C. fuscoviridis*, and *C. kuesteriana* Regel. In preparation for the integrative taxonomy analysis, morphological comparisons revealed that the taxon from Guerrero has epigeous stems, median leaflets of 1.9–2.8 cm width, and long ovulate strobili. These data strongly suggest affinity of the Guerrero taxon to the second group.

In this context, the Guerrero taxon has a close morphological similarity to *Ceratozamia subroseophylla* and *C. robusta*; however, it has differences when compared to all species within the second group (see the taxonomic key below, for more details). Further morphological comparisons between these three entities reveal that only a few vegetative morphological characters provide support for species delimitation. The detailed morphological differences between *C. subroseophylla*, *C. robusta* and the Guerrero taxon are listed in Tables 1, 2. In this regard, the leaves of the Guerrero taxon show great morphometric affinity with *C. robusta* and *C. subroseophylla* with a leaf length of ranging from 1.20 to 2.80 meters. In contrast, the leaf position of the new taxon is descending, whereas in the other two species it is erect (Table 1). Additionally, the Guerrero taxon bears linear and membranaceous leaflets in contrast to *C. robusta* and *C. subroseophylla*, which have lanceolate and papyraceous to subcoriaeous leaflets

Table 1. Comparison of diagnostic qualitative morphological characters between *Ceratozamia leptoceras* and morphologically similar species.

Characters	Species		
	C. leptoceras	***C. robusta***	***C. subroseophylla***
Leaf color at emergence	*Green with copperish-green petiole*	Dark brown	Yellowish-brown
Leaf position	*Descending*	Ascending	Ascending
Prickles on petiole	*Thin*	Robust	Robust
Leaflet shape	*Linear*	Lanceolate	Lanceolate
Leaflet texture	*Membranaceous*	Papyraceous	Papyraceous
Leaflet base color	Green (brown only in articulation)	Green (yellow in juvenile leaves)	Green (brown in juvenile leaves)
Ovulate strobilus color	*Copperish-green with greyish-black pubescence*	Dark green with dark trichomes	Rosaceous-green with brown trichomes
Ovulate strobilus apex	*Acute*	Acuminate	Mucronate
Megasporophyll horns shape	Straight	Straight	Straight
Megasporophyll distal face form	Prominent	Prominent	Prominent
Microsporophylls horns direction	Straight	Recurved	Recurved
Microsporophylls horns shape	*Thin*	Robust	Robust
Infertile portion of microsporophylls	*Linear*	Rounded	Rounded
Fertile portion of microsporophylls	Deeply lobate	Deeply lobate	Lobate

Autapomorphic character states are shown in italics.

Table 2. Comparison of diagnostic quantitative morphological characters between *Ceratozamia leptoceras* and morphologically related species; values are given in centimeters. The reproductive structures were measurements at maturity.

Characters	Species		
	C. leptoceras	*C. robusta*	*C. subroseophylla*
Pairs of leaflets*	22–61	13–58	15–40
Distance between median leaflets	1.8–2.8	2–3.9	0.9–3.9
Length of median leaflets	28–43.5	30.5–44.5	23.5–44.5
Width of median leaflets	1.9–2.8	3.1–3.9	2.4–4
Length of ovulate strobilus	23.5–28	26–40	15.5–23.5
Diameter of ovulate strobili	9.5–11	11.5–14.5	7–10
Length of ovulate strobili peduncle	11–16	5–6.2	9.8–17.5
Number of orthostichies*	8–9	9–12	9–11
Number of megasporophylls per column*	*7–9*	17–20	11–13
Length of megasporophyll horns	*0.60–0.81*	0.38–0.50	0.41–0.62
Length of pollen strobili	*42–45*	60–70	15–30
Diameter of pollen strobili	6.0–7.8	7–8.5	6.2–8
Length of pollen strobili peduncle	13–19	14–17	10–15
Width of microsporophylls	1.09–1.35	1.14–1.80	1.01–1.24
Length of microsporophylls	2.21–2.55	2.33–3.0	1.47–2.80
Length infertile portion of microsporophylls	*0.83–0.96*	0.45–0.65	0.49–0.59
Distance between microsporophyll horns	0.44–0.56	0.55–0.75	0.55–0.42
Length of microsporophyll horns	*0.1–0.23*	0.26–0.40	0.27–0.38

Diagnostic character states are in italics. *Meristic characters.

(Fig. 3). Furthermore, the shape of prickles on the petiole are robust in *C. subroseophylla* and *C. robusta*, whereas in the Guerrero taxon the prickles are thin.

The primary diagnostic morphological characters lie in the reproductive structures. However, quantitative characters traditionally used for species distinction overlap in this case, both in length and diameter of ovulate strobili and peduncle length of ovulate strobili (Table 2). The most relevant and diagnostic characters are, instead, the number of megasporophylls per orthostichy and the length of horns in the megasporophylls (Table 2). Moreover, the pollen strobilus in the Guerrero taxon has several differences when compared to species in the second group, especially in terms of microsporophyll morphology: in the Guerrero taxon, the horns of microsporophylls are straight, thin, the infertile portion is linear, and the fertile portion is deeply lobate (Fig. 4).

Character-based DNA barcoding

Sequences of *matK* and ITS allowed the molecular identification of 19 out of 31 *Ceratozamia* species (Table 3). The ITS region alone granted 45% identification success of recognized species, which increased to 61% after the inclusion of *matK*. Surprisingly, molecular diagnosability determined through character-based DNA barcoding in CAOS indicated that the taxon from Guerrero has two autapomorphies within *matK* [i.e. 'simple pure' (sPu) characteristic attributes *sensu* Sarkar et al. 2008] in all

Figure 3. Leaflet variation at the population level **A** *Ceratozamia leptoceras*, a.1, a.2 San Pedro Cuitlapan, a.3 Riverbank "Chipili" **B** *C. robusta*, b.1 Cañón del Sumidero, b.2, b.3 Cuchumbak **C** *C. subroseophylla*, c.1 Sinapan, c.2 "El Vigía". All leaflets were collected from middle and right side of leaf with exception of two first leaflets for *C. leptoceras* (left).

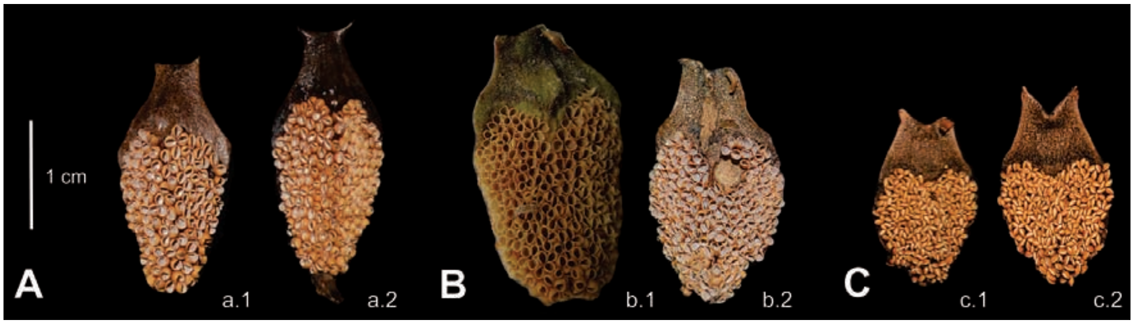

Figure 4. Abaxial view of microsporophylls **A** *Ceratozamia leptoceras*, a.1, a.2 San Pedro Cuitlapan **B** *C. robusta*, b.1 Cuchumback, b.2 Cañón del Sumidero **C** *C. subroseophylla*, c.1 "El Vigía", c.2 Sinapan. For more detail of differences in character states see Table 1.

five sequenced individuals (G: 1592; C: 1807). No interindividual or intrapopulation polymorphic sites were detected.

Among the species with morphological affinity to the Guerrero taxon, *Ceratozamia robusta* and *C. subroseophylla* were diagnosable with ITS and ITS+*matK*, respectively. Notably, *C. subroseophylla* has a 15 nucleotide deletion in region 569–583 of the

Table 3. Species identification using the candidate combination of loci for character-based DNA barcoding in *Ceratozamia*. The dash indicates absence of diagnostic sites.

Species	ITS region	*matK*	Total DNA diagnostic sites
C. alvarezii	1	–	1
C. brevifrons	–	–	–
C. chamberlainii	–	1	1
C. chimalapensis	–	–	–
C. decumbens	–	–	–
C. delucana	–	–	–
C. euryphyllidia	1		1
C. fuscoviridis	–	–	–
C. hildae	–	–	–
*C. hondurensis**	–	–	–
C. huastecorum	–	–	–
C. kuesteriana	6	3	9
C. latifolia	1	–	1
C. leptoceras	–	2	2
C. matudae	4	2	6
C. mexicana*	1	–	1
C. miqueliana	–	–	–
C. mirandae	–	2	2
C. mixeorum	3	–	3
C. morettii	–	3	3
C. norstogii	–	–	–
C. robusta	1	–	1
C. sabatoi	1	1	2
C. santillanii	2	–	2
C. subroseophylla	1	1	2
C. tenuis	2	–	2
C. totonacorum	–	–	–
C. vovidesii	–	3	3
C. whitelockiana	–	–	–
C. zaragozae	1	–	1
C. zoquorum	8	–	8

Gray squares indicate the presence of at least one DNA diagnostic site for the corresponding locus. For species with an asterisk, only ITS sequences are available.

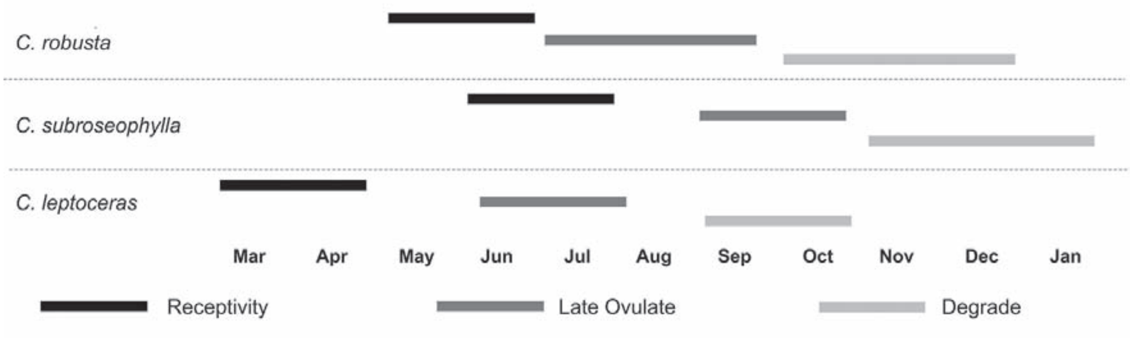

Figure 5. Phenological patterns of ovulate strobili in *Ceratozamia leptoceras* and morphologically similar species.

aligned matrix; this type of deletion is not present in any other *Ceratozamia* species (Table 3; Suppl. material 4: Table S1) and, thus, represents an autapomorphy. Our samples from Guerrero are more distant genetically from other phenotypically similar species such as *C. mexicana*, *C. mixeorum*, *C. delucana*, *C. whitelockiana* and *C. brevifrons*. The latter three species did not have any diagnostic sites; however, a closer visual inspection of groups generated by CAOS showed divergences with the taxon from Guerrero and greater similarities with *C. miqueliana* and *C. morettii*.

Ceratozamia kuesteriana had the greatest number of diagnostic sites (nine in total). This species was followed by *C. matudae* and *C. zoquorum*, with six and five diagnostic characters, respectively. The remaining species of the genus had low values of DNA diagnostics; the number of diagnostic sites by species ranges from one to three DNA diagnostics, and for nine species diagnostic sites were consistently absent (Table 3). In the case of *C. chamberlainii* only a gap in *matK* was recovered as a potential diagnostic site and it was included as a diagnostic character due to its location in a coding region.

Ovuliferous reproductive phenology

Overall, the Guerrero taxon has a phenological reproductive pattern that differs from its most morphologically related species (Fig. 5). The phenological cycle of *Ceratozamia robusta* and *C. subroseophylla* is mutually more similar; however, in the first species, the R phase occurs from May to June, whereas in *C. subroseophylla* this phase occurs from June to July. OT and D phases for these species have different offsets (Fig. 5). In contrast, the R phase in the Guerrero taxon occurs from March to April, while the D phase runs from September to October.

Integrative taxonomic inference of a new *Ceratozamia* species

According to the inferential rules of the ‘taxonomic circle’ in our integrative taxonomy approach, the specimens collected from Guerrero were marked with a ‘red flag’ – i.e. as a hypothetical species demanding test. This hypothesis was corroborated after detection of morphological diagnostic characters, DNA diagnostics and phenological differences, and after the establishment of the particular geographic locality of the collected specimens. Formally, these specimens are recognized here as belonging to a new species based on the presence of (i) distinct morphological qualitative characters, particularly in reproductive structures; (ii) exclusive DNA diagnostic sites in *matK*; (3) distinctions in the phenological pattern in comparison to similar species; and (4) a separate geographical range, which suggests allopatric geographic isolation (i.e. barriers to gene flow). In summary, the new *Ceratozamia* species from Guerrero is diagnosable according to all tested criteria.

Taxonomic treatment

Ceratozamia leptoceras **Mart.-Domínguez, Nic-Mor., D.W. Stev. & Lorea-Hern., sp. nov.**
urn:lsid:ipni.org:names: 77211166-1
Figures 6–8

Type. Mexico. Guerrero: Tlacoachistlahuaca, 3 Km NW of San Pedro Cuitlapan, 1,400 m, 26 Jun. 2019, *L. Martínez-Domínguez & F. Nicolalde-Morejón 1867* ♀ (holotype CIB; isotypes MEXU, NY).

Ceratozamia leptoceras is most similar to *C. robusta*, but can be distinguished by its linear membranaceous leaflets and petioles with thin prickles. In addition, *C. leptoceras* is easily distinguished from its congeners by having obconic microsporophylls with a long, linear infertile portion (0.83–0.96 cm), and two thin horns; ovulate strobilus with abundant pubescence at base of megasporophylls, 8–9 orthostichies, and 7–9 sporophylls per orthostichy.

Additional specimens examined (paratypes). Mexico. **Guerrero:** Cochoapa El Grande, 4 km to W-NW of San Pedro by a logging road, 1,170 m, 4 Feb 1984, *F. Lorea-Hernández 2928* (FCME); Tlacoachistlahuaca, San Pedro Cuitlapan, riverbank "Chipili", 1,200 m, 29 May 2019, *L. Martínez-Domínguez et al. 1756* (CIB), *1757* (CIB, MEXU), *1758* (XAL), *1759* (CIB, MEXU); *F. Nicolalde-Morejón et al. 3173* (XAL), *3174* (FCME), *3175* (CIB); 3 km NW of San Pedro Cuitlapan, 1,400 m, 26 June 2019, *L. Martínez-Domínguez & F. Nicolalde-Morejón 1860*, *1861* (MEXU), *1862–1866* (CIB).

Description. *Stem* epigeous, erect to decumbent, 30–150 cm in length, 11–35 cm in diameter, covered with leaf bases. *Cataphylls* persistent, reddish-brown, densely brownish tomentose abaxially at emergence, pubescent at maturity, triangular, apex acuminate, 9–11 × 2.5–3 cm at base. *Leaves* 7–50, descending, 93.5–281 cm, green at emergence with sparse reddish-brown pubescence, glabrous at maturity. *Petiole* terete, linear, 45–85 cm, armed with long (0.48–0.68 cm) and thin prickles, copperish-green in mature leaves. *Rachis* terete, linear, 75–196 cm, armed with long and thin prickles, green in mature leaves. *Leaflets* 22–61 pairs, linear, abaxially curved, not basally falcate, membranaceous, flat, opposite to subopposite, plane, green, adaxial and abaxial surfaces glabrous, acuminate and symmetric apex, attenuate at base, with conspicuous and green veins; median leaflets 28–43.5 × 1.9–2.8 cm, 1.8–2.8 cm between leaflets; articulations generally copperish-green. *Pollen strobilus* generally solitary (rarely 2), cylindrical, erect, 40–45 cm in length, 6.0–7.8 cm in diameter, brownish-yellow at emergence, yellowish-green with brownish trichomes at maturity; peduncle tomentose, reddish-brown to brown, 13–19 cm in length, 1.5–2.0 cm in diameter; microsporophylls 2.1–2.45 × 1.09–1.30 cm, obconic, non-recurved distal face, fertile portion deeply lobate, infertile portion 0.83–0.96 cm, linear, horns 0.1–0.23 cm, straight, thin, 0.44–0.56 cm between horns, 180–230 sporangia on abaxial side. *Ovulate strobilus* solitary, cylindrical, erect,

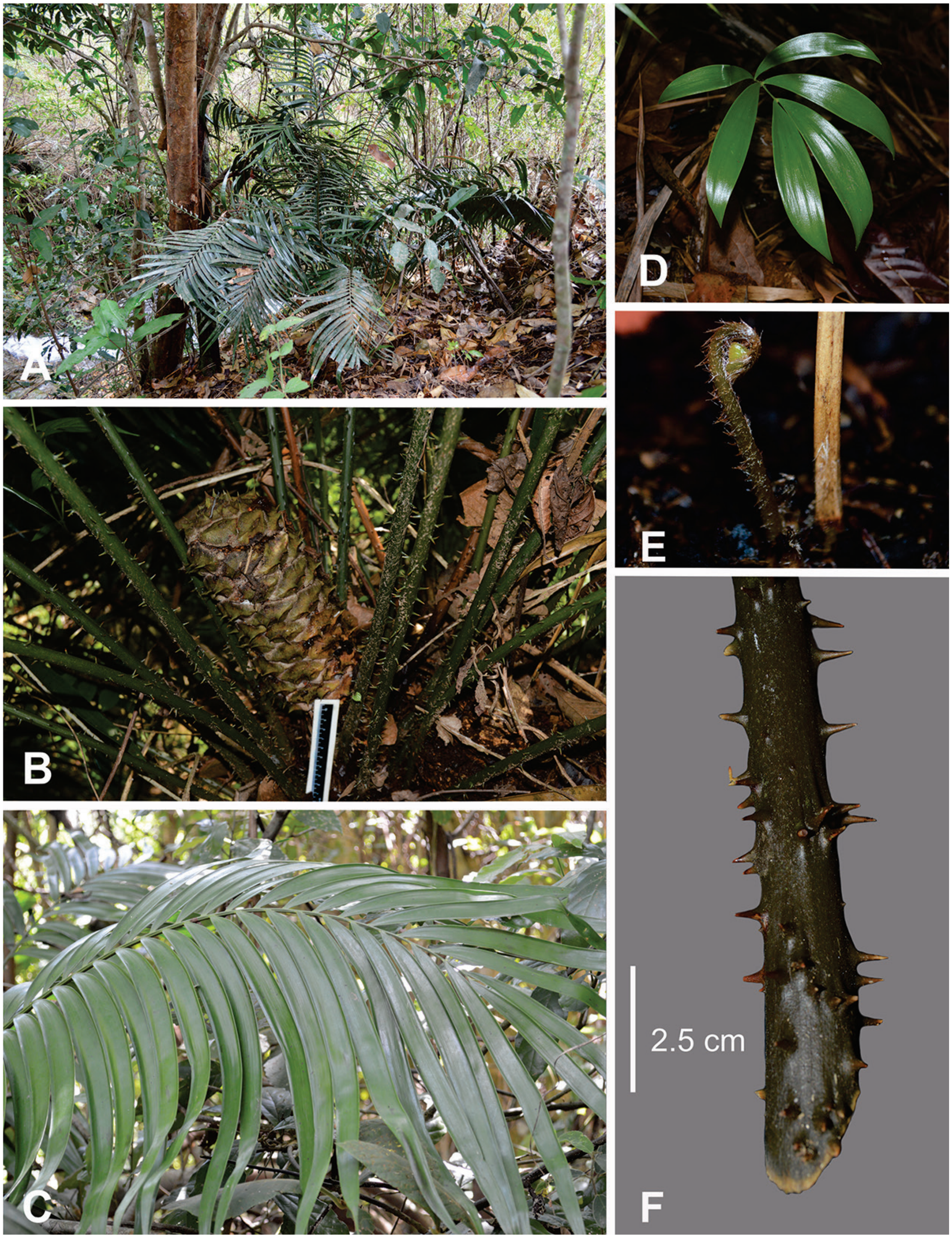

Figure 6. *Ceratozamia leptoceras* **A** adult plant in habit **B** ovulate strobilus **C** detail of leaflets **D** seedling **E** ptyxis **F** prickles on petiole.

23.5–28 cm in length, 9.5–11 cm in diameter, brownish-green with greyish-black trichomes at emergence, copperish-green with greyish-black pubescence at maturity, acute apex; peduncle tomentose, brown, 11–16 cm in length, 1.5–2.0 cm in diameter; megasporophylls 56–81, 8–9 orthostichies (column), 7–9 sporophylls per column, 4.9–5.6 ×

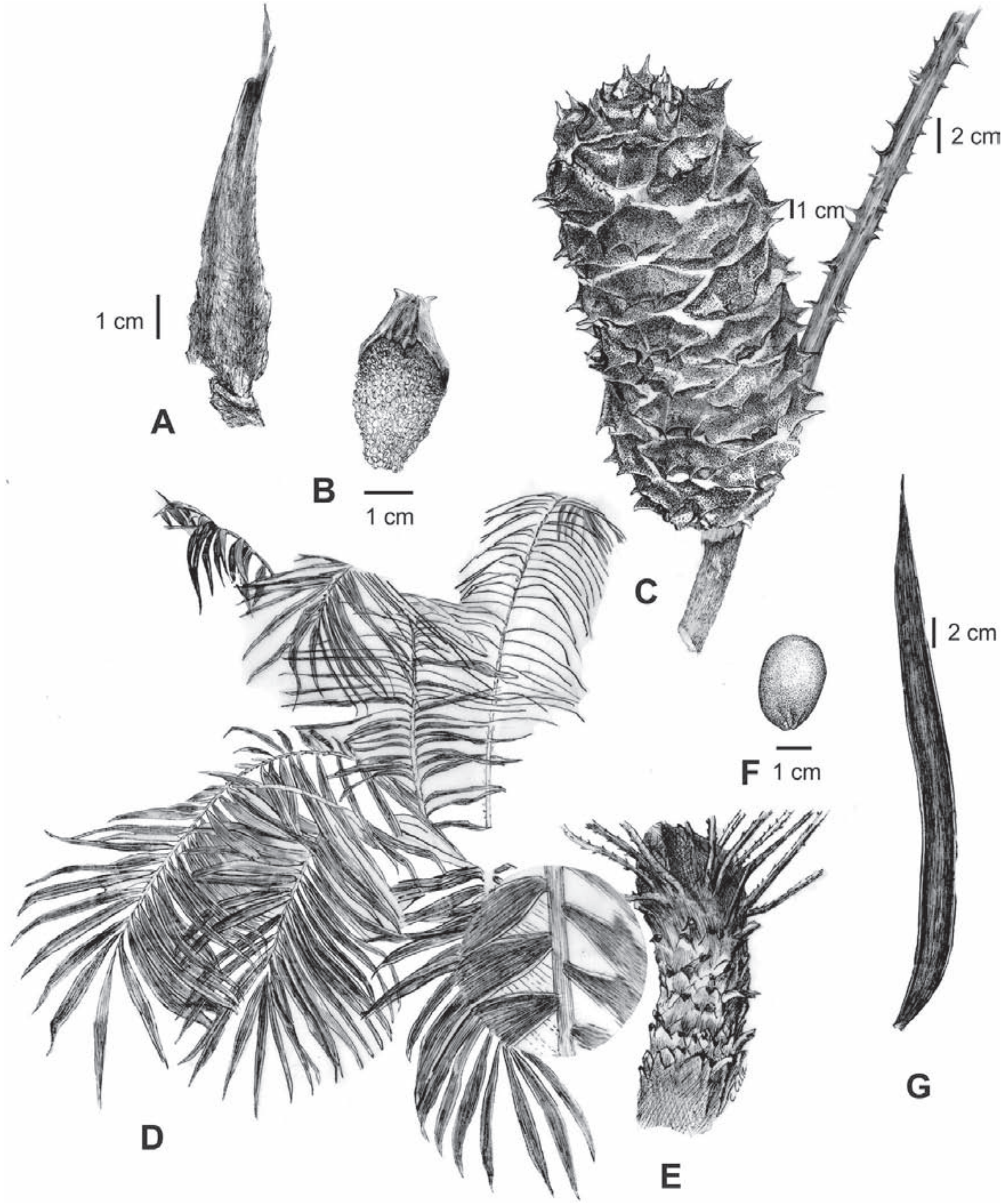

Figure 7. Illustration of *Ceratozamia leptoceras* **A** cataphyll **B** microsporophyll **C** ovulate strobilus **D** leaves and detail of leaflets **E** stem **F** seed **G** leaflet. This illustration is based on *L. Martínez-Domínguez & F. Nicolalde-Morejón 1867*, with exception microsporophyll, which is based on *L. Martínez-Domínguez et al. 1757.*

2.2–2.6 cm, prominent distal face, horns 0.63–0.81 cm, straight, 0.95–1.35 cm between horns, straight angle between horns. *Seeds* ovoid, 2.43–2.71 cm in length, 1.4–1.8 cm in diameter, sarcotesta whitish-pink when immature, light brown at maturity.

Etymology. The specific epithet alludes to microsporophylls horns shape, which are short and thin. This name comes from the Greek “lepto”, which means thin or fine, and “ceras” in reference to horns.

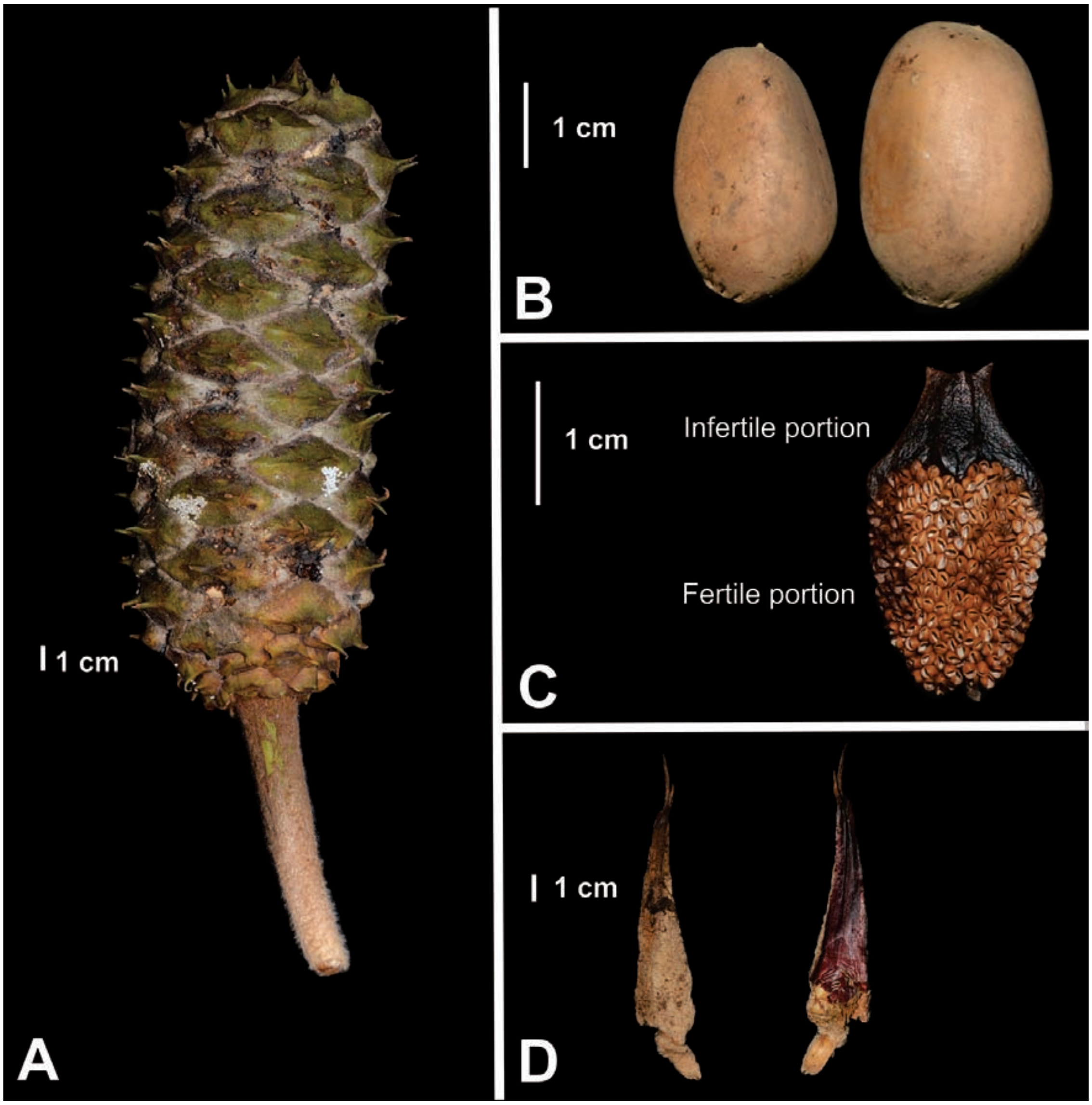

Figure 8. *Ceratozamia leptoceras* **A** detail of ovulate strobilus and megasporophylls **B** seeds **C** abaxial view of microsporophylls **D** cataphylls **A, B, D** are based on *L. Martínez-Domínguez & F. Nicolalde-Morejón 1867*; C is based on *L. Martínez-Domínguez et al. 1757*.

Distribution and habitat. Only known from Guerrero, Mexico, on the karstic rocks within the elevation range of 1,170–1,400 m of the Sierra Madre del Sur subprovince of the Guerreran district (Morrone 2017) (Fig. 1). This species occurs in cloud forest. The climate type is (A) C (w2)-semi-warm temperate subhumid with summer rains, and the annual precipitation is from 2, 000 to 2, 500 mm (García 2004).

Phenology. The leaves are produced in groups of 9 to 15 and mature almost simultaneously. Ovulate strobili mature from June to July; seeds mature from August to September. Pollen strobili mature from January to May.

Common names. The common local name for this species by the "Mixteco" ethnic group is *Shalukaá*.

Taxonomic keys to *Ceratozamia leptoceras* and morphologically related species

Key for vegetative plants

1 Leaflets coriaceous..**2**
– Leaflets membranaceous to papyraceous..**3**
2 Leaflets keeled; petiole with long (0.3–0.6 cm) prickles robust and abundant (> 50)... ***C. brevifrons***
– Leaflets flat; petiole with short (0.1–0.2 cm) prickles thin and sparse (< 40) **4**
4 Leaves ascending; new leaves yellowish-green at emergence. Cataphylls pubescent to tomentose .... ***C. delucana***
– Leaves descending; new leaves light-green at emergence. Cataphylls pubescent to scarcely pubescent .... ***C. mexicana***
3 Leaves descending; petiole unarmed to armed with thin prickles.......................**5**
– Leaves ascending; petioles armed with robust prickles **8**
5 Leaflets linear, membranaceous. New leaves green with copperish-green petiole and rachis... ***C. leptoceras***
– Leaflets lanceolate, papyraceous. New leaves green **6**
6 Petiole unarmed to armed with scarce prickles (< 15)............. ***C. whitelockiana***
– Petiole armed with abundant prickles (> 20) **7**
7 Leaflets with adaxial side glabrous; leaflets in median portion inserted at acute angle along rachis.. ***C. mixeorum***
– Leaflets glaucous; leaflets in median portion inserted at right angle along rachis .... ***C. delucana***
8 New leaves dark brown at emergence. Leaflets in median and apical portion abaxially curved .... ***C. robusta***
– New leaves yellowish brown at emergence. Leaflets abaxially curved in median and apical portion mostly planar... ***C. subroseophylla***

Key for microsporangiate plants

1 Microsporophylls discoid, infertile portion orbicular........................ ***C. delucana***
– Microsporophylls obconic, infertile portion rounded to linear **2**
2 Microsporophylls with straight horns..**3**
– Microsporophylls with recurved horns **4**
4 Pollen strobili 60–70 cm long; microsporophylls with infertile portion deeply lobate.. ***C. robusta***
– Pollen strobili < 50 cm long; microsporophylls with infertile portion lobate **6**
6 Peduncle of pollen strobili > 3.5 cm, tomentose reddish-brown to light brown .7
– Peduncle of pollen strobili <3 cm, glabrous to scarcely pubescent.. ***C. whitelockiana***
7 Pollen strobili green with black trichomes, peduncle 3.5–5 cm long ***C. mexicana***
– Pollen strobili light green with brown-reddish trichomes, peduncle 10–15 cm long .... ***C. subroseophylla***

3 Microsporophylls with infertile portion linear **5**

– Microsporophylls with infertile portion rounded .... ***C. mixeorum***

5 Microsporophylls with infertile portion of 0.4–0.61 cm long, horns 0.24–0.33 cm long .... ***C. brevifrons***

– Microsporophylls with infertile portion of 0.83–0.96 cm long, horns 0.1–0.23 cm long .... ***C. leptoceras***

Discussion

In this synthesis of *Ceratozamia*, we constructed a database for the genus based with geographic, morphological, and molecular data through the use of comparative and character-based DNA barcoding methods. In addition, we investigated the potential value of phenological reproductive patterns for species delimitation; this type of ecological information has been scarcely studied in cycads (Stevenson 1981; Clugston et al. 2016) or in integrative taxonomy studies of gymnosperms. Considering the different male plants in a population, the phenology of pollen strobili shows a broader cycle at the population level; the lifespan of a pollen strobilus is shorter than for ovulate strobili, and an open pollen phase is prolonged in different individual plants (Martínez-Domínguez et al. 2018b). In contrast, duration of ovulate strobili exhibits high specificity in the receptivity phase, which suggests that these data are taxonomically informative. Our findings on the length of the lifespan of reproductive structures and phenological phases coincide with our previous observations in *C. tenuis* (Martínez-Domínguez et al. 2018b). *Ceratozamia leptoceras* has a different phenological pattern for ovulate strobili compared to its morphologically similar congeners, which perhaps represents a reproductive barrier in the field (Fig. 5). Unfortunately, these data are not available for all *Ceratozamia* species.

Despite the species diversity of *Ceratozamia* and its restricted geographic distribution, a formal infrageneric classification for the genus has not been proposed. This is mainly because previously reconstructed phylogenetic relationships display remarkable differences in the number of clades and the members of the clades (cf. González and Vovides 2002, 2012; Medina-Villarreal et al. 2019). This situation has led to an incorrect identification of specimens that unfortunately has limited the inference of evolutionary relationships, with contradictory results inexplicably unrecognized (cf. Medina-Villarreal and González-Astorga 2016; Medina-Villarreal et al. 2019). The groups within the genus are based on similarities in geographic distribution and morphology (Vovides et al. 2004); however, *Ceratozamia* species complexes defined by these authors are more influenced by geography. In this work, we follow the proposal of Stevenson et al. (1986) to address morphology in this genus. Our morphological description for all *Ceratozamia* species recognized tree groups based on the affinity of morphological characters, both vegetative and reproductive, which facilitates comparisons between species.

Under integrative taxonomy criteria (*sensu* De Salle et al. 2005), we have proposed a new *Ceratozamia* species from the Sierra Madre del Sur. This biogeographic province is a salient area in terms of gymnosperm diversity in the country (Contreras-Medina 2016). This new record for the genus in Guerrero represents an expansion of the distribution pattern for the genus on the Pacific seaboard, which opens new questions on the influence of mountain systems on the diversity of the Mexican flora. Our survey shows that the vast majority of *Ceratozamia* species grow at relatively high elevations (Fig. 2); the genus has standard elevation gradient patterns and does not exhibit occurrences at higher elevations or lower elevations. Therefore, it appears that *Ceratozamia* is poorly adapted at lower and very high elevations (Fig. 2). Species with the lowest elevation distributions occur in southern Mexico and Central America, and those at higher elevations occur in the SMO, Highlands of Chiapas, and SMS. This atypical distribution pattern seems to be favored by mountain regions, mainly in areas with cloud forest.

Historically, taxonomic research in the genus *Ceratozamia* has been characterized by difficulties in species identification (Miquel 1868). Recently, new species have been described (e.g. Martínez-Domínguez et al. 2018a) but only some of them have been evaluated. Particularly, in relation to DNA datasets, only 19 species could be diagnosed with ITS+*matK*. Other loci already tested (*psbK/I*, *rpoC1*, *rbcL* and *atpF/H*) have not provided enough variation for improved resolution (see Nicolalde-Morejón et al. 2011; Martínez-Domínguez et al. 2017). As an approach to molecular species identification, character-based DNA barcoding is susceptible to the addition of new sequences (Nicolalde-Morejón et al. 2010); therefore, further sequencing of new loci (e.g. single-copy nuclear genes; Salas-Leiva et al. 2014) could contribute new molecular diagnostic characters and improved resources for automated species identification in *Ceratozamia* and other cycad genera.

In terms of morphological taxonomic evidence, vegetative characters have been widely used in the genus to identify species (Vovides et al. 2004; Whitelock 2004; Pérez-Farrera et al. 2009). However, these characters are polymorphic in some species, which hinders the construction of dichotomous keys (Martínez-Domínguez et al. 2017). The classification of strictly allopatric taxa, as is the case in some *Ceratozamia* species, will always remain arbitrary to some degree so that the evaluation of new characters is a pressing task. In this work, we introduced previously unreported reproductive characters such as the internal parts of the strobilus, which have not been described before in detail for the genus *Ceratozamia* (Fig. 4; Table 1). Comparisons of pollen strobili and the microsporophylls among different species indicated strong morphological divergence limited to certain groups of related species (Fig. 4). For this reason, we only present a key for the species group that includes the newly described taxon. Thus, it is necessary to explore these reproductive characters for the rest of the species in the genus. Keys based on vegetative features still preserve their relevance due to the dioic condition of these gymnosperms, and the lack of reproductive structures in materials deposited at the consulted herbaria (Morettii et al. 1989). However, we consider that, in view of the complexity of vegetative morphological variation, keys based on reproductive characters could function as excellent support tools in the systematics of cycad genera such as *Ceratozamia*.

Species delimitation in *Ceratozamia* exhibits a high degree of complexity (Whitelock 2004). The morphological and molecular evidence available for the genus at present does not allow the diagnosability of all species. For example, *C. hildae* is a morphologically distinctive species, but lacks molecular diagnostics; in contrast, *C. vovidesii* and *C. mirandae* are species that share many morphological affinities, but variation at the molecular level facilitates their taxonomic differentiation (Table 3). This taxonomic-systematic scenario points to the need to explore new molecular markers and the evaluation of variation between populations with morphological, molecular, and phenological evidence for some closely related species. In particular, there are some populations in Oaxaca and Chiapas that still await closer inspection. In the present work, 31 species have been recognized for the genus (Stevenson et al. 1986; Nicolalde-Morejón et al. 2014; Vovides et al. 2016; Pérez-Farrera et al. 2017; Martínez-Domínguez et al. 2017, 2018a), with *C. becerrae* and *C. microstrobila* treated as synonyms of *C. zoquorum* and *C. latifolia*, respectively (Stevenson et al. 1986; Martínez-Domínguez et al. 2017, 2018a).

Conclusions and future directions

This study adds to recent research that suggests the significant role of topography in SMS as a speciation driver in shaping its high species diversity (Santiago-Alvarado et al. 2016). The geographical barriers and ecological changes in this biogeographic province could have allowed the intermingling of different species; these processes could have favored the development of a number of centers of endemism (Morrone 2010; Santiago-Alvarado et al. 2016).

Our integrative taxonomic assessment provided support for the recognition of a new species, *Ceratozamia leptoceras*. However, the taxonomic complexity of the genus indicates the need of further systematic revisions using multiple sources of evidence, particularly in some groups of species with problematic boundaries. In addition, we have demonstrated the value of investigating ovulate and pollen strobili – particularly, microsporophylls – for the construction of refined morphological matrices for *Ceratozamia*; and finally, that the construction of dichotomous keys with vegetative characters should consider variation at the population level.

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Appendix 1

Localities of sampled populations for morphologically similar *Ceratozamia* species to Guerrero taxon.

Species	State	Municipality	Population	Elevation (m)
C. robusta	Chiapas	Berriozábal	Cuchumbak	1,129
C. robusta	Chiapas	Tuxtla Gutiérrez	Cañón del Sumidero	1,263
*C. robusta**	Chiapas	San Fernando	Cuauhtémoc	1,200
C. subroseophylla	Veracruz	Santiago Tuxtla	Hill El Vigía	474
C. subroseophylla	Veracruz	Santiago Tuxtla	Sinapan	425
Guerrero taxon	Guerrero	Tlacoachistlahuaca	Near Riverbank “Chipili”	1,200
Guerrero taxon	Guerrero	Tlacoachistlahuaca	Near San Pedro Cuitlapan	1,400

* Population not monitored in field for reproductive phenology data.

Supplementary material 1

File S1. GenBank accession numbers of sequences used in the analyses for ITS and matK, respectively. Sequences were generated by this study are in bold.

Authors: Lilí Martínez-Dominguez, Fernando Nicolalde-Morejón

Data type: GenBank accession numbers of sequences used in the analyses

Link: https://doi.org/10.3897/phytokeys.156.53502.suppl1

Supplementary material 2

Fig. S1. Strict consensus tree of nine equally parsimonious trees

Authors: Lilí Martínez-Dominguez, Fernando Nicolalde-Morejón

Data type: Strict consensus tree

Explanation note: Heuristic searches were conducted with 1000 random-addition, tree bisection-reconnection (TBR) branch swapping, and collapse zero-length branches off. All characters were treated as equally weighted and unordered.

Link: https://doi.org/10.3897/phytokeys.156.53502.suppl2

Supplementary material 3

File S2

Authors: Lilí Martínez-Dominguez, Fernando Nicolalde-Morejón

Explanation note: Herbarium specimens consulted for phenology data.

Link: https://doi.org/10.3897/phytokeys.156.53502.suppl3

Supplementary material 4

Table S1

Authors: Lilí Martínez-Dominguez, Fernando Nicolalde-Morejón

Data type: DNA diagnostic sites

Link: https://doi.org/10.3897/phytokeys.156.53502.suppl4

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Research

A new Mexican *Ceratozamia* (Zamiaceae) species with notes on reproductive phenology

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Ceratozamia, a genus of Zamiaceae, is one of the most species rich genera of gymnosperms in Mexico with a high level of species endemism. Over the last two decades, their known diversity has increased considerably because of extensive field work. Recently, 16 new species were described as a result of revision of species complexes and new explorations in southern Mexico. Here, we describe a new species from Oaxaca (Mexico) which inhabits an evergreen tropical forest. We collected this species during fieldwork in a little studied area located in the karst region of northern Oaxaca. The new species, *C. martinezii* sp. nov., is described and illustrated, and its phenological pattern is characterized. The species is compared with geographically close and morphologically similar species. Additionally, we present notes on the reproductive phenology of the new species and a taxonomic key for diagnosability.

Keywords: endemism, gymnosperms, Oaxaca flora, reproductive phenology, Zamiaceae

Introduction

The order Cycadales is a monophyletic group of gymnosperms, which consists of 10 genera with 365 species (Calonje et al. 2021). *Ceratozamia* Brongn., is a Neotropical genus with the vast majority of its 33 species occurring in Mexico and only three species in the rest of Central America (Calonje et al. 2021). This genus includes plants with both semihypogeous and epigeous stems, and occurring in shady habitats on karstic rocks or on shallow soils, at 10–2050 m elevation (Martínez-Domínguez et al. 2020). Clarifying the taxonomy and systematics of *Ceratozamia* has been challenging due to the complex and continuous variability among species and incomplete knowledge of their morphological characters (Vovides et al. 2004, Martínez-Domínguez et al. 2018a). In the past 20 years of cycad research and exploration, 48 percent of all presently known *Ceratozamia* species have been described (Nicolalde-Morejón et al. 2014, Martínez-Domínguez et al. 2018a).

Currently, *Ceratozamia* in Mexico comprises 33 species, of which 90 percent are endemic (Calonje et al. 2021). Most of the species are known to be restricted to cloud forest and evergreen tropical rain forest restricted to the karst (Martínez-Domínguez et al. 2018a, 2020). The distribution range of this genus encompasses two biogeographic provinces with high species diversity: the Sierra Madre Oriental and the Veracruzan province (Morrone et al. 2017). In particular, the Sierra Madre del Sur located along to the Pacific Ocean from Jalisco to the Oaxaca highlands is the most diverse in Mexico with the largest number of species of vascular plants (Morrone 2017, Aragón-Parada et al. 2021). Despite the high species richness, this biogeographic province has low *Ceratozamia* diversity with only three species currently known for this area (Martínez-Domínguez et al. 2020, Aragón-Parada et al. 2021).

Revision of herbarium specimens and fieldwork was carried out in Sierra Madre del Sur in Oaxaca State as part of ongoing taxonomic and phylogenetic research on *Ceratozamia*. These works based upon detailed morphological comparisons resulted in identifying specimens from 'La Cañada' region as a new taxon. This new species is described and illustrated with comments on geographically and morphologically comparable species, namely: *C. mixeorum* Chemnick, T.J. Greg. & Salas-Mor., *C. whitelockiana* Chemnick & T.J. Greg. , *C. decumbens* Vovides, Avendaño, Pérez-Farr. & Gonz.-Astorga and *C. subroseophylla* Mart.-Domínguez & Nic.-Mor.. In particular, the last species was included because the populations in the North of Oaxaca and the region of the Tuxtlas have historically been considered as part of the *C. robusta* complex (Stevenson et al. 1986, Chemnick and Gregory 1996). However, *C. robusta* was recently more narrowly circumscribed to include just material from Chiapas to Belize and Guatemala (Martínez-Domínguez et al. 2016, Gutiérrez-Ortega et al. 2021).

Phenology is a relevant topic in the life history of cycads; however, it has been poorly explored. Recently published research on this topic has focused on characterizations of phenological variation and stages through data from living collections and herbarium specimens (Griffith et al. 2012, Clugston et al. 2016). Phenological studies have been hampered by poor basic data of coning events for most species in *Ceratozamia*. Here, we have compiled detailed reproductive phenology notes on the characters during phenophases and time of events for this new species.

Material and methods

The new species was recognized by a unique combination of diagnostic character states inferred under the 'taxonomic circle' assumptions of integrative taxonomy sensu DeSalle et al. (2005). The circumscription was based on a combination of geographical, qualitative and quantitative morphological, and ecological data through comparisons with morphologically similar taxa.

For assessing morphological variation, we collected at the population level from 1) three populations of the new species, 2) two populations for *Ceratozamia whitelockiana* and 3) three populations for *C. mixeorum* (Fig. 1). In each population, we evaluated 20 adult specimens from which characters were recorded. The seeds were measured without the sarcotesta. Also, we reviewed specimens deposited in CIB, ENCB, FCME, IBUG, MEXU, NY and XAL. The type locality for each species was included in our data gathering. The distribution map was elaborated using ArcMap Geographic Information System 10 (ESRI 2011). Additionally, systematic records were made in two populations of the new species during 2020 and 2021. The directed sampling was conducted to record individuals in reproductive stages with reproductive structures (pollen and ovulate strobili). The number of pollen and ovulate strobili recorded was 48 and 24, respectively. The records were carried out once a month during September and October 2020, February to May 2021. All observations were categorized according to Martínez-Domínguez et al. (2018b).

Results

Taxonomic circumscription of *Ceratozamia* in Sierra Norte of Oaxaca (Mexico)

Generally, the specimens of *Ceratozamia* from this region have been associated and/or assigned to *C. mexicana*, which has a long history of taxonomic confusion caused by the ambiguity of the type locality ('Mexico' sensu Brongniart 1846). The taxonomic application of *C. mexicana* and associated historic names were recently established (Vovides et al. 2016, Martínez-Domínguez et al. 2018a, b). As now defined, *C. mexicana* is endemic to the central region in Veracruz State (Vovides et al. 2016). In the Sierra Norte de Oaxaca, *Ceratozamia mixeorum* and *C. whitelockiana* were described from the Sierra Mixe and the drainage in the Río Valle Nacional, respectively (Chemnick and Gregory 1996, Chemnick et al. 1998). Review of protologues, herbarium specimens and specimens collected for these species in their type localities and close areas allowed to describe the reproductive structures (Table 1, 2).

The new species inhabits an area at the north of the Sierra Norte de Oaxaca in evergreen tropical forest, between 458 and 800 m elevation near the Santo Domingo river. *Ceratozamia whitelockiana* occurs in La Chinantla region of Sierra Norte de Oaxaca, between 500 and 1800 m a.s.l. (Appendix 1). It inhabits an altitudinal gradient of evergreen tropical forest with *Quercus* sp. and cloud forest. *Ceratozamia mixeorum* is endemic to the southern portion of the Sierra Norte of Oaxaca in the Mixe region on karstic rocks. This species occurs in cloud forest and oak forest between 1170 and 2150 m a.s.l. (Appendix 1).

Ceratozamia martinezii Mart.-Domínguez, Nic.-Mor. & D.W. Stev. sp. nov. (Fig. 2–5)

A species most similar to *C. whitelockiana*, but distinguished by its oblong leaflets with an abruptly acuminate apex,

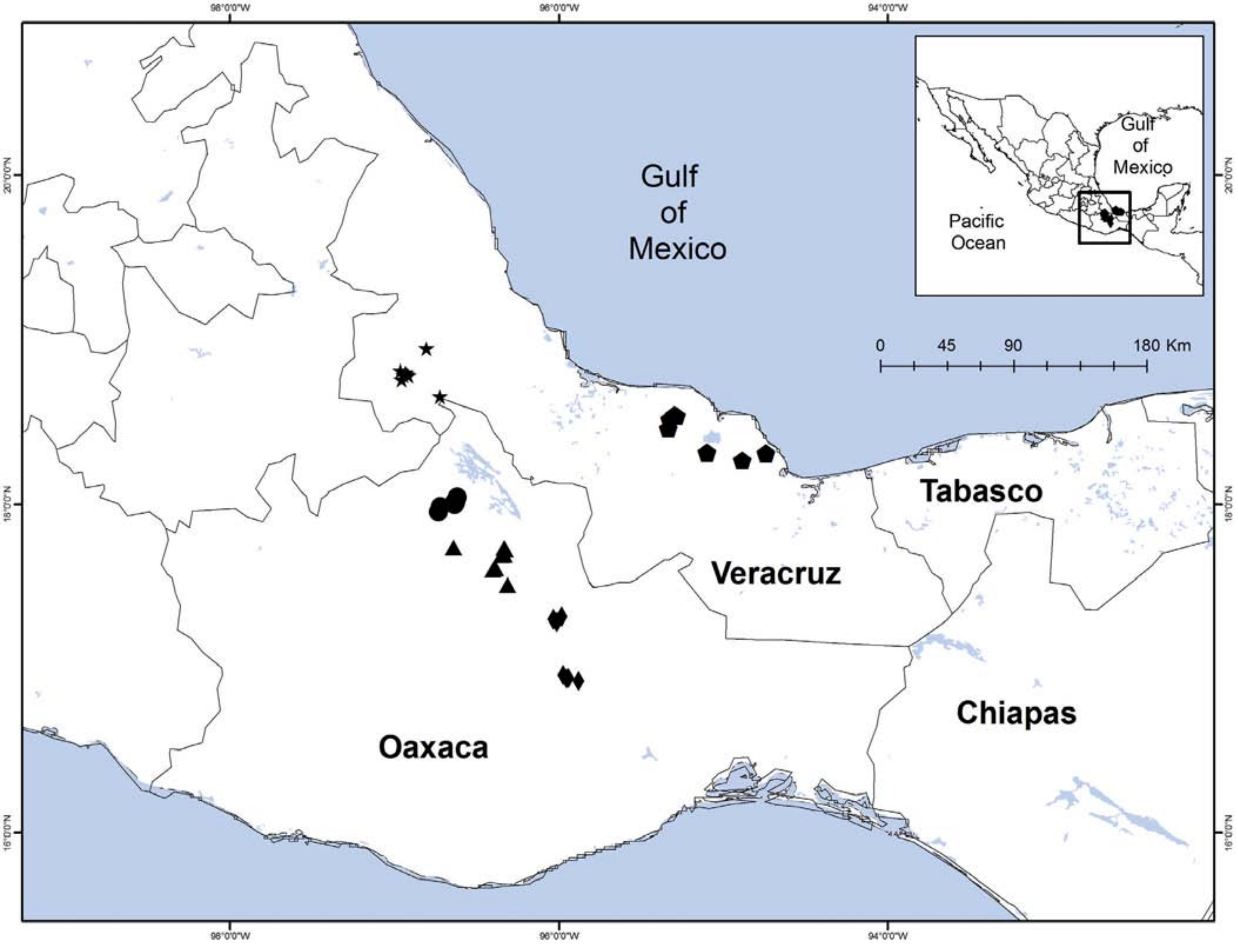

Figure 1. Distribution map of *Ceratozamia martinezii* (dots) and its morphologically and geographically close species: *C. whitelockiana* (triangle), *C. mixeorum* (diamond), *C. decumbens* (star) and *C. subroseophylla* (pentagon).

whereas the leaflets are lanceolate with a long acuminate apex in *C. whitelockiana*. The leaves are reddish-brown at emergence, the petiole has abundant (16–24) and long prickles (0.32–0.55 cm) and the rachis has prickles in the lower third of its length, whereas *C. whitelockiana* has light green and glaucous leaves at emergence, and petiole with scarce (3–22) and short prickles (0.06–0.39 cm). In addition, *C. whitelockiana* is distinguished from its congeners by having a combination of ovulate strobili with an acute apex and megasporophylls with recurved horns, and pollen strobili with obconic microsporophylls and a rounded infertile portion.

Type: Mexico, Oaxaca: San Pedro Teutila, El Faro, alt. 615 m a.s.l., 28 Sep 2020, Martínez-Domínguez et al. 2049 ♀ (holotype: CIB, isotypes: MEXU, NY).

Description

Stem 30–250 cm long, 10–15 cm in diameter, epigeous, erect or sometimes decumbent. Cataphylls 3.6–6.5 × 2.0–3.3 cm wide at base, persistent, triangular, reddish-brown, densely brown tomentose at emergence, glabrous at maturity, acuminate at apex. Leaves 7–30 (50), 117–240 cm long, ascending, reddish-brown at emergence, with whitish trichomes, glabrous at maturity. Petiole 45–85 cm long, terete, linear, reddish-brown or greenish brown at emergence, dark green in mature leaves; prickles 16–47, 0.30–0.66 cm long, thin. Rachis 70–160 cm long, terete, linear, dark green in mature leaves, with prickles in lower third. Leaflets 12–38 pairs, opposite to subopposite, plane, oblong, generally planar, not basally falcate, papyraceous, flat, green, with adaxial and abaxial side glabrous, abruptly acuminate and with symmetric apex, attenuate at base; veins conspicuous and light-green; median leaflets 21.0–42.5 × 3.3–4.8 cm, with 3.2–6.0 cm between leaflets; articulations 0.9–1.5 cm wide, green. Pollen strobilus 20–35 cm long, 3.1–4.6 cm in diameter, solitary, cylindrical, erect, greenish with reddish trichomes at emergence, greenish-yellow with reddish-brown trichomes at maturity; peduncle 7.0–15 cm long, 1.5–2.1 cm in diameter, reddish brown pubescent; microsporophylls 1.50–2.20 × 0.98–1.30 cm, with 120–210 sporangia on the abaxial side,

Table 1. Qualitative morphological characters to distinguish *Ceratozamia martinezii* from the geographically and morphologically similar species of the genus.

Character	*C. martinezii*	*C. mixeorum*	*C. whitelockiana*	*C. subroseophylla*	*C. decumbens*
Type of prickles	thin	thin	thin	robust	thin
Trichomes color of leaves	whitish	light brown	whitish gray	whitish	whitish gray
Leaf color at emergence	reddish brown	light green and glaucous	light green and glaucous	yellowish-brown	reddish brown
Leaf position	ascending	descending	descending	ascending	descending
Shape of leaflets	oblong	lanceolate	lanceolate	lanceolate	oblong
Appearance of leaflet apex	abruptly acuminate	long acuminate	long acuminate	long acuminate	acuminate
Arrangement of prickles along rachis	from basal to apical end of leaf	up to middle of leaf	unarmed	from basal to apical end of leaf	basal end of leaf or unarmed
Microsporophylls shape	obconic	obconic	elliptic	obconic	obconic
Infertile portion shape	rounded	linear	linear	rounded	linear
ovulate strobilus color at maturity	green with reddish trichomes	green with brown to blackish trichomes	green with brown to blackish trichomes	green with pale pink base and dark brown to reddish-brown trichomes	wine with blackish trichomes
Position of ovulate strobilus at maturity	erect	pendulous	erect	erect	erect
Ovulate strobilus apex	acute	apiculate	apiculate	mucronate	acute
Megasporophyll horns type	recurved	straight	straight	straight	straight

obconic, with non-recurved distal face; fertile portion lobate; infertile portion 0.47–0.84 cm long, rounded; horn 0.16–0.38 cm, straight, with 0.50–0.80 cm distance between horns forming an acute angle. Ovulate strobilus 11–36 cm long, 9.4–12.5 cm in diameter, solitary, cylindrical, erect, greenish-yellow with abundant reddish trichomes at emergence, green with brown trichomes at maturity, apex at acute; peduncle 5.0–19.5 cm long, 1.3–2.3 cm in diameter, pubescent, light brown; megasporophylls 20–200,with 5–11 orthostichies with 4–19 sporophylls per row, 2.25–2.68 × 3.6–4.0 cm; distal face truncate; horns 0.54–0.90 cm long, non-recurved, with 1.05–1.77 cm distance between horns forming an acute angle between horns. Seeds 2.4–3.0 cm long, 1.4–1.7 cm in diameter, ovate; sarcotesta whitish-yellow to yellow when immature, light brown at maturity.

Etymology

The specific epithet is in memory of Mr Agustín Martínez Casanova, an excellent father and friend.

Distribution and habitat

Ceratozamia martinezii is known only from the highlands between the south of the Río Santo Domingo to the north of the Sierra Norte in Oaxaca. According to the cultural regionalization and topographic characteristics of Oaxaca State, this area is known as 'Cañada' (Arellanes 1996). The species is found in evergreen tropical forest on karstic rocks, at between 458 and 800 m a.s.l.

Uses

The leaves of this species are used as ornaments. People make flower arrangements in wreaths using the leaves.

Similar species

Ceratozamia martinezii is morphologically and geographically close to *C. mixeorum* and *C. whitelockiana*. However, the last two species have light green and glaucous leaves at emergence, with lanceolate leaflets; whereas, *C. martinezii* has leaves that are reddish brown and lustrous at emergence, with

Table 2. Quantitative morphological characters to distinguish *Ceratozamia martinezii* from the geographically and morphologically compared species of the genus. Values are given in cm. * Meristic characters.

Character	*C. martinezii*	*C. mixeorum*	*C. whitelockiana*	*C. subroseophylla*	*C. decumbens*
Length of stem	30–250	30–100	20–80	10–150	10–40
Number of leaves*	7–30	3–17 (30)	3–10	10–83	2–7
Number of prickles on petiole*	16–47	30–50	3–22	25–50	2–22
Length of prickles on the petiole	0.30–0.66	0.20–0.38	0.06–0.39	0.40–0.85	0.02–0.23
Length of petiole	45–85	46–132	60–140	50–104	40–100
Width of leaflets	3.3–4.8	1.9–2.9	2.0–3.7	2.5–5.0	2.8–5.0
Distance between leaflets	3.2–6.0	1.7–3.0	1.6–3.1	1.7–3.9	2.6–6.5
Length of pollen strobili (fertile portion)	20–30	22–30	20–30	15–20	20–23
Length of pollen strobilus peduncle	7–15	10–15	15–25	9.5–11.0	8.0–10.8
Length of microsporophylls	1.5–2.2	1.4–2.4	1.5–3.0	1.8–2.0	1.0–1.9
Width of microsporophylls	0.98–1.30	0.7–1.3	0.8–1.5	1.3–1.5	1.0–1.4
Length of ovulate strobilus (fertile portion)	11–36	23–32	14–20	15.5–30.0	9–11
Length of ovulate strobilus peduncle	5.0–19.5	12.0–23.5	1–4	9.8–17.5	3–4
Number of orthostichies	5–11	6–9	4–5	9–12	6–7
Number of megasporophylls	20–200	60–112	24–40	110–168	18–49

Figure 2. *Ceratozamia martinezii* sp. nov. (A) adult plant in habitat, (B) leave at maturity (C) leave at emergence, (D) vernation, (E) juvenile plant.

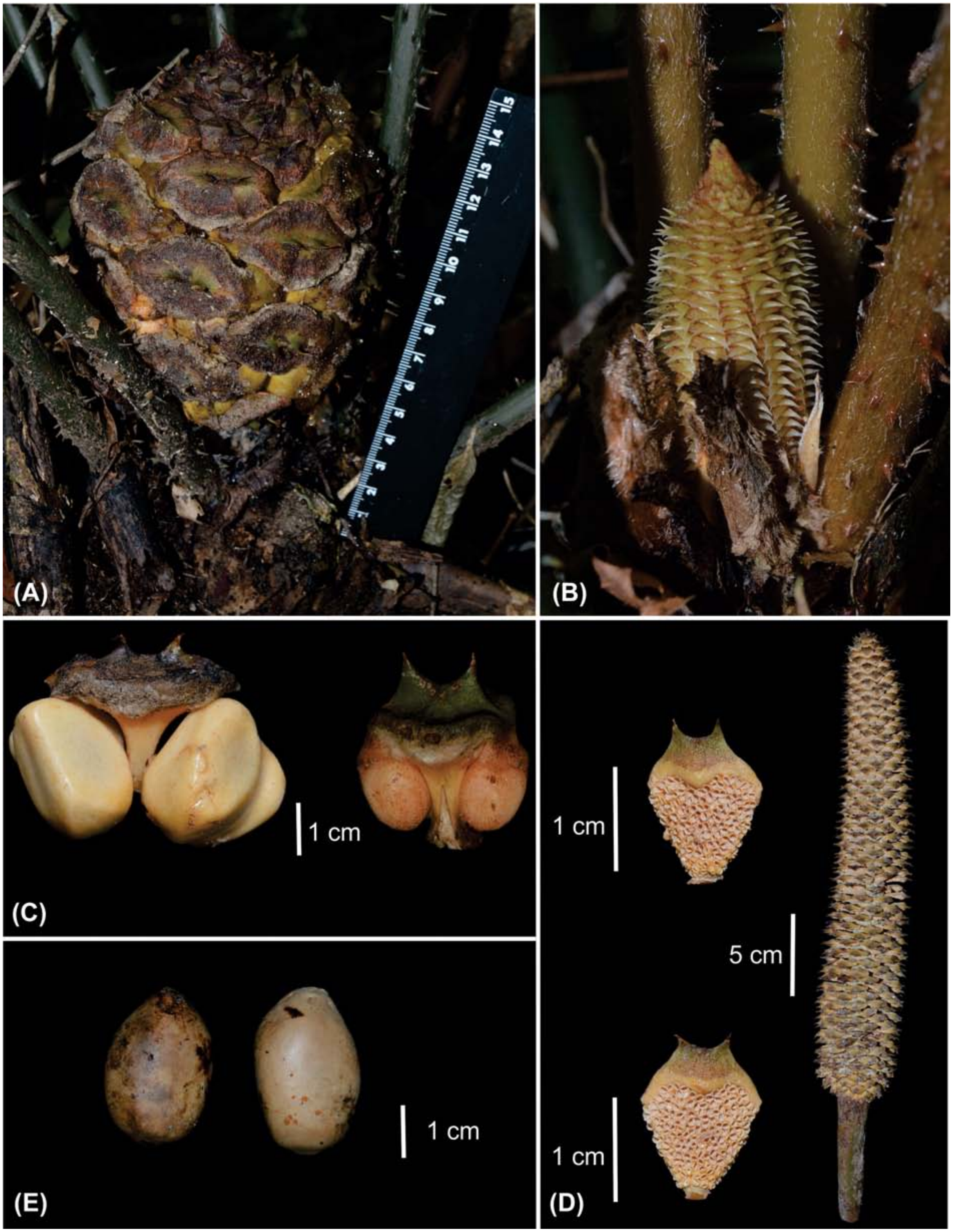

Figure 3. Reproductive structures of *Ceratozamia martinezii* sp. nov. (A) ovulate strobilus at maturity, (B) pollen strobilus at maturity, (C) megasporophylls with mature and immature ovules (left and right, respectively), (D) microsporophylls, (E) seeds.

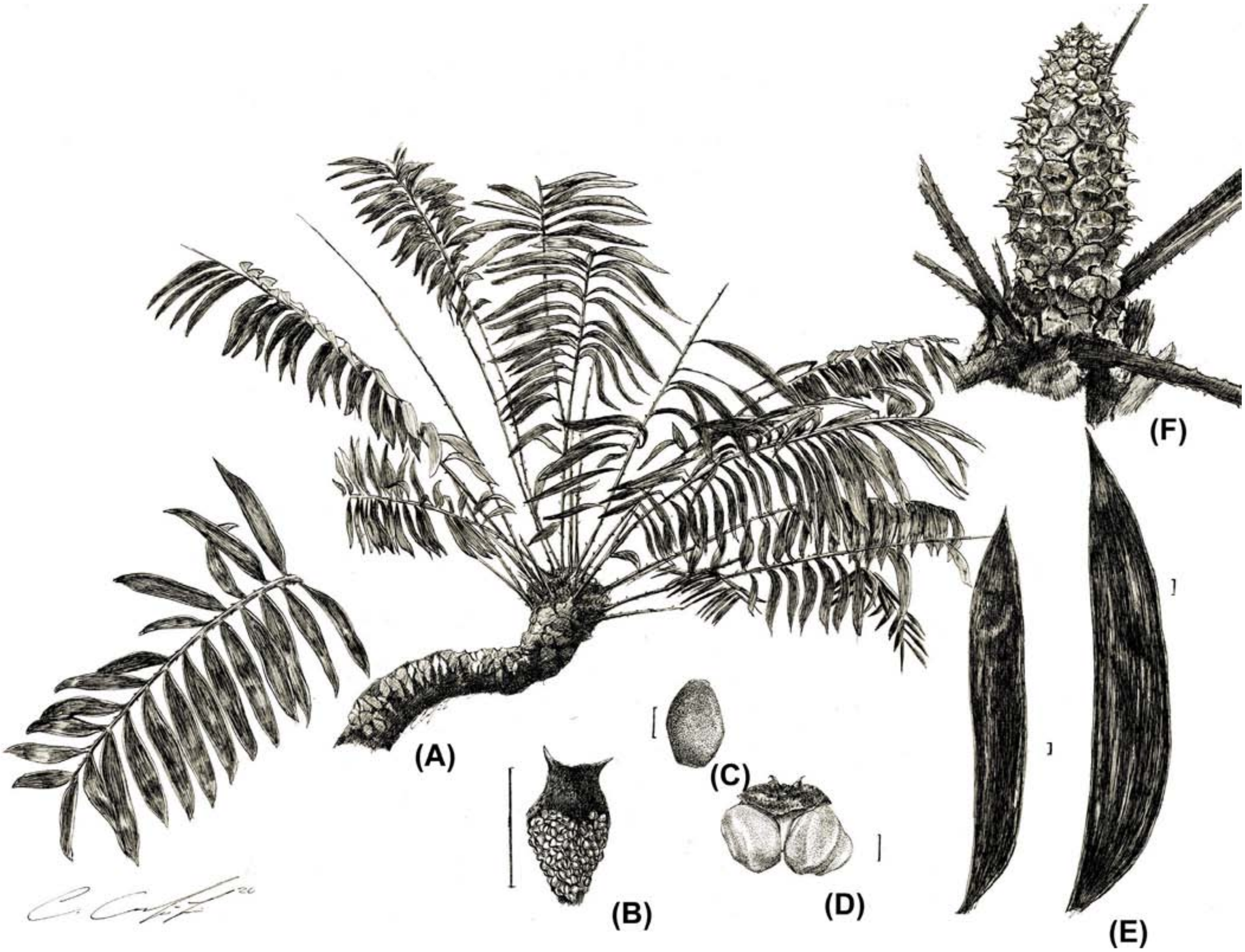

Figure 4. Illustration of *Ceratozamia martinezii* sp. nov. (A) adult plant, (B) microsporophylls, (C) seeds, (D) megasporophylls, (E) leaflets, (F) ovulate strobilus at maturity. Scale bar = 1 cm.

oblong leaflets (Table 1). In addition, the distance between leaflets is greater in *C. martinezii*, 3.2–6.0 cm versus 1.6–3.1 cm in *C. mixeorum* and *C. whitelockiana* (Table 2). *C. martinezii* has longer, thin stems (up to 250 cm long and 15 cm in diameter), which are arborescent and when the stems are decumbent, they renew their erect growth.

Other species with morphological and geographical affinity to the new species are *C. subroseophylla* and *C. decumbens*. These species have leaves brown at emergence with whitish trichomes. *Ceratozamia subroseophylla* and *C. martinezii* are similar in vegetative appearance with their long leaves and stems, ascending leaves, papyraceous leaflets and several long prickles. However, the leaflets are oblong with an abruptly acuminate apex in *C. martinezii*, whereas in *C. subroseophylla* they are lanceolate with a long acuminate apex (Table 1, Fig. 6).

Ceratozamia decumbens also has oblong leaflets, but it differs from *C. martinezii* by leaflet consistency wich is coriaceous in the former and papyraceous in the latter. Additionally, *C. decumbens* has prickles only in the basal part of rachis or is unarmed, in contrast to the new species that has prickles from basal to apical end of leaf. Furthermore, these species show differences in characters of pollen and ovulate strobili (Table 1). In particular, *C. martinezii* differs by having green megasporophylls with brown trichomes at maturity, horns recurved and microsporophylls with a rounded infertile portion (Table 1) while *C. decumbens* has vinaceous with blackish trichomes at maturity and straight horns of megasporophylls, and a linear infertile portion of the microsporophylls.

Additional specimens examined (paratypes)

Mexico, Oaxaca: Chiquihuitlán de Benito Juárez, 861 m a.s.l., 26 Nov 2004, C. A. Cruz-Espinosa and G. Juárez-García 1945 (MEXU). San Felipe Jalapa de Díaz, 500 m a.s.l., 28 Jun 2008, J. A. Pérez de la Rosa et al. 1966 (IBUG). San Pedro Teutila, 708 m a.s.l., 10 Jun 2004, G. Juárez-García 425 (MEXU); 458 m a.s.l., 23 Sep 2020, F. Nicolalde-Morejón et al. 3323–3333 (CIB); 500 m a.s.l., 23 Sep 2020, F. Nicolalde-Morejón et al. 3334–3337 (CIB); 615 m a.s.l., 28 Sep 2020, F. Nicolalde-Morejón et al. 3415–3419 (CIB); 458 m a.s.l., 23 Sep 2020, L. Martínez-Domínguez et al. 1946–1950 (CIB), 1951 (CIB,

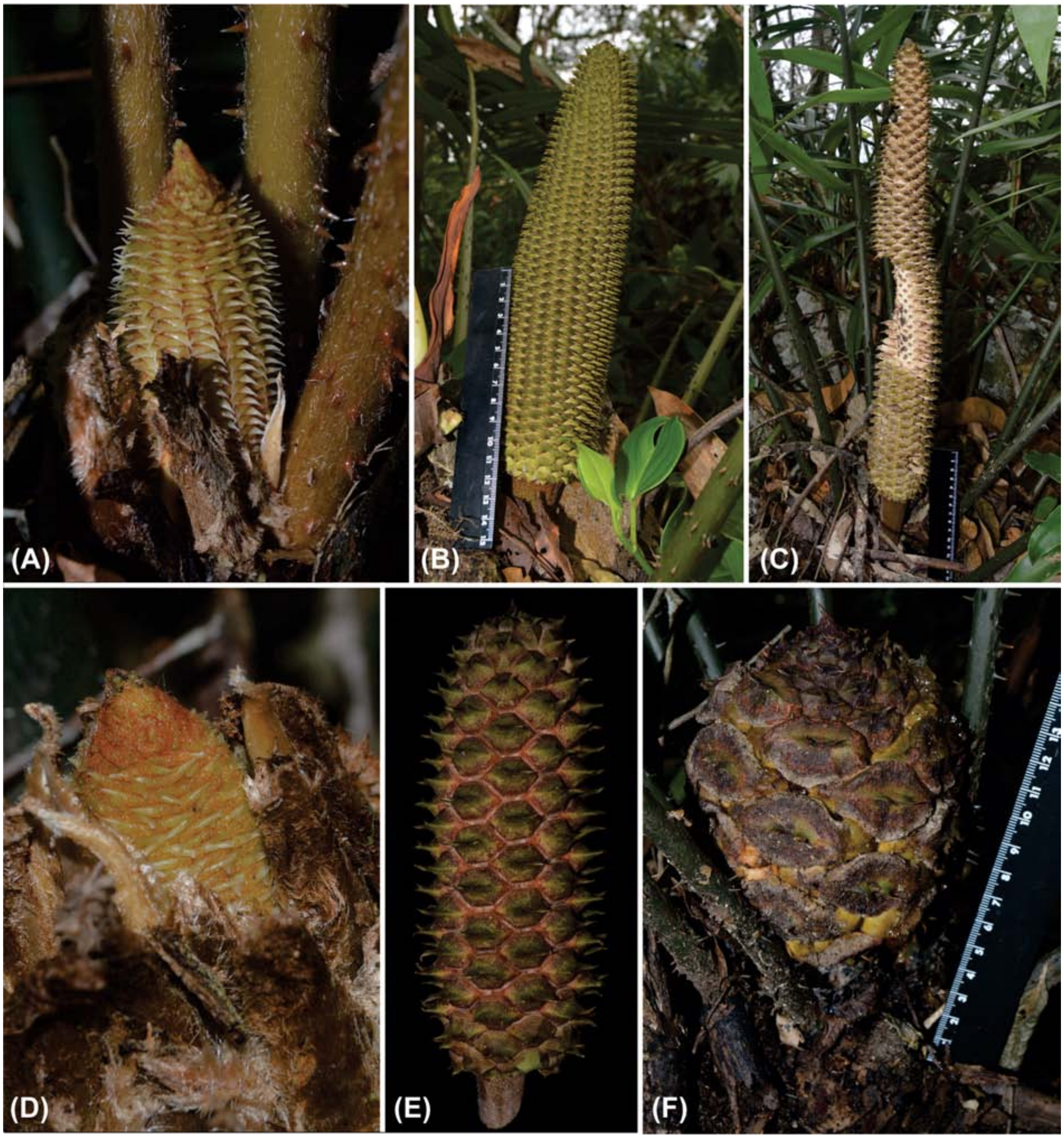

Figure 5. Reproductive phenology for pollen and ovulate strobili of *Ceratozamia martinezii*. (A) to (C): phenophases for pollen strobili: (A) emergence, (B) closed pollen, (C) open pollen. (D) to (F): phenophases for ovulate strobili: (D) emergence, (E) receptivity, (F) late ovulate.

MEXU), 1952–1957 (CIB); 500 m a.s.l., 23 Sep 2020, L. Martínez-Domínguez et al. 1958–1960 (CIB, MEXU), 1961 (CIB); 615 m a.s.l., 28 Sep 2020, L. Martínez-Domínguez et al. 2050, 2052 (CIB, MEXU), 2051, 2053 (CIB); 539 m a.s.l., 6 Apr 2021, L. Martínez-Domínguez et al. 2141, 2142, 2144 (CIB), 2143 (CIB, MEXU, NY). Santa María Tlalixtac, 675 m a.s.l., 25 Nov 2004, G. Juárez-García and C. A. Cruz-Espinosa 868 (MEXU).

Notes on the reproductive phenology of the new species

The Emergence (E) phases of pollen strobili occurs during August and September and ovulate strobili in September (Fig. 5A). The megasporophylls and microsporophylls in this phase are yellow to greenish-yellow with orange trichomes. In ovulate strobili, the phases are: 1) receptivity (R), which occurs at the end of March up to May; 2) late ovulate (LO) phase during approximately the six months between April and September; and 3) disintegration (D) in October and November (Fig. 5). In the last phase, the megasporophylls detach from the central axis of the ovulate strobilus. The R phase is characterized by a slight separation of the megasporophylls from each other (i.e. the ovulate strobilus opens) and the base of the megasporophylls change color (reddish to pinkish).

The phases for pollen strobili are: 1) closed pollen (CP); 2) open pollen (OP); and 3) senescence (S). The first phase

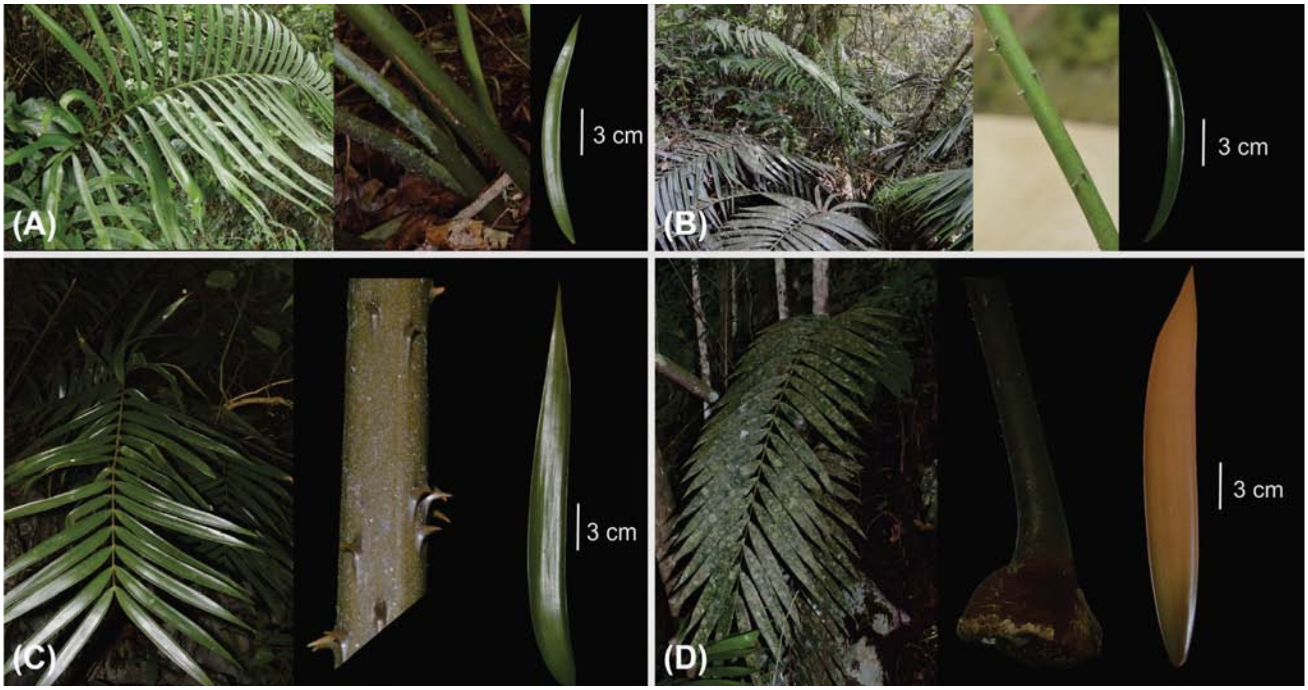

Figure 6. Comparison among morphologically and geographically close species: (A) *C. whitelockiana*, (B) *C. mixeorum*, (C) *C. subroseophylla*, (D) *C. decumbens*.

lasts approximately 4–5 months depending on the individual; however, this phase occurs during most of the year at the population level and this allows for a long period of potential pollen dispersal. The OP phase occurs for one to two weeks depending on the individual and it is characterized by the elongated central axis and the release of pollen (Fig. 5). Finally, in the S phase, the central axis become senescent, is curved and soon drops. Synchrony between R and OP takes place during March to May.

Identification key to morphologically and geographically close species of *Ceratozamia*

1. Leaflets oblong ………………………………..……......2
   – Leaflets lanceolate……………………………….…..….3
2. Leaflets coriaceous; prickles on petiole short (0.02–0.23 long); rachis unarmed or with prickles on basal end of leaf; leaves descending; ovulate strobili vinaceous with blackish trichomes.……………………….*C. decumbens*
   – Leaflets papyraceous; prickles on petiole long (0.30–0.66 long); rachis with prickles from basal to apical end of leaf; leaves ascending; ovulate strobili green with reddish trichomes………..……..……..……..……*C. martinezii*
3. Petiole with robust prickles; leaves ascending, yellowish brown at emergence; infertile portion of microsporophylls rounded……………………………..*C. subroseophylla*
   – Petiole with thin prickles; leaves descending, green at emergence; infertile portion of microsporophylls linear……………………………………...……….4
4. Rachis unarmed; petiole with sparse prickles (up to 22); ovulate strobilus with up to 5 orthostichies……………… ……....…....…....…....…....…....…..*C. whitelockiana*
   – Rachis armed with prickles; petiole with abundant prickles (30–50); ovulate strobilus with 6–9 orthostichies…….……....…….……....…..*C. mixeorum*

Discussion

Biogeographically, the new species is distributed in Sierra Madre del Sur in Oaxaca State. Oaxaca has a complex orogeny of mountain chains and valleys with climatic and floristic diversity (García-Mendoza and Meave 2011). The 'Cañada' region is an area in Oaxaca that unlike the other regions of the State has been scarcely studied (García-Mendoza et al. 1994, Munn-Estrada 2017). The botanic record of *Ceratozamia* in this region dates only from 20 years ago, suggesting that this relevant region of Mexico may still harbor unknown species. The karst forest is dominant in this region of Oaxaca, which together with Chiapas is considered one of the centers of diversity for cycads (Vovides et al. 2003). The karsts are characterized by shallow soils which are poor nutrients (Wendt 1989); nevertheless, these habits represent a relevant refuge for *Ceratozamia*. Unlike most of the species of the genus that inhabit cloud forests between 800 and 1400 m, *C. martinezii* occurs in evergreen tropical forest on karstic rocks with abundant organic matter and at a low elevation (458–800 m a.s.l.) (Martínez-Domínguez et al. 2020).

Reproductive phenology is a sequence of annual events related to the production of reproductive structures (pollen and ovulate strobili). Such event series are generally well known at the genus level (Stevenson 1981). Recently, the subject has become relevant because phenology is useful for studying the relationship between plants and their environment, and could provide evidence for species delimitation and an inability to hybridize (Clugston et al. 2016). However, observations on the duration of phenophases and their contributions to the taxonomy of cycads are scarce (Ornduff 1991, Griffith et al. 2012). The basic data in reproductive phenology for most species of *Ceratozamia* are still unknown (Martínez-Domínguez et al. 2018a). Here, we provide field notes documenting the reproductive phenology in this species. Considering the lack of fertile specimens in herbaria, this type of field data is important for studies of reproductive phenology, periodicity of timing, and as a source of evidence to contrast with other species or genera, especially for species with higher levels of similarity or degrees of relatedness. Moreover, phenological patterns could be relevant data to unravel the evolutionary history within the genus *Ceratozamia* as well as reproductive biology in the genus.

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Author contributions

Lilí Martínez-Domínguez: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (equal); Methodology (equal); Resources (equal); Writing – original draft (equal); Writing – review and editing (equal). **Fernando Nicolalde-Morejón**: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (equal); Resources (equal); Writing – original draft (equal); Writing – review and editing (equal). **Francisco Vergara-Silva**: Conceptualization (equal); Writing – review and editing (equal). **Dennis Wm. Stevenson**: Conceptualization (equal); Writing – original draft (equal); Writing – review and editing (equal).

Data availability statement

There are no new data for this publication.

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Ceratozamia oliversacksii (Zamiaceae), a new species of gymnosperm from western Oaxaca, Mexico

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Summary. *Ceratozamia* is a neotropical genus in the Cycadales that is particularly diverse in Mexico. In this genus, most species are endemic to Mexico and most of these species have a restricted distribution pattern. We propose and describe a new species from western Oaxaca State, Mexico, which was previously subsumed under the concept of *C. robusta*. The circumscription of this new species derived from a review of two associated taxa: *C. robusta* Miq. and *C. leptoceras* Mart.-Domínguez, Nic.-Mor., D.W.Stev. & Lorea-Hern. We conducted an extensive review of herbarium specimens, complemented with *ex profeso* fieldwork, during which we monitored populations in Oaxaca for phenological characterisation. The new species is described and illustrated. This species occurs in a wide distribution range of cloud forest in western Oaxaca. We provide a taxonomic key for all species with morphological and geographical affinity to *C. oliversacksii* and all species distributed in Oaxaca. The description of *C. oliversacksii* increases diversity in this cycad genus to six species in Oaxaca. This research highlights the study and monitoring of reproductive structures for robust taxonomic work.

Key Words. Cycadales, cloud forest, endemism, phenology, Sierra Madre del Sur.

Introduction

Ceratozamia Brongn. is a genus in the Zamiaceae with approximately 33 species (Calonje *et al.* 2013 – 2021). Morphological studies in *Ceratozamia* have focused on quantitative vegetative characters (Vovides *et al.* 2003; Whitelock 2004), but these characters are highly variable (Martínez-Domínguez *et al.* 2017, 2018a). Leaflet morphology has contributed most of the useful qualitative characters (Stevenson *et al.* 1986). As all cycads are dioecious, combined with their long phenological cycle, analyses of morphology in reproductive structures have been scarce (Stevenson 1981). Additionally, cycad specimens in international herbaria are usually infertile or, if fertile, the structures have been poorly preserved.

Ceratozamia spp. inhabit humid ecosystems, particularly in cloud forest and evergreen tropical forest. Mexico is considered a centre of diversification for this cycad genus (Vovides *et al.* 2004). The highest diversity is in the eastern region of Mexico and Central America, with Veracruz State encompassing the richest flora for this genus (Nicolalde-Morejón *et al.* 2014). In western Mexico, only five species have been reported between Guerrero and Chiapas States as compared to 12 in Veracruz.

Some populations of *Ceratozamia* from western Oaxaca have been reported and assigned to *C. robusta*. Historically, this species has had a controversial circumscription; populations from Belize, Guatemala, southern Veracruz, Chiapas and Oaxaca in Mexico were considered part of a *C. robusta* complex (Vovides *et al.* 2004). Recently, this species complex has been partially disentangled and re-circumscribed (Martínez-Domínguez *et al.* 2016; Gutiérrez-Ortega *et al.* 2021). Consequently, populations from Oaxaca have been identified as *C. leptoceras*. However, a closer review of reproductive structures throughout its distribution in Oaxaca have allowed us to recognise a new species within the range of the observed morphological variation. With this description, the number of species reported for the State of Oaxaca is increased to 6 and the total number of species for Mexico rises to 33.

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Material and Methods

All relevant specimens from western Oaxaca available in the CIB, ENCB, FCME, IBUG, MEXU, MICH, MO, NY and XAL herbaria were reviewed. Acronyms of the herbaria follow Thiers (2021). Geographic coordinates were registered using the ArcMap Geographic Information System 10.2 (Esri, Redlands, USA). We conducted fieldwork in three populations located in western Oaxaca to evaluate the morphological variation in vegetative and reproductive structures (Map 1). We collected 20 adult specimens in each population; this material *ex profeso* for this study was processed and deposited in the CIB and MEXU herbaria. Additional fieldwork was conducted through June 2019 and March to June 2021 to monitor and observe the developmental stages of reproductive structures for phenological data and analyses. The reproductive timing was described according to Martínez-Domínguez *et al.* (2018b). The prickles were counted along 10 cm from the base to the distal end of the petiole. Our circumscription is based on comparisons with the morphologically and geographically close species. The digitised holotype of this new species was deposited at https://www.uv.mx/herbariocib/tipos-nomenclaturales/.

Taxonomic Treatment

Ceratozamia oliversacksii *D.W.Stev., Mart.-Domínguez & Nic.-Mor.* **sp. nov.** Type: Mexico, Oaxaca, Candelaria Loxicha, alt. 1616 m, 10 June 2021, *L. Martínez-Domínguez, F. Nicolalde-Morejón & M. Ríos-Méndez* 2261 ♀ (holotype: CIB, isotypes: K, MEXU, NY).

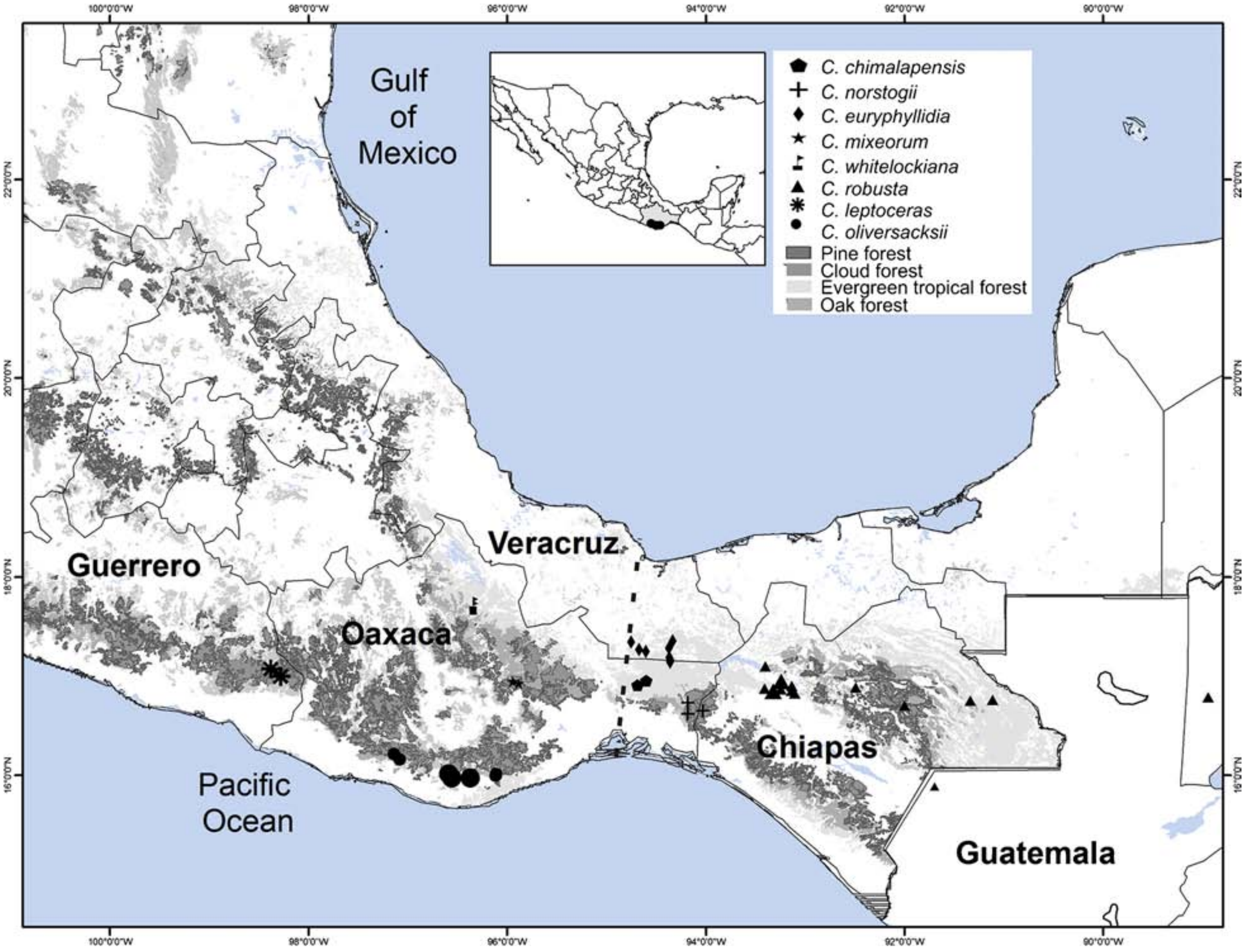
http://www.ipni.org/urn:lsid:ipni.org:names:77221531-1

Stem 30 – 80 cm long, 10 – 40 cm in diam., epigeous, decumbent. *Cataphylls* 6.5 – 7.5 × 1.6 – 2.0 cm wide at base, persistent, triangular, reddish-brown, brown tomentose at emergence, glabrous at maturity, apex acuminate. *Leaves* 7 – 36 (50), 124 – 258 cm long, descending, green at emergence with brown trichomes, glabrous at maturity. *Petiole* 45 – 110 cm long, terete, linear, green at emergence, dark green in mature leaves with long (0.50 – 0.70 cm), abundant (40 – 65) and thin prickles. *Rachis* 85 – 182 cm long, terete, linear, dark green in mature leaves with prickles. *Leaflets* 24 – 47 pairs, opposite to subopposite, linear, planar and abaxially curved, not basally falcate, papyraceous, flat, green, adaxial and abaxial sides glabrous, acuminate and symmetric apex (rarely asymmetric), attenuate at base, with conspicuous and light-green veins; median leaflets 25 – 40 × 2.3 – 3.6 cm, 2.0 – 3.3 cm between leaflets; articulations 0.5 – 1.1 cm wide, green. *Pollen strobilus* 20 – 30 cm long, 3.0 – 4.5 cm in diam., solitary, cylindrical, erect, greenish with reddish trichomes at emergence, greenish-yellow with reddish-brown trichomes at maturity; peduncle 10 – 15 cm long, 1.2 – 1.8 cm in diam., pubescence, reddish-brown; microsporophylls 2.5 – 2.75 × 0.99 – 1.28 cm, 200 – 260 sporangia in abaxial side, obconic, non-recurved distal face, fertile portion lobate, infertile portion 0.68 – 0.80 cm long, rounded, horn 0.08 – 0.18 cm long, straight, 0.50 – 0.70 cm distance between horns with an acute angle. *Ovulate strobilus* 33 – 36 cm long, 11.5 – 14 cm in diam., solitary, cylindrical, erect, greenish-yellow with few reddish trichomes at emergence, green with brown to reddish-brown trichomes or glabrous at maturity, acuminate apex; peduncle 8.0 – 10.0 cm long, 1.4 – 1.6 cm in diam., pubescence, light brown; megasporophylls 80 – 210, 9 – 10 orthostichies, 11 – 13 sporophylls per row, 3.9 – 5.0 × 2.3 – 2.9 cm, truncate distal face, horns 0.31 – 0.53 cm long, non-recurved, acute, 1.33 – 1.40 cm distance between horns, right angle between horns. *Seeds* 2.45 – 2.65 cm long, 1.4 – 1.6 cm in diam., ovate, sarcotesta whitish-yellow to yellow when immature, light brown at maturity. Figs 1, 2.

RECOGNITION. *Ceratozamia oliversacksii* is similar to *C. leptoceras* and *C. robusta*, but can be distinguished by the combination of linear and papyraceous leaflets, green leaves at emergence, and petiole with abundant (40 – 65) and long prickles (0.50 – 0.70 cm); ovulate strobili with 11 – 13 megasporophylls per row and an acute apex; megasporophylls with acuminate horns up to 0.53 cm long and few brown to reddish-brown trichomes on distal end; and microsporophylls with a rounded infertile portion and straight horns.

DISTRIBUTION AND HABITAT. *Ceratozamia oliversacksii* is endemic to Oaxaca State along the Eastern Sierra Madre del Sur subprovince (Oaxacan Highlands district) (Map 1). It inhabits cloud forest and the transition zone between pine forest with cloud forest. It occurs between 1040 – 1850 m of elevation on karstic rocks with abundant organic matter.

ADDITIONAL SPECIMENS EXAMINED: **MEXICO**. Oaxaca, Candelaria Loxicha, alt. 1380 m, 3 May 2005, *A. Luna José & B. Rendón Aguilar* 1472, 1473 (XAL); alt. 1630 m, 23 June 2019, *F. Nicolalde-Morejón & L. Martínez-Domínguez* 3231 – 3243 (CIB); alt. 1630 m, 23 June 2019, *L. Martínez-Domínguez & F. Nicolalde-Morejón* 1832 – 1839 (CIB), 1840 (CIB, MEXU); alt. 1418 m, 20 April 2021, *L. Martínez-Domínguez et al.* 2160, 2161 (CIB); alt. 1616 m, 10 June 2021, *M. Ríos-Méndez et al.* 105 (CIB). San Agustín Loxicha, alt. 1760 m, 12 Oct. 2003, *A. Luna José & B. Rendón Aguilar* 518 (XAL); alt. 1400 m, 23 June 2019, *F. Nicolalde-Morejón & L. Martínez-Domínguez* 3229, 3230 (CIB); alt. 1400 m, 23 June 2019, *L. Martínez-Domínguez & F. Nicolalde-Morejón* 1829 – 1831 (CIB). Santa Catarina Juquila, alt. 1850 m, 13 April 1965, *J. Rzedowski* 19557 (ENCB); alt. 1450 – 1700 m, 11 Feb. 1965, *R. McVaugh* 22346 (ENCB; MICH). San Miguel del Puerto, alt. 1040 m, 1



Map 1. Distribution map of *Ceratozamia* species in Oaxaca. The localities from fieldwork and herbarium specimens for *C. oliversacksii* and its morphologically and geographically close species are represented by large symbols and small symbols, respectively. The dashed black line shows the Isthmus of Tehuantepec, which could represent a barrier limiting the geographical distribution of the species.

April 2003, *L. Schibli et al.* 152 (SERO); alt. 1430 m, 17 April 2000, *J. Rivera H. et al.* 2378 (FCME, SERO); alt. 1060 m, 25 June 2019, *F. Nicolalde-Morejón & L. Martínez-Domínguez* 3244 – 3249 (CIB); alt. 1060 m, 25 June 2019, *L. Martínez-Domínguez & F. Nicolalde-Morejón* 1847 – 1849, 1851, 1852 (CIB), 1850 (CIB, MEXU).

CONSERVATION STATUS. based on current evidence from fieldwork and herbarium specimens, this species is endemic to Oaxaca State in Mexico. This new species inhabits the highlands of western Oaxaca where it is apparently abundant. We sampled four populations, of which one population has been subject to loss of its natural habitat by coffee plantations. Most populations occur in cloud forest on steep slopes; these populations are in areas of good quality (undisturbed forest) or with low degree of disturbance. The average number of seeds per ovulate strobilus is 300. Additionally, we found a remarkable proportion of seedlings and juveniles in each population. Given this distribution range and relatively large population size, this species should be considered as "Vulnerable (VU)" according to the IUCN Red list Categories and Criteria (IUCN 2012).

PHENOLOGY. Leaves are produced in groups of 6 – 9. The receptivity of ovulate strobili and pollen shedding by pollen strobili last a short period of time, approximately three to four weeks. The receptivity phase has been registered in May and then the ovulate strobili start to mature in June. At the population level, the pollen strobili undergo an open pollen shedding phase from January to June.

ETYMOLOGY. The specific epithet is in memory of the distinguished neurologist and historian of science Oliver Sacks (1933 – 2015), who loved cycads and publicised them through a popular book, *Island of the Color Blind and Cycad Island* (Sacks 1997) and his love of Oaxaca as exemplified in his book, *Oaxaca Journal*, a

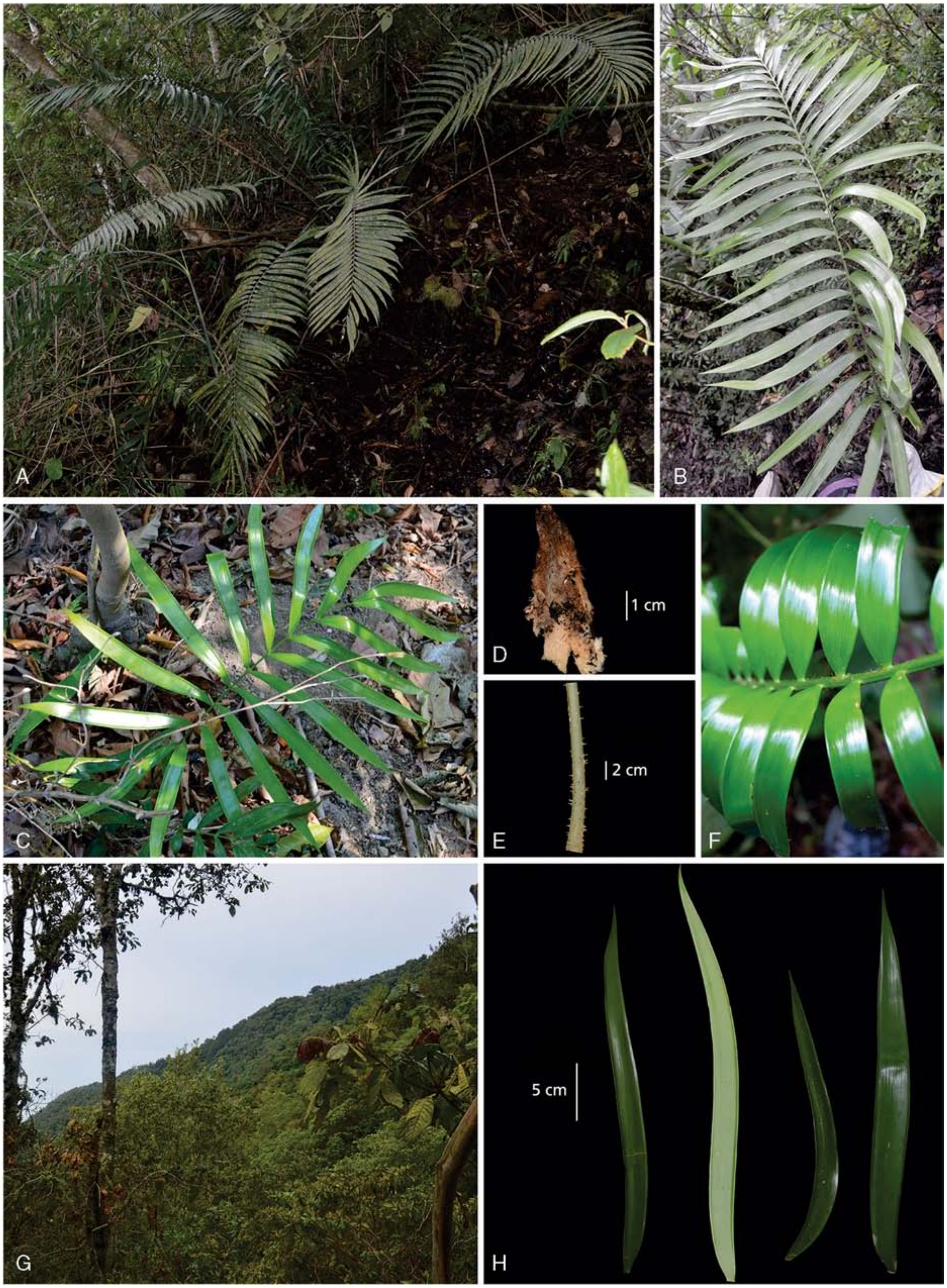


Fig. 1. Vegetative morphology of *Ceratozamia oliversacksii*. **A** adult plant; **B** leaf at maturity; **C** juvenile plant; **D** cataphylls; **E** petiole; **F** new leaves; **G** habitat; **H** variation of leaflets. PHOTOS: L. MARTÍNEZ-DOMÍNGUEZ.

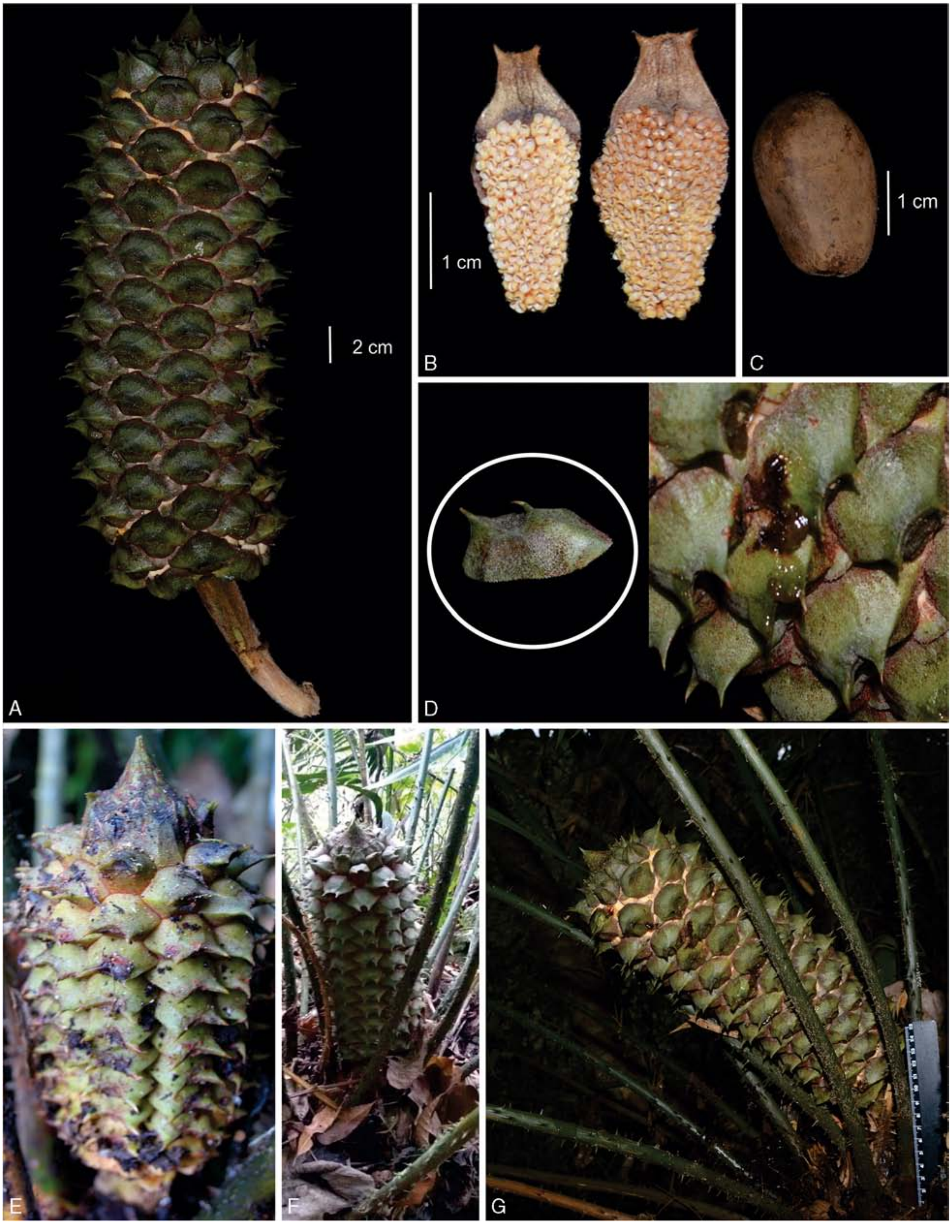


Fig. 2. Reproductive morphology of *Ceratozamia oliversacksii*. **A** ovulate strobilus; **B** microsporophylls; **C** seed; **D** details of megasporophylls and horns; **E** ovulate strobilus at emergence; F ovulate strobilus at receptivity; **G** ovulate strobilus at maturity. PHOTOS: **A – D** L. MARTÍNEZ-DOMÍNGUEZ, **E – G**. F NICOLALDE-MOREJÓN.

Fig. 3. Comparison between closely related species. **A** *Ceratozamia oliversacksii*; **B** *C. robusta*; **C** *C. leptoceras*. PHOTOS: **A, B** L. MARTÍNEZ-DOMÍNGUEZ, **C** F. NICOLALDE-MOREJÓN.

book on the natural and cultural history of Southern Mexico with an emphasis on ferns (Sacks 2002).

Discussion

This new species is morphologically most similar to *Ceratozamia robusta* and *C. leptoceras* (Fig. 3). The first species, *C. robusta,* occurs in Chiapas from the geological monument "Cañón del Sumidero" to Guatemala and Belize (Gutiérrez-Ortega *et al.* 2021), and bears brown leaves at emergence, robust prickles on the petiole, and lanceolate leaflets, whereas in the new species leaves are green at emergence, prickles on the petiole are thin, and the leaflets are linear. In terms of reproductive structures, *C. oliversacksii* differs from *C. robusta* by its short (up to 0.18 cm long), straight and thin microsporophyll horns; in addition, the number of megasporophylls per row in ovulate strobili of *C. robusta* is greater than this new species (17 – 20 megasporophylls) (Fig. 4; Table 1).

Ceratozamia oliversacksii is most similar in vegetative morphological characters and geographical distribution to *C. leptoceras* (Fig. 3). The latter species occurs in cloud forest of the Guerreran district of Sierra Madre del Sur between an elevation of 1170 – 1400 m (Martínez-Domínguez *et al.* 2020). Despite the similarity between these species, the newly-described species can be recognised principally by its reproductive structures (Table 1; Fig. 4). The ovulate strobili of both species have acute apices, but *C. oliversacksii* has acuminate megasporophyll horns and relatively short, up to 0.53 cm long, compared to *C. leptoceras* which has apiculate megasporophyll horns more than 0.60 cm long (Table 1); in turn, the megasporophylls in *C. leptoceras* are pubescent at the base, whereas those of *C. oliversacksii* have scarce trichomes or are glabrous. Microsporophylls of both species are obconic and similar in length; however, *C. leptoceras* has a linear infertile portion 0.83 – 0.96 cm long, in contrast to *C. oliversacksii* which has a rounded infertile portion of 0.68 – 0.80 cm long. In terms of vegetative characters, these species are close, but *C. leptoceras* has membranaceous leaflets.

In northeast Oaxaca, five species of *Ceratozamia* were recorded in an ethnobotanical study, namely: *C. chimalapensis* Pérez-Farr. & Vovides, *C. mixeorum* Chemnick, T.J.Greg. & Salas-Mor., *C. norstogii* D.W. Stev., *C. robusta* and *C. zoquorum* Pérez-Farr., Vovides & Iglesias (Bernal-Ramírez *et al.* 2019). After extensive review of botanical specimens of this genus, no records were found for *Ceratozamia zoquorum*; this species remains endemic to Chiapas State. Given that *C. whitelockiana* Chemnick & T.J.Greg. is similar to *C. robusta* and occurs in San Juan Bautista Valle Nacional where the authors sampled, this record should be considered a misidentification. Regarding the distribution of *Ceratozamia* species in Oaxaca, three are restricted to the southern part of the Isthmus of Tehuantepec (*C. euryphyllidia* Vázq.Torres, Sabato & D.W.Stev., *C. norstogii* and *C. chimalapensis*) and three to its northern region (*C. whitelockiana, C. mixeorum* and *C. oliversacksii*). Of these three taxa, *C. whitelockiana* and *C. mixeorum* occur in the east, whereas *C. oliversacksii* is located in western Oaxaca. Finally, this work attempts to contribute to the understanding of the patterns of variation in the region, and to the clarification of the taxonomic identity of *C. robusta.*

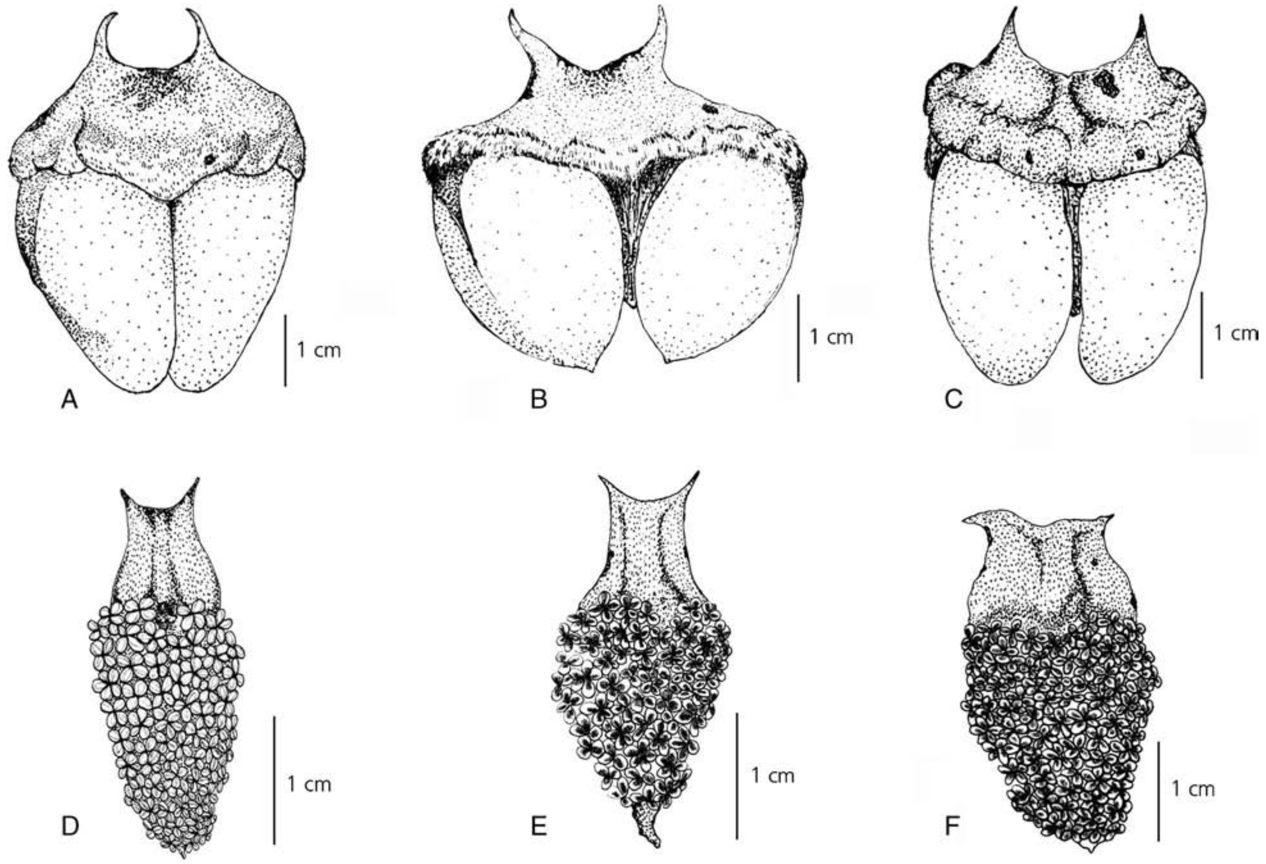


Fig. 4. Illustration of megasporophylls and microsporophylls of *Ceratozamia oliversacksii* and closely related species. **A, D** *C. oliversacksii*; **B, E** *C. leptoceras*; **C, F** *C. robusta*. DRAWN BY GERARDO QUINTOS.

Table 1. Morphological characters of *Ceratozamia oliversacksii* compared with its morphologically and geographically close species. * Meristic characters.

Character	*C. oliversacksii*	*C. robusta*	*C. leptoceras*
Type of prickles	thin	robust	thin
Leaf colour at emergence	green	brown	green
Leaf position	descending	ascending	descending
Shape of leaflets	linear	lanceolate	linear
Leaflet consistency	papyraceous	papyraceous	membranaceous
Width of median leaflets (cm)	2.0 – 3.3	2.5 – 4.0	1.9 – 2.8
Horn shape of microsporophylls	straight	recurved	straight
Infertile portion of microsporophylls	rounded	rounded	linear
Length of microsporophylls (cm)	2.5 – 2.75	2.33 – 3.0	2.1 – 2.45
Width of microsporophylls (cm)	0.99 – 1.28	1.14 – 1.91	1.09 – 1.30
Horn length of microsporophylls (cm)	0.08 – 0.18	0.26 – 0.40	0.1 – 0.23
Length of infertile portion of microsporophylls (cm)	0.68 – 0.80	0.45 – 0.65	0.83 – 0.96
Ovulate strobilus apex	acute	acuminate	acute
Megasporophyll horn type	acuminate	acuminate	apiculate
Trichomes on megasporophylls	scarce brown to reddish-brown trichomes or glabrous	scarce blackish or glabrous	pubescence at base of megasporophylls
Number of orthostichies*	9 – 10	8 – 14	8 – 9
Number of megasporophylls per row*	11 – 13	17 – 20	7 – 9
Distance between horns of megasporophylls (cm)	1.33 – 1.40	1.35 – 1.60	0.95 – 1.35
Horn length of megasporophylls (cm)	0.31 – 0.53	0.38 – 0.50	0.63 – 0.81

Taxonomic key to the *Ceratozamia* species of Oaxaca and those morphologically close to *C. oliversacksii*

1. Petiole and rachis twisted . . . . **C. norstogii**
1. Petiole and rachis straight 2
    2. Leaflets obovate, ≥8.5 cm wide, veins prominent . . . . **C. euryphyllidia**
    2. Leaflets lanceolate to linear, up to 4.0 cm wide, veins not prominent 3
        3. Leaflets linear; infertile portion of microsporophylls ≥0.68 cm long and horns ≤0.23 cm long . . . 4
            4. Leaflets papyraceous; ovulate strobili with ≥ 11 sporophylls per row; megasporophylls green with scarce brown to reddish-brown trichomes or glabrous; microsporophylls with infertile portion rounded . . . . **C. oliversacksii, sp. nov.**
            4. Leaflets membranaceous; ovulate strobili with ≤ 9 sporophylls per row; megasporophylls green with abundant trichomes at maturity (pubescence at base); microsporophylls with infertile portion linear . . . . **C. leptoceras**
        3. Leaflets lanceolate; infertile portion of microsporophylls < 0.68 cm long and horns ≥0.30 cm long 5
            5. Petiole with robust prickles, >0.45 cm long . . . . **C. robusta**
            5. Petiole with thin prickles, < 0.40 cm long 6
                6. Leaflets ≤ 1.8 cm wide; leaves ascending, brown to brownish at emergence . . . . **C. chimalapensis**
                6. Leaflets >1.8 cm wide; leaves descending, green at emergence 7
                    7. Petiole with abundant prickles and shorter than rachis; rachis with prickles . . . . **C. mixeorum**
                    7. Petiole with few prickles and nearly or the same length as the rachis; rachis unarmed . . . . **C. whitelockiana**

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ORIGINAL ARTICLE

Species delimitation in *Ceratozamia* (Zamiaceae) from Southwestern Mexico, in light of reproductive and climatic diversification

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Abstract

Multidisciplinary taxonomic studies have enhanced the delimitation of species within complex taxonomic groups. In the cycad genus *Ceratozamia*, the taxonomy is still not fully resolved for the several species complexes that have been proposed. Five *Ceratozamia* species occur in geographic proximity in the Soconusco region of southwestern Mexico. Because of similarity and variable morphology across these species, their taxonomy is a source of confusion. Based on integrative taxonomy, including morphological (qualitative and quantitative), ecological, and molecular data, we aimed to diagnose the species from the Soconusco region. We carried out extensive field sampling, collecting 225 individuals from a total of 12 populations, and reviewed one hundred herbarium specimens to evaluate whether the species are diagnosable. We carried out morphometric analyses at the population level on vegetative and reproductive characters and present the first analyses of phenotypic variability of pollen and ovulate strobili in neotropical cycads. Additionally, we evaluated the influence of climatic factors on morphological variation. Morphological characters of ovulate strobili were correlated with climatic conditions. In contrast, there was no significant correlation between the morphology of pollen strobili and the environment. In the pollen strobili, we found fixed characters that allow for the identification of the species. The haplotype networks displayed several groups of related haplotypes, which in some species corresponded to mixed population clusters. We detected a discordance between phenotypic and genetic evidence. The integration of evidence offers grounded views for clarifying species boundaries and outlining the differences among species, which we incorporated into a taxonomic key. In general, the mixed relations within this group of species may be due to historical hybridization events favored by common phenology and sharing of biotic pollination vectors. Finally, based on our results, we discuss the use of morphology for species delimitation and inference of evolutionary relationships in cycads.

Keywords Co-inertia · Cycads · Cycadales · Gymnosperm · Multivariate morphometrics · Phenotype

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Introduction

Species circumscription and taxonomic revisions are critical tasks for taxonomy (Wheeler, 2020). Therefore, robust taxonomic frameworks are essential for successfully testing species delimitation. These frameworks include the species concept, the definition of diagnostic characters, and a battery of analytical methods. The integrative taxonomy approach considers multiple sources of biological evidence and specific analytical techniques in the circumscription of species (Dayrat, 2005; DeSalle et al., 2005; Padial et al., 2010) and has proved beneficial for unraveling taxonomic limits within species complexes (Carstens et al., 2013; Prata et al., 2018).

Species complexes are groups of populations of two or more species with unclear taxonomic limits (Prata et al., 2018) because of high or low variation in their taxonomic characters, such as morphological, molecular, and ecological (Duminil et al., 2012). Generally, species in species complexes are indistinguishable by their morphological characters. Moreover, different sets of characters, e.g., morphological, genetic, ecological, may result in different grouping patterns and diagnosable species. Such discrepancies in the taxonomic delimitation of species suggest discordant character evolution (De Queiroz, 2007; Padial et al., 2010).

Cycadales is an order of dioecious gymnosperms with at least 356 species arranged in ten genera (Calonje et al., 2013–2022). Many species complexes have been suggested within the Cycadales. Within the genus *Ceratozamia* Brongn., with a long history of synonymies and high morphological similarity among species, up to seven species groups have been proposed (Miquel, 1868; Vovides et al., 2004a, b; Whitelock, 2004). *Ceratozamia* is restricted to the Neotropics, with most of its distribution in Mexico. Vovides et al. (2004a) used vegetative morphology and anatomy and geographic affinities to define seven species complexes in *Ceratozamia*. Taxonomic limits of most species complexes in *Ceratozamia* are based on morphological characters, especially those of a quantitative nature (Pérez-Farrera et al., 2009, 2014). The morphology of reproductive organs in *Ceratozamia* may prove helpful for the taxonomy of the genus and clarifying elements within these seven groups. However, little evidence of this kind has been systematically collected, partly because of the dioecious nature of cycads and the scarcity of phenological studies.

Because of the reasons given above, identification of many species in *Ceratozamia* remains problematic, which calls into question their delimitation. Some of these species show great variation in vegetative morphology (Martínez-Domínguez et al., 2017). Nonetheless, vegetative morphology and reproductive morphology of both ovulate and pollen strobili are crucial for identification. Unfortunately, studies of patterns of intraspecific molecular variation are restricted to a few species (Martínez-Domínguez et al., 2016, 2017). Several studies have tested species limits in the genus (Martínez-Domínguez et al., 2016, 2017, 2018a; Pérez-Farrera et al., 2009, 2014). However, circumscription efforts are still needed in species complexes such as those in the Soconusco region of southwestern Mexico, where up to five species of *Ceratozamia* occur: *C. norstogii* D.W.Stev., *C. vovidesii* Pérez-Farr. & Iglesias, *C. matudae* Lundell, *C. alvarezii* Pérez-Farr., Vovides & Iglesias, and *C. mirandae* Vovides, Pérez-Farr. & Iglesias. It is especially difficult to differentiate between *C. alvarezii* and *C. mirandae* because of their blurred morphological boundaries. Furthermore, *C. alvarezii* is only known from the type locality, whereas *C. mirandae* is widely distributed along the southern mountains in Chiapas (Pérez-Farrera et al., 2017).

Infrageneric relationships in *Ceratozamia* are not fully resolved; molecular phylogenetic studies disagree in the number of clades and the relationships among them (c.f. Condamine et al., 2015; González & Vovides, 2002, 2012; Liu et al., 2022; Medina-Villarreal et al., 2019). González and Vovides (2002, 2012) found clades congruent with geographic distribution, in which *C. alvarezii, C. mirandae, C. vovidesii*, and *C. norstogii* were recovered as monophyletic. However, the phylogenetic hypothesis of Medina-Villarreal et al. (2019) supported only two main clades, and these lacked geographic or morphological correspondence among species; the Soconusco species were recovered in different clades where *C. alvarezii* and *C. norstogii* are sisters, *C. mirandae* was sister to a clade that includes *C. vovidesii* and *C. santillanii* Pérez-Farr. & Vovides, while *C. matudae* was only distantly related to these taxa and sister to *C. whitelockiana* Chemnick & T.J. Greg. Although infrageneric relationships were not discussed by Liu et al. (2022), their phylogeny based on transcriptome data is the most complete for *Ceratozamia* and recovered a phylogenetic pattern more similar to that of González and Vovides (2012), particularly for Soconusco species. In summary, *C. alvarezii*, *C. mirandae, C. vovidesii*, and *C. norstogii* seem to be phylogenetically closely related species despite some discrepancies among studies (c.f. Medina-Villarreal et al., 2019; Vovides et al., 2004a). These four species show similar morphology and occur mainly in the cloud forest of the Soconusco region, a biogeographic province comprising a narrow strip that includes a small area of southern Oaxaca and most of the Pacific coast of Chiapas (Arriaga et al., 1997). These species belong to the *C. norstogii* complex, although the members are currently under debate (Vovides et al., 2004b). Furthermore, *C. chimalapensis* Pérez-Farr. & Vovides was proposed as part of this complex (Vovides et al., 2008); however, this species is endemic to Oaxaca in the disjunct portion of the Soconusco and has morphological characters that allow it to be recognized (Pérez-Farrera et al., 2014; Vovides et al., 2008).

In this study, we aimed to test the species limits of the species of *Ceratozamia* that inhabit the cloud forest of the Soconusco region, with particular attention to *C. alvarezii* and *C. mirandae* because of their morphological similarity. The approach is to implement the taxonomic circle in which multiple lines of evidence are analyzed independently for taxonomic hypothesis testing, corroboration, and review (DeSalle et al., 2005). Considering that the environment plays a role in phenotypic expression and could relate to local ecological adaptions (Limón et al., 2016), we test the relationship between the climatic conditions where the species occur and variation of vegetative and reproductive morphological characters. We emphasize analyzing phenotypic variation because reproductive characters have not been studied in either an evolutionary or taxonomic context. In addition, we explore molecular variation at the population level, which is informative and contributes substantially to the investigation of evolutionary processes in this genus. Based on these results, we propose a circumscription with qualitative and quantitative vegetative and reproductive morphological evidence and molecular and phenological evidence. The results are discussed in light of clinal variation in phenotype and speciation hypotheses.

Material and methods

Sample collections and geographic distributions

We collected 225 *Ceratozamia* individuals in the field from 12 populations (Supplementary Material 1; Fig. 1). These specimens were collected under scientific collection permit SGPA/DGVS/5506 from SEMARNAT, Mexico. Leaf vouchers were prepared for all individuals and deposited in the CIB and MEXU herbaria. Based on this material, we analyzed morphological variation within and among populations and species. In addition, leaf tissue from five individuals per population was preserved in silica gel for DNA sequence analysis.

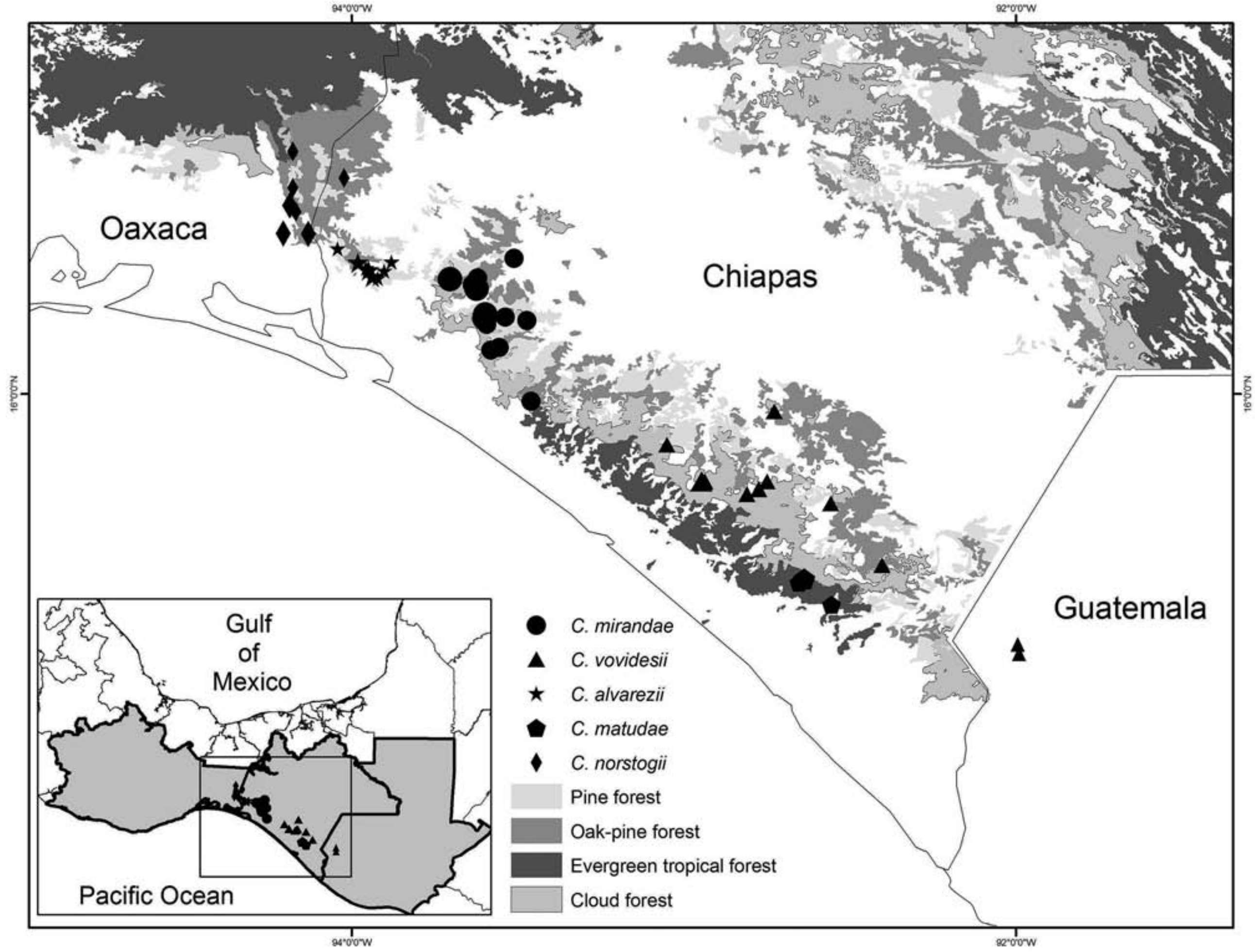

Fig. 1 Distribution of *Ceratozamia* in the Soconusco region. The small symbols indicate species records from herbaria, whereas the large symbols indicate sampled populations

Character selection and coding

Morphological characters were examined from a selection of individuals covering the full spectrum of geographical and habitat variation expressed in these species. For morphological studies, we carried out the characterization of reproductive structures that had attained reproductive maturity, i.e., during pollen liberation in pollen strobili and post-pollination ovulate strobili. The parts of the pollen and ovulate strobili were described in detail. In total, 20 qualitative and 22 quantitative reproductive characters were evaluated (Supplementary Material 2, 3) in 54 ovulate strobili and 73 pollen strobili (from 5 to 10 per population of each strobilus type). These characters were measured in the wild. In addition, 18 qualitative and 22 quantitative vegetative characters were evaluated (Supplementary Material 2, 3).

Criteria of species delimitation and exploration of evolutionary scenarios

Working under the phylogenetic species concept in the context of the integrative taxonomy approach (Wheeler & Platnick, 2000), we applied three criteria for species delimitation and speciation hypotheses within these morphologically similar entities: (1) diagnosability, applied to qualitative morphological characters through population aggregation analysis; (2) phenetic, applied to quantitative morphological characters using multivariate methods (between-class analysis and co-inertia); and (3) statistical parsimony, based on molecular data. Additionally, we used phenological and geographic data to delimit species. The evidence was used to define diagnostic characters and clarify the identity of the species. We also reviewed approximately one hundred herbarium specimens housed in BIGU, CH, CHAPA, CHIP, ENCB, IBUG, MEXU, MO, NY, SERO, USCG, and XAL. Based on the results of our data analyses and the informative characters detected, these herbarium specimens were critically examined and re-determined when necessary. We used geographical coordinates from herbarium specimens when available and our own collections to obtain a characterization of the habitat and distribution range for each species. We projected the geographic coordinates onto a digital map of Mexico using ArcMap 10.2 (ESRI, 2011) and included soil use and vegetation type layers (VI series, 1:250,000 scale; INEGI, 2013). Further, considering the patterns of variation, we assessed divergence among these complementary axes and explored the main drivers of speciation in this region.

Qualitative morphological characterization

A matrix with qualitative characters was constructed at the population level to compare and analyze the morphological patterns. We applied population aggregation analysis to test for species diagnosability (Davis & Nixon, 1992). This method searches for fixed character differences among populations. These data were used to develop the diagnoses for the species.

Quantitative morphological characterization

To address whether morphometry discriminated among *Ceratozamia* species of the Soconusco, we used a between-class analysis based on principal components analysis. The grouping factor was the identity of each of the 12 populations, and we used 18 vegetative characters. To establish the significance of the separation of the centroids in the multivariate space, we used a permutation Monte Carlo approach in which the values for each variable were shuffled at every iteration. For each iteration the distance among the centroids was calculated. We performed 1000 iterations and calculated the empirical probabilities as the number of times the distance between each pair of centroids was equal to or greater than the distances estimated from the actual data.

Phenotype and environment

To explore the correlation among measurements of vegetative and reproductive characters with the environmental conditions we used co-inertia analysis (Antoniazzi et al., 2021; Doledec & Chessel, 1994; Dray et al., 2003). Environmental data were the 19 variables of the WorldClim project (Hijmans et al., 2005) at a spatial resolution of 30 arc-sec. Because cycads are dioecious, we performed co-inertia analyses for vegetative, pollen strobilus, and ovulate strobilus characters separately.

In each case, we included a model selection procedure to identify the best possible correlation on subsets of morphometric and climatic variables. The model selection was automated in a machine learning algorithm (Bies et al., 2006) that selected the subsets of variables that maximized the multivariate correlation. To establish the significance of the correlation of the final model, we used a Monte Carlo randomization test (Heo & Gabriel, 1998). All tests were run with the R software environment v.3.1.0 (R Core Team, 2014).

Molecular techniques

We extracted genomic DNA from 10 mg of leaf tissue for three specimens per population using the DNeasy Plant Mini Kit (QIAGEN, Germantown, MD, USA). All samples were amplified for the plastid *matK* gene, the nuclear ribosomal ITS region (internal transcribed spacer region, nrITS), and a single-copy (SC) nuclear gene, CyAG. We used the amplification protocols described in Nicolalde-Morejón et al. (2011) and Salas-Leiva et al. (2014). PCR products were purified using a QIAquick PCR Purification Kit (QIAGEN). Purified products were then sent to Macrogen Inc. (Seoul, South Korea) for automated DNA sequencing.

Molecular analyses

Electropherograms were edited and assembled using Sequencher v.4.8 (Gene Codes Corp., Ann Arbor, MI, USA). All sequences were aligned in BioEdit v.7.0.9 (Hall, 1999) using the "multiple alignment" option in Clustal X (Thompson et al., 1997) and visually checked to assure there were no ambiguously aligned sites. The alignments from each molecular marker were concatenated into a single dataset using SequenceMatrix v.1.7.8 (Vaidya et al., 2011). We conducted a statistical parsimony analysis to identify the relationships among the haplotypes of each population using TCS v.1.2.1 and tcsBU to visualize the haplotype network (Clement et al., 2000; Dos Santos et al., 2016). The analysis was conducted with a connection limit of 95%. The analyses were carried out separately for each molecular marker. We explored genetic admixture and potential conflicting signal using the split decomposition method. The parsimonious split was calculated using SplitsTree v.4.17.0 (Bandelt & Dress, 1992; Huson & Bryant, 2006). The amount of molecular variation was estimated with DnaSP v.5.1 (Rozas et al., 2003).

Phenology

The characterization of reproductive phenology was carried out through observation of reproductive structures in the field and herbarium specimens. Because the phases in ovulate plants are critical for determining reproductive isolation between phenological entities, the phenophases are focused on plants bearing ovulate strobili (Martínez-Domínguez et al., 2018b). Ovulate strobili have four phenophases: (1) emergence, characterized by development of ovules; (2) receptivity, which occurs at the separation of megasporophylls, permitting pollination; (3) late ovulate, during which the pollinated ovules develop; and (4) disintegration, which results in liberation of seeds (Martínez-Domínguez et al., 2018b).

Results

Qualitative morphological variation patterns

The vegetative morphological patterns show few unique and consistent differences among species. Populations within species have fixed patterns of variation that allow them to be diagnosed. The most informative characters were (i) arrangement of petiole and rachis, (ii) leaflet apex shape, (iii) leaf color at emergence, (iv) color of the leaflet base at maturity, and (v) leaf position (Fig. 2). The morphological differences between *Ceratozamia norstogii* and the remaining species were remarkable because this species has a twisted petiole and rachis, and leaflets with an acute apex. The leaf at emergence is brown for all species except *C. matudae*, in which it is light green. Additionally, this species bears a yellow leaflet base at maturity whereas the others have a brown or green leaflet base. The populations of *C. alvarezii*, *C. mirandae*, and *C. vovidesii* do not represent morphologically distinct units using these characters (Fig. 2).

The reproductive morphological characters were the most consistent and useful for delimiting species. The pollen strobili exhibit relevant microsporophyll characters (i) microsporophyll shape, (ii) infertile apical portion shape, (iii) angle between the microsporophyll horns, and (iv) microsporophyll horn shape (Fig. 3). Four species exhibited a unique combination of character states useful for their diagnosability. *Ceratozamia norstogii* and *C. matudae* have discoid microsporophylls and an orbicular apical infertile portion with an acute angle between the two horns. In contrast, *C. norstogii* has a lobate apical infertile portion and *C. matudae* has a deeply lobate apical infertile portion (Fig. 3). *Ceratozamia vovidesii* differs from *C. mirandae* and *C. alvarezii* in possessing obconic microsporophylls with a linear apical infertile portion (Fig. 3). Populations of *C. alvarezii* and *C. mirandae* showed the same reproductive morphological pattern in ovulate and pollen strobili (Figs. 3 and 4). The ovulate strobili were very similar in all species, with the exception of *C. matudae*. This species has an aristate strobilus apex and recurved horns.

Quantitative morphological variation patterns

The between-class analysis based on vegetative characters showed segregation of the five species at the population level, but there were some significant overlaps between populations of different species (Fig. 5). *Ceratozamia norstogii*, including the three populations analyzed, was differentiated from the other *Ceratozamia* species of the Soconusco region ($p < 0.001$). At the same time, there were no significant differences between pairs of *C. norstogii* populations ($p > 0.495$). The population of *C. matudae* showed marginal overlap with some populations of *C. mirandae* ($p = 0.094$ and $p = 0.052$) and differed significantly from all other species ($p < 0.001$). Of the populations for *C. alvarezii*, population 2 ("Corralito") differed significantly from populations 1 ("El Cafetal") and 3 ("Valle Corzo") ($p < 0.01$) whereas populations 1 and 3 were not significantly differentiated ($p = 0.374$). Nonetheless, the three populations of *C. alvarezii* differed significantly from the populations of the other four species ($p < 0.009$). *Ceratozamia mirandae* showed significant differentiation among populations. Still, populations of *C. mirandae* showed only marginal overlap

Fig. 2 Vegetative characteristics of *Ceratozamia* species in habitat. **A** *C. alvarezii* (population 1 from Cintalapa), **B** *C. mirandae* (population 4 from Villaflores), **C** *C. norstogii* (population 1 from Cintalapa), **D** *C. matudae* (population 1 from Acacoyagua), **E** *C. vovidesii* (population 1 from La Concordia)

with populations of *C. matudae* and significant overlap ($p = 0.351$) with the only population of *C. vovidesii*. This was the only significant overlap for *C. vovidesii*.

The reproductive characters for pollen strobili showed strong patterns of species clustering in morphological space (Fig. 5B). Populations of *C. matudae* and *C. norstogii* showed significant differentiation; these species have wider microsporophylls than the other species studied here (WMi; Fig. 5B). Populations of *C. mirandae* and *C. alvarezii* exhibited more similarity to each other with their longer microsporophylls (LMi and LIpMi). Some populations in *C. alvarezii* differed marginally from others, and one of these populations is similar to *Ceratozamia vovidesii.* Phenotypic variation in ovulate strobili showed morphological overlap among most species (Fig. 5C). *Ceratozamia alvarezii* has the widest megasporophylls, although *C. alvarezii* and the two populations of *C. mirandae* show significant overlap. *Ceratozamia matudae* was separated clearly from all populations of the other species by having a long ovulate strobilus peduncle and the longest megasporophylls (Fig. 5C).

Environment and phenotype

Co-inertia analysis showed a significant correlation between vegetative characters and prevailing climatic conditions ($r = 0.61$; $p = 0.01$). *Ceratozamia vovidesii* had the highest values of PL (pairs of leaflets), LT (total length of leaf), LP (length of petiole), and NVFM (number of veins in median leaflets), which were correlated with the high seasonality of precipitation (Bio4). Also, *C. alvarezii* and *C. norstogii* had the smallest values for these characters except PF and

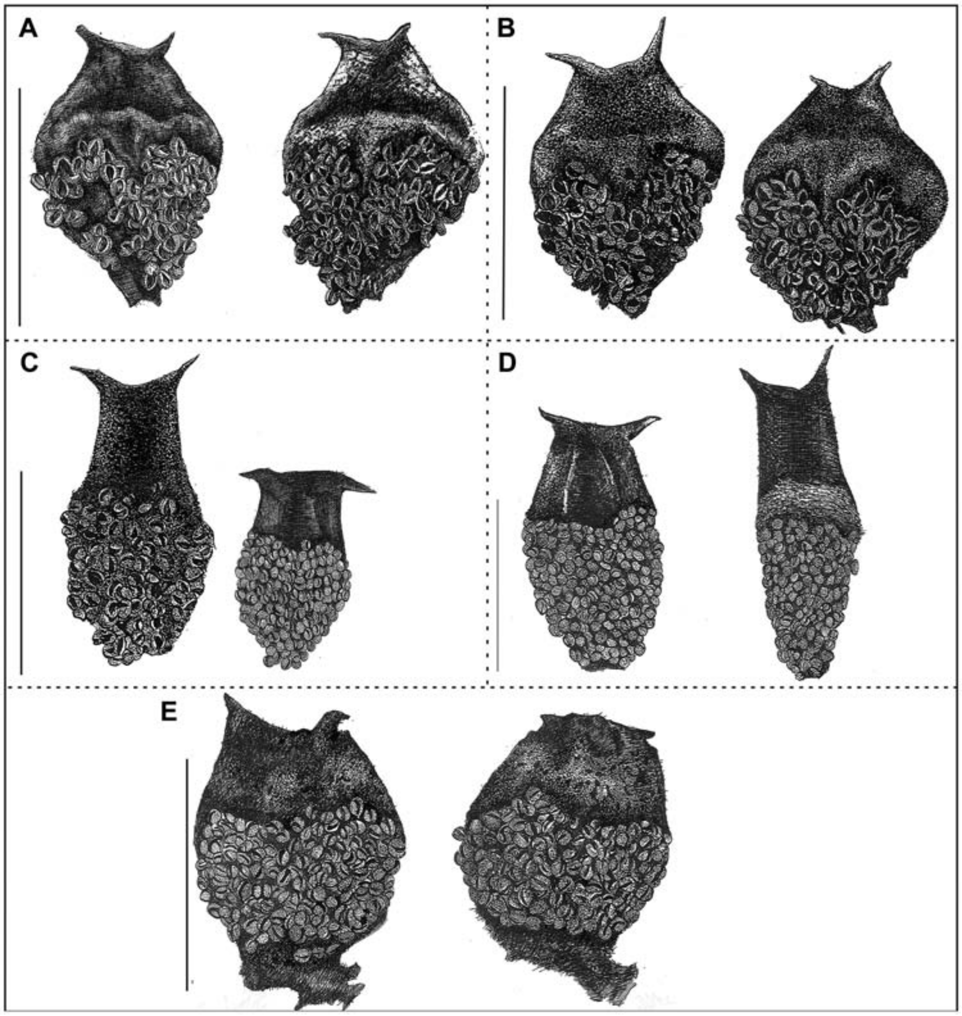

Fig. 3 Line drawing of microsporophylls. **A** *C. vovidesii*, **B** *C. matudae*, **C** *C. alvarezii*, **D** *C. mirandae*, **E** *C. norstogii*. Scale bar = 1 cm

occurred at sites with relatively low seasonality in temperature (Fig. 6A). *Ceratozamia norstogii* occurred at sites with high precipitation in the wettest quarter of the year (Bio16), whereas *C. alvarezii* occurred at sites with relatively low precipitation in the wettest quarter of the year. Comparatively, between *C. norstogii* and *C. alvarezii*, *C. norstogii* had greater PF and LT, and *C. alvarezii* had greater LP and NVFM. Also, among populations of *C. matudae* and *C. mirandae*, there was a continuous increase in PL and LT that correlated positively with the amount of rain during the wettest quarter of the year (Fig. 6A).

There was also a significant co-inertia between the morphology of ovulate strobili and the environment ($r = 0.65$; $p = 0.04$), but there was no significant correlation between the morphology of pollen strobili and the environment ($r = 0.37$; $p = 0.08$). For ovulate strobili, there was a clear separation of most species' populations (Fig. 6C). *Ceratozamia matudae* had narrow ovulate strobili (DEO) and occurred in sites with the highest maximum temperature (Bio5), the most significant annual range in temperature (Bio7), and the highest precipitation during the wettest month of the year (Bio13). As maximum temperature, annual temperature range, and precipitation during the wettest month decreased, the diameter of the ovulate strobili increased for *C. vovidesii*, *C. mirandae*, and *C. norstogii*. Only *C. alvarezii* showed separation of its populations in multivariate space, but it was consistent with the increase of the diameter of the ovulate strobili as Bio5, Bio7, and Bio13 decreased. The width of the megasporophylls (WMe) increased with the seasonality of precipitation (Bio15) and minimum temperature (Bio6). In contrast, the width of the megasporophylls decreased as the precipitation during the driest month of the year increased.

Phenology

We found a distinction between plants in phenophase "disintegration" (Fig. 7). In *Ceratozamia matudae*, this phenophase occurred from May to July, whereas for *C. norstogii* and *C. alvarezii* it occurred from July to September. This

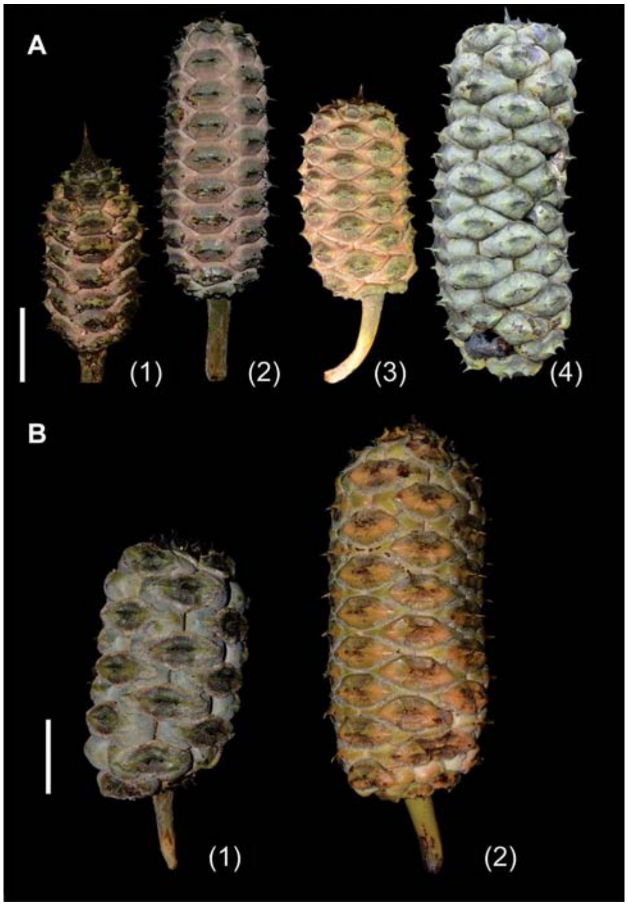

Fig. 4 Phenotypic variation of ovulate strobili. **A** *Ceratozamia mirandae* from Villaflores (1), (2) population 4; (3) population 1; (4) population 3. **B** *C. alvarezii* from Cintalapa (1), (2) populations 1 and 2. Scale bar = 5 cm

phase in *C. vovidesii* was from September to November. *Ceratozamia mirandae* exhibits more variability between phases at the population level. Most ovulate plants in the "La Sombra" population were in this phenophase from August to September, whereas in "Tres Picos" populations it was from September to November. In the latter populations, two ovulate plants were recorded in receptivity at the end of May, which is out of sync with seed shedding.

Molecular DNA variation

The concatenated matrix was 3088 bp in length: 1250 bp for CyAG, 806 bp for *matK*, and 1031 bp for the ITS region. The CyAG gene accounted for 40.5% of the alignment length. The ITS region and CyAG were the most variable with 21 and 11 parsimony-informative sites, respectively. The ITS region had the highest average nucleotide diversity per site (π) of 0.00353 m = , compared to 0.00215 for CyAG, and 0.00253 for *matK*, with 9 parsimony-informative sites. The haplotype diversity (Hd) was highest in CyAG with 0.917, followed by *matK* with 0.732, and ITS with 0.557. The haplotype network for CyAG showed the variable sites distributed among 12 different haplotypes. *Ceratozamia alvarezii*, *C. mirandae*, and *C. norstogii* shared haplotypes (Fig. 8A). The other shared haplotypes were between *C. alvarezii* and *C. norstogii* and *C. mirandae* and *C. alvarezii*. The two populations for *C. mirandae* in "Tres Picos" had unique haplotypes ("Tres Picos North" had two haplotypes and "Tres Picos South" had one). The network for *matK* with 6 haplotypes had one haplotype shared by most individuals of *C. norstogii* and *C. alvarezii*; the latter species shared another common haplotype with *C. mirandae* (Fig. 8B). The ITS region only had 4 haplotypes. *Ceratozamia matudae* had an exclusive haplotype, a common haplotype is shared by all populations of *C. mirandae*, two of *C. alvarezii* and *C. vovidesii*, and one population of *C. alvarezii* shared a haplotype with *C. norstogii* (Fig. 8C).

Overall, the haplotype networks showed an intricate structure. *Ceratozamia matudae* did not show a direct relationship with the remaining species of the Soconusco (Figs. 8 and 9). Groups of related haplotypes correspond to population clusters. In particular, *C. vovidesii* had a clear structure. All sequences of *C. norstogii* collapsed into different haplotypes, whereas *C. matudae* has one haplotype (Fig. 8). The *matK* network indicated interconnected haplotypes in *C. alvarezii* and *C. mirandae* (Fig. 8B). For the CyAG network, these species shared most of their haplotypes with each other (Fig. 8A). The populations of *C. alvarezii* that are geographically close to *C. mirandae* ("Los Alpes" and "La Sombra") were more genetically related than the other populations of *C. mirandae* in "Tres Picos". Intra- and interspecific variations were observed in both species, *C. alvarezii* and *C. mirandae*. In particular, *C. alvarezii* showed the most intraspecific variation in all populations, and the two populations for *C. mirandae* in "Tres Picos" had a greater difference with respect to the other populations of this species; however, we observed different haplotypes for one population in "Tres Picos" (Fig. 8).

The taxonomic distribution of haplotypes indicated a relationship between geographically close species in the north of the Soconusco region, particularly between *Ceratozamia norstogii* + *C. alvarezii* and *C. alvarezii* + *C. mirandae*. The greatest differences were in *C. mirandae* + *C. matudae* and *C. vovidesii* + *C. matudae* (Table 1). *Ceratozamia matudae* was the most genetically dissimilar species in the region. Data showed that the variation value within some populations was similar to the variation between different species (Supplementary Material 4). The differences between the two populations of *C. mirandae* in "Tres Picos" were low whereas between other populations of *C. mirandae* they were higher; however, the differences were not consistent, which indicated that there was no evidence that this population was genetically subdivided from the rest of this species.

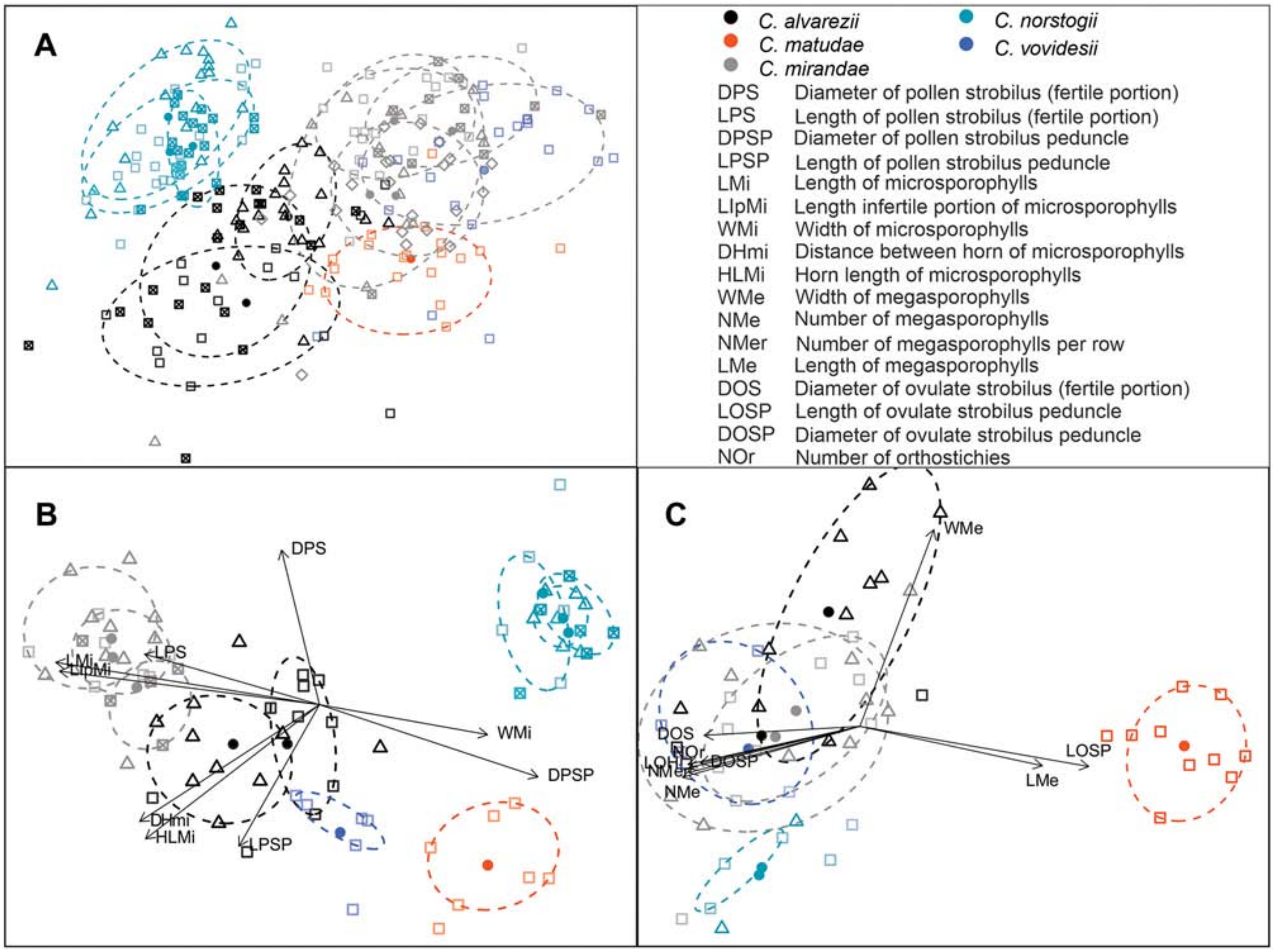

Fig. 5 Phenotypic variation in quantitative characters. **A** Vegetative characters. **B** Reproductive characters for pollen plants. **C** Reproductive characters for ovulate plants

The split decomposition analysis showed strong conflicting splits in the sequences for *Ceratozamia mirandae* and *C. alvarezii* (Fig. 9). In *C. norstogii*, some individuals from two populations were in a group with individuals of *C. alvarezii*. The other group contained a mixture between the individuals of *C. alvarezii* and *C. mirandae*; this haplotype network showed subgroups for two populations of *C. mirandae* closely related geographically and the other populations for this species in subgroups with *C. alvarezii*.

How many species?

Considering a unique combination of character states, mainly reproductive characters, this integrative approach allowed us to recognize and clarify limits among the five species found in the Soconusco region. *Ceratozamia alvarezii* and *C. mirandae* can only be recognized by evaluating several individuals at the population level. The herbarium specimens recorded from Guatemala were identified as *C. vovidesii.* This represents a southwards distribution range extension for *C. vovidesii*, whereas *C. matudae* should now be considered endemic to Mexico.

Geographic distribution patterns

Field work and review of herbarium specimens resulted in a range expansion of *Ceratozamia alvarezii* to 5 populations (Fig. 1), some in proximity to *C. norstogii* and *C. mirandae*. *Ceratozamia alvarezii* inhabits pine-oak forest and oak forest at elevations of 900 to 1200 m. *Ceratozamia norstogii* was thought to be limited to southern Oaxaca and northern Chiapas in mountainous region with pine to pine-oak forest at 800–1600 m. *Ceratozamia mirandae* and *C. vovidesii* occur in a broader area in the southern part of the Soconusco province at 850–1500 m and 800–1850 m of elevation,

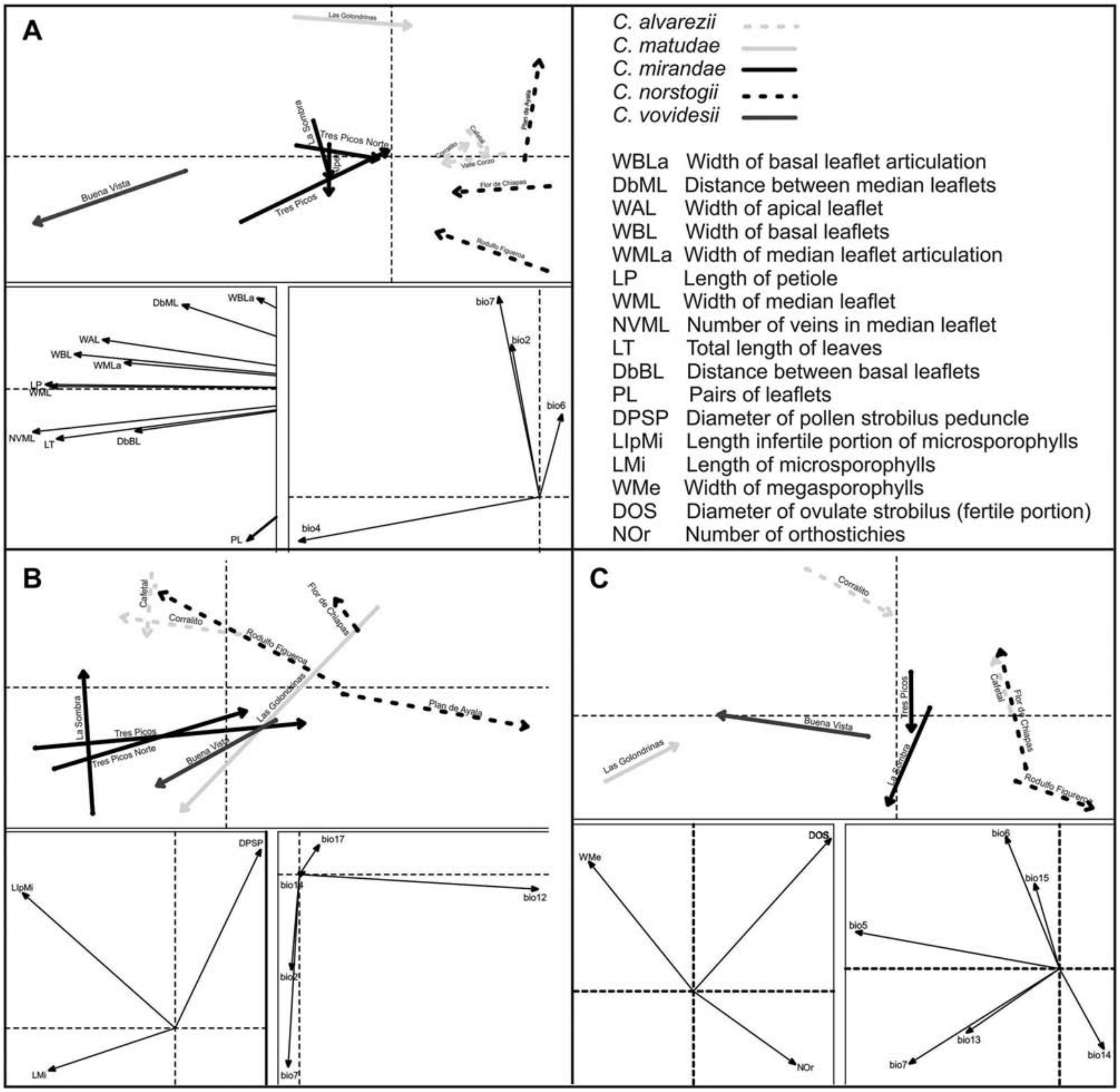

Fig. 6 Co-inertia analysis of five species of *Ceratozamia* in Soconusco. **A** Vegetative characters. **B** Reproductive characters for pollen plants. **C** Reproductive characters for ovulate plants. Circles indicate populations based on morphological characters, and arrowheads represent the environments where populations occur

respectively. The first showed more ecological amplitude; this species inhabits oak forest, transition oak forest between pine oak forest, and cloud forest and the second only cloud forest. *Ceratozamia vovidesii* had the widest distribution but inhabits only cloud forest from 700 to 1000 m in Mexico and Guatemala. *Ceratozamia matudae* occurred in a wide area of cloud forest, pine-oak, and oak forest at elevations of 800–1500 m in Mexico. In contrast to all others, *C. matudae* had a narrow distribution in evergreen tropical forest at 1000–1500 m (Fig. 1).

Discussion

Taxonomic scheme

In this study, the evidence for species recognition is based on careful evaluations of taxonomic characters. The protocol begins with at least one putative difference in a character to test species hypotheses by corroboration of this character in conjunction with other evolutionary evidence of lineage separation (or a specific character) or just congruence (i.e.,

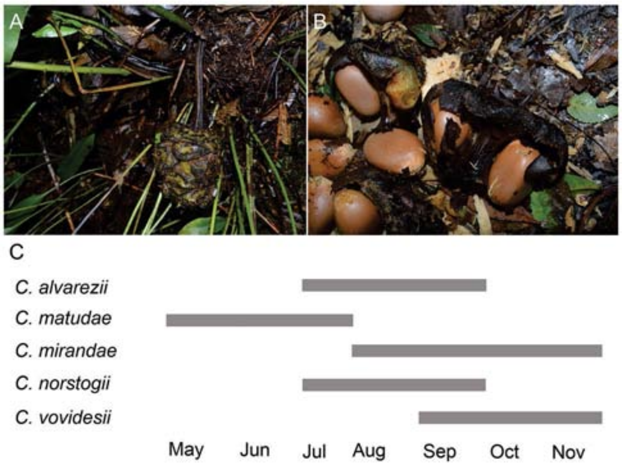

Fig. 7 Disintegration phenophase of *Ceratozamia* in Soconusco. **A** Beginning of disintegration phenophase of *C. matudae*. **B** Seed release of *C. matudae*. **C** Timing of disintegration of *Ceratozamia*

consensus protocol; Padial et al., 2010). Using this approach, we recognize five biological entities as species in this region. The different types of data contributed robustly to the recognition of these species.

The main characters used to distinguish species in *Ceratozamia* have been quantitative vegetative such as WML and LML (c.f. Pérez-Farrera et al., 2014; Whitelock, 2004). In this group from Soconusco, we found that these characters are highly variable within and among populations (Figs. 2 and 5A). The high morphological variation observed in vegetative characters and the use of only this type of evidence has led to taxonomic ambiguities in species of *Ceratozamia*, including underestimation of diversity and inconsistent taxonomic proposals (e.g., Medina-Villarreal et al., 2016).

In particular, *Ceratozamia alvarezii*, *C. vovidesii*, and *C. mirandae* exhibit a remarkable morphological similarity (c.f. Pérez-Farrera et al., 1999); this led to the confusion associated with the morphometric characters historically used as diagnostic within this group. Comparative studies including one or two of these species sampled single populations and found discontinuous patterns (Pérez-Farrera et al., 2014, 2017). Here, we sampled more than one population for the focal taxa, which have high similarity and convergent vegetative features that can lead to incorrect identification. Our vegetative morphometric analyses of these samples show that the populations of each species have wide variation. It is important to highlight that except for *C. norstogii*, there is no clear separation of vegetative characters at the population or species level; instead, variation is continuous (Fig. 5A).

Cycads produce large pollen and ovulate strobili, which are considered "model organs" for research related to the evolution from leaf-like reproductive structures to integrated reproductive organs (Brenner et al., 2003). However, strobili are rarely used for taxonomy at the species level although some taxonomic recommendations have been made for characters useful within *Zamia* (Calonje et al., 2019). This is in part because all cycads are dioecious and often lack strobili. Recently, the reproductive morphology of *Ceratozamia* has been noted as an important source of evidence for taxonomic decisions (Martínez-Domínguez et al., 2020). However, these characters were not the main objective of that work. Our results indicate that both quantitative and qualitative reproductive characters are taxonomically informative.

The ovulate strobili dimensions in *Ceratozamia matudae* and *C. norstogii* show no overlap (Fig. 5C), whereas they do in the remaining species. The pollen strobili show low levels of morphological variation among populations considered as belonging to the same species. *Ceratozamia alvarezii*,

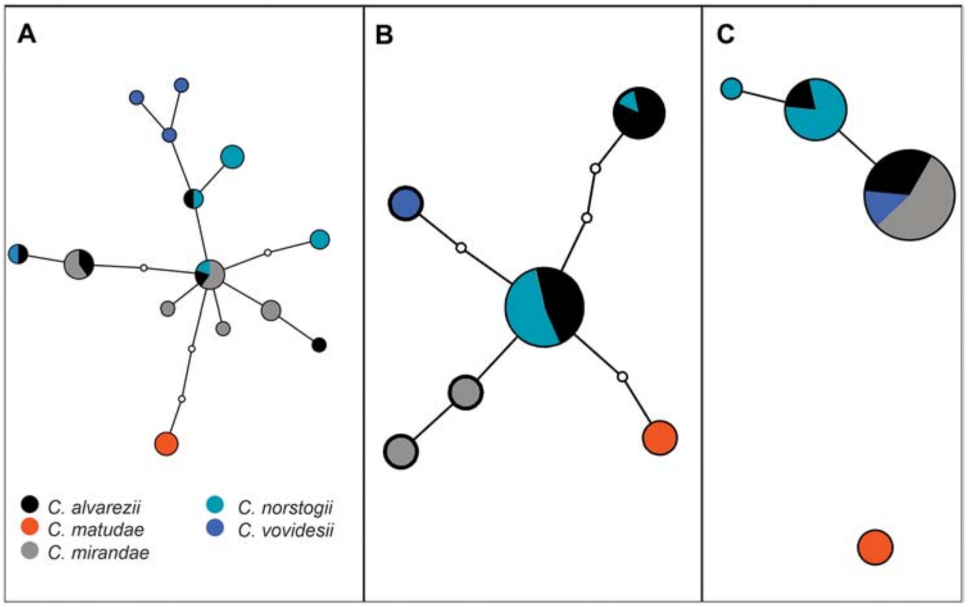

Fig. 8 The haplotype network for *Ceratozamia* species. **A** CyAG. **B** *matK*. **C** ITS region. The area of the circles is proportional to haplotype frequency

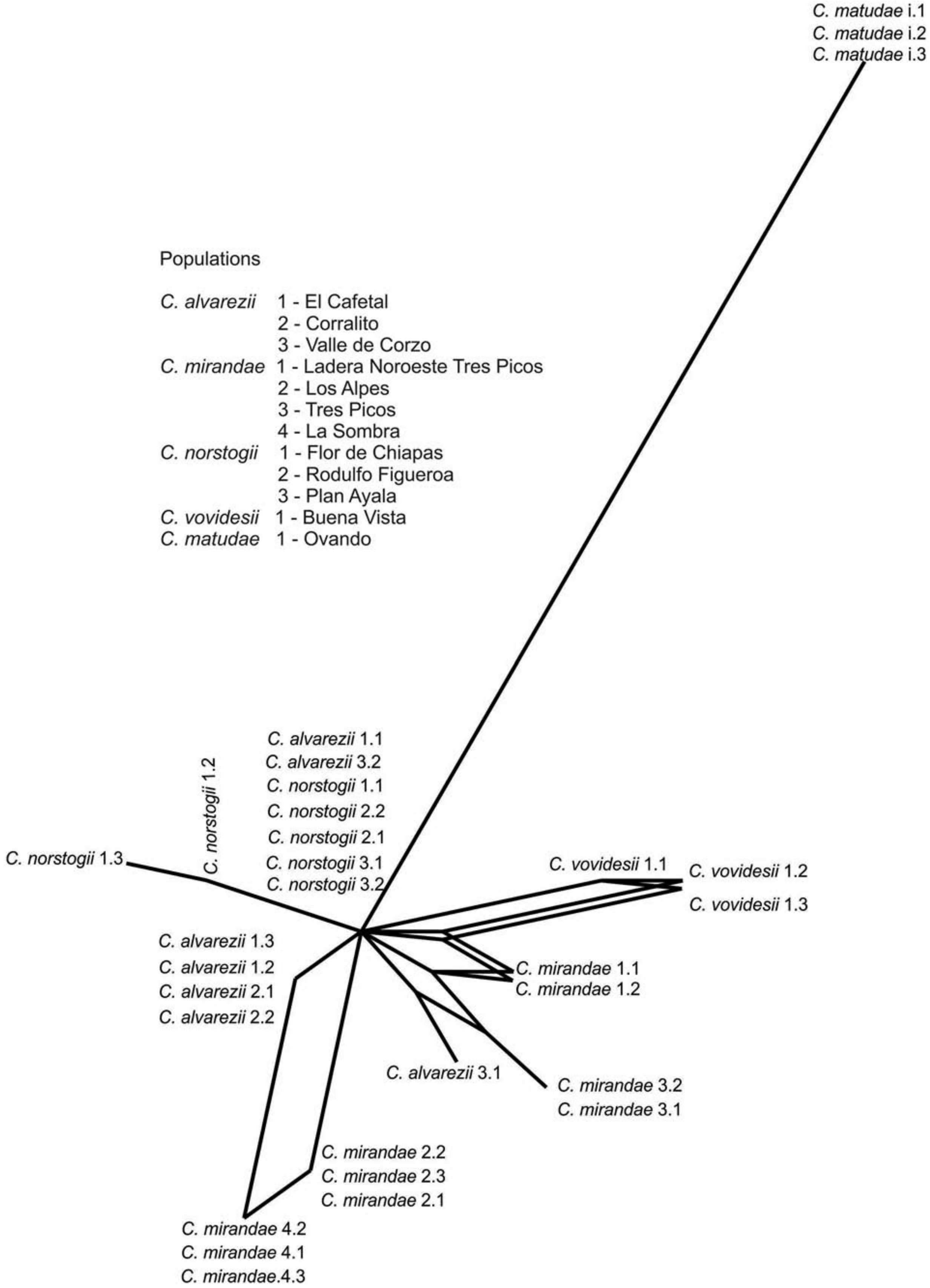

Fig. 9 Split graph for *Ceratozamia* species based on the concatenated matrix

Table 1 Genetic diversity values of *Ceratozamia* species

Species	*C. alvarezii*	*C. mirandae*	*C. norstogii*	*C. vovidesii*	*C. matudae*
C. alvarezii	-	4.700	3.592	6.190	27.143
C. mirandae	17	-	5.729	7.567	28.300
C. norstogii	14	1	-	5.619	27.429
C. vovidesii	3	4	4	-	29.333
C. matudae	25	25	26	29	-

Number of fixed differences below the diagonal; average number of nucleotide differences between populations above the diagonal

C. matudae, and *C. vovidesii* are morphologically cohesive taxa, but the analysis indicates morphological differentiation in populations of the same species and between populations of different species (Fig. 5B). In general, our comparisons between pollen and ovulate strobili at the population level resulted in the recognition of differences between the parts of microsporophylls such as horn types and infertile portion shape. Notably, the qualitative characters of the pollen strobilus are distinctive in most of the species (Fig. 3). These characters could be relevant in other cycad genera where they have been little explored such as *Encephalartos* Lehm., in which subspecies have recently been described from small areas. Recently, a natural hybrid in *Zamia* has been proposed by means of leaflet anatomy analyzed by morphometric analysis (Pérez-Farrera et al., 2016). This could be explored further in the context of diversity of methods used here. Additionally, applying this approach could contribute to a discussion of the infraspecific ranks such as varieties and subspecies that are still used in some cycad species.

Generally, genetic differentiation among species of *Ceratozamia* is low (González & Vovides, 2002; Nicolalde-Morejón et al., 2011; Pérez-Farrera et al., 2017). Despite the low genetic diversity in these species, we detected variability among individuals from the same population. Besides, we found patterns of discordant genetic differentiation among populations and species (Supplementary Material 4). The haplotypes in all markers were connected by a central loop with *C. alvarezii*, which indicates hidden relationships among them. Despite geographic proximity and vegetative morphological similarity, the haplotype data show that *C. matudae* is highly divergent from all other *Ceratozamia* species in the region. *Ceratozamia norstogii* is the species in this region with the highest morphological uniqueness, particularly because of its consistent and unique twisted rachis (Fig. 2C), but it has high genetic similarity with the other species, particularly with *C. alvarezii*. *Ceratozamia matudae* has the highest genetic divergence from other species in the region despite its phenotypic similarity in vegetative characters to other species such as *C. vovidesii*; in contrast, both ovulate and pollen strobili exhibit a pattern of phenotypic differentiation congruent with genetic evidence (Figs. 3, 5C and 8).

Some authors proposed that phenological patterns could help delimit species in *Zamia* (Clugston et al., 2016). In *Ceratozamia*, a few studies have included or described phenological data of species. We have provided a preliminary general description of reproductive timing found in the Soconusco region. The species boundaries between *C. alvarezii* and *C. mirandae* correspond with their phenological patterns; in the populations of *C. alvarezii*, the receptivity phase occurs before that of *C. mirandae*. In this context, the late ovulate phase starts in July for *C. mirandae*, whereas in *C. alvarezii*, this phase concludes in July. Despite the usefulness of phenological data, the long-life cycle of cycad strobili and lack of data on their periodicity have hindered phenological studies (Clark & Clark, 1987). This information is not a particularly practical character for identification of species, but it could be valuable in species delimitation. Our data in turn demonstrate the need for more analyses and monitoring to address the phenological patterns and discontinuities among species (Clugston et al., 2016; Griffith et al., 2012).

Evolutionary scenario

In *Ceratozamia*, there are similar environmental pressures favoring a common phenotype (Medina-Villarreal et al., 2019; Stevenson et al., 1986). Here, the evidence suggests that *Ceratozamia* in the Soconusco region has a complex evolutionary history. Moreover, low genetic divergence and discordance between genetics and morphology were detected (Figs. 8 and 9). The shared haplotypes among *Ceratozamia alvarezii*, *C. mirandae*, and *C. norstogii* could be explained by the retention of ancestral polymorphisms and incomplete lineage sorting (ILS), or gene flow. Recently, sequence data for gymnosperms (Mudannayake et al., 2019; Stull et al., 2021) have shown high levels of genomic conflict in which ILS could be the causes of gene discordance. This could explain shared haplotypes across species boundaries in *Ceratozamia*. Further research with analytical methods focusing on this question could provide insight into the patterns of genetic diversity within cycads.

The mixed relations within this species group including the phenotypic similarity could be an artifact of historical hybridization events. In *Ceratozamia alvarezii*, *C. mirandae*, and *C. norstogii*, the climatic, morphological, and distributional relationships suggest a strong shared biogeographical history (Figs. 5 and 6). The cloud forest is distributed throughout the Soconusco region, in which climatic cycles drove elevational migration and fragmentation in the distribution of species in the Mesoamerican area (Ornelas et al., 2013). In this context, the limited correspondence detected through analysis of haplotypes could represent the isolation of some populations in Soconusco (Figs. 8 and 9). The current geographic distribution of *Ceratozamia* in this region could be a climate-driven response of a few remanent populations that survived Pleistocene glacial cycles and subsequently speciated.

Pollination in cycads is an event mediated by insects that requires thermogenesis and volatiles to be effective (Marler, 2010; Terry et al., 2007). *Ceratozamia alvarezii*, *C. mirandae*, *C. norstogii*, and *C. vovidesii* share two species of insect pollinators, *Ceratophilla picipennis* and *C. chiapensis* (Tang et al., 2018), which opens the possibility of gene flow. Because climatic conditions influence phenological cycles in plants (Elzinga et al., 2007), geographically close species can have similar phenological patterns. It is possible that phenology could play a relevant role in speciation within this group. In particular, the overlap in phenological stages among species may be facilitated gene flow. We observed a slight disruption in the receptivity stage of ovulate strobili for *Ceratozamia alvarezii* and *C. mirandae*. Thus, this short temporal overlap could permit some gene flow between populations that would have been reproductively isolated in the past.

Is morphology dead in *Ceratozamia*? Illuminating taxonomy and evolution

Historically, morphological data have been fundamental for studying the evolution of biological groups. However, the integration of molecular techniques unintentionally caused a decrease in the use and evaluation of this type of data and, finally, morphology has been underappreciated (Wanninger, 2015). Recently, new approaches have highlighted the importance of not losing sight of morphology (Adams & Collyer, 2019; Daly et al., 2001; Wanninger, 2015). High variation in vegetative morphological characters and convergent morphology question the use of morphology for taxonomic approaches (Medina-Villarreal et al., 2019). However, our results have demonstrated the usefulness of these types of characters, and surprisingly, the quantitative evidence of pollen reproductive structures was relevant for taxonomic identification and not affected by environmental factors (Figs. 3, 5, and 6). Based on reproductive morphology, the strobili contributed to the clarification of species, complimenting the clustering of the vegetative variation of each species and finally, in the identification of herbarium specimens. Despite the great phenotypic complexity in *Ceratozamia*, the description of reproductive architecture provides valuable information for the identification and description of the species in the genus. Additionally, our findings have conservation implications because all these species are included in the IUCN Red List. For example, *C. mirandae* has a broad distribution but is categorized as Endangered. In contrast, following our results, *C. matudae* only occurs in a small area in Mexico, but is categorized also as Endangered. This suggests that the extinction risk of the latter species should be re-evaluated, and could be assigned to Critically Endangered.

Key to the species

1a. Rachis and petiole twisted; leaflets coriaceous, strongly involute..............*C. norstogii.*

1b. Rachis and petiole linear; leaflets papyraceous, slightly concave to flat..2.

2a. Articulation and base of leaflets yellow; new leaves green; peduncle of ovulate strobilus longer or the same length as the fertile portion..................................
.........*C. matudae.*

2b. Articulation and base of leaflets green; new leaves brown; peduncle of ovulate strobilus shorter than the fertile portion...3.

3a. Leaflets flat; microsporophylls obconic, apical infertile portion rounded and proportional to the length of the fertile portion (0.37–0.50 cm long)...............*C. vovidesii.*

3b. Leaflets slightly concave; microsporophylls elliptic, apical infertile portion linear and longer than fertile portion (0.50–0.81 cm long)..................................4.

4a. Plants from Cintalapa and Jiquipilas municipalities in Chiapas (Mexico); ovulate strobilus usually 17–27 cm long with 5–10 sporophylls per row....................*C. alvarezii.*

4b. Plants from Sierra Morena and Tres Picos in Chiapas (Mexico); ovulate strobilus usually 13.5–30 cm long with 7–10 sporophylls per row..........................*C. mirandae.*

Taxonomic treatment

Ceratozamia alvarezii Pérez-Farr., Vovides & Iglesias. Novon. 9: 410. 1999 — Holotype: Mexico. Chiapas: Cintalapa, Rancho El Cafetal, 950 m, 4 Mar 1996, *M. A. Pérez-Farrera 889* (CHIP!; isotypes: F!, HEM!, MEXU!, MO!).

Description. Leaves ascending, 36–125 cm long, brown at emergence with whitish trichomes; petiole and rachis linear, brown to greenish brown in mature leaves, with prickles. Leaflets 25–68 pairs, papyraceous, slightly adaxially concave, articulations brown. Pollen strobilus 15–40 cm long, 3.5–5.1 cm in diameter; peduncle 4–10 cm long, 1.5–2.1 cm in diameter, pubescence, reddish-brown to brown; microsporophylls 1.24–1.56 × 0.59–0.83 cm, yellowish cream with blackish trichomes, elliptic, fertile portion lobate, apical infertile portion 0.50–0.81 cm long, linear, horn 0.14–0.30 cm. Ovulate strobilus 17–27 cm long, 7.2–12.3 cm in diameter with acuminate apex; peduncle 4.0–10 cm long, 1.1–2.2 cm in diameter, erect, with scarce trichomes, brownish; megasporophylls 20–80, glaucous green with reddish brown to blackish trichomes at maturity, 4–8 orthostichies (rows), 5–10 sporophylls per row, 2.15–2.80 × 3.90–6.30 cm wide, horns 0.32–0.51 cm long, 0.69–1.80 cm distance between horns, straight. Seeds 2.5–3.0 cm long, 2.3–2.9 cm in diameter, spherical.

Specimens examined. Mexico. Chiapas: Cintalapa, 1100 m, 10 Mar 1993, *A.P. Vovides 1234* (XAL); 900 m, 10 Jul 1994, *M.A. Pérez-Farrera 71* (CIB, CHIP, MEXU); 950 m, 5 Sep 1995, *776* (CIB, XAL); 920 m, 21 Jun 2018*, F. Nicolalde-Morejón & al. 2791–2799* (CIB); 980 m, 22 Jun 2018, *F. Nicolalde-Morejón & al. 2830* (CIB); 1107 m, 18 Jun 2019, *F. Nicolalde-Morejón & L. Martínez-Domínguez 3177–3183* (CIB); 1350 m, 18 Jun 2019, *F. Nicolalde-Morejón & L. Martínez-Domínguez 3186–3196* (CIB); 1450 m, 11 Oct 1994, *J. Castillo & al. 445* (CHIP); 920 m, 21 Jun 2018, *L. Martínez-Domínguez & al. 1359–1369* (CIB), *1370* (CIB, MEXU); 980 m, 22 Jun 2018, *L. Martínez-Domínguez & al. 1402* (CIB, MEXU); 925 m, 18 Jun 2019, *L. Martínez-Domínguez & F. Nicolalde-Morejón 1770* (CIB); 1107 m, 18 Jun 2019, *L. Martínez-Domínguez & F. Nicolalde-Morejón 1771,1772* (CIB, MEXU), *1773* (CIB), *1774* (CIB, MEXU), *1775–1777* (CIB); 1350 m, 18 Jun 2019, *L. Martínez-Domínguez & F. Nicolalde-Morejón 1784–1794* (CIB); 1000 m, 27 Mar 2000, *O. Farrera S. 2068* (CHIP). Jiquipilas, 1200 m, 6 Jun 2002, *A. Reyes-García 5017* (MEXU); 1,380 m, 17 Feb 2000, *E. Palacios E. 2469* (CHIP); 1170 m, 7 Jul 1994, *M.A. Pérez-Farrera 68* (CIB).

Ceratozamia matudae Lundell. Lloydia. 2: 75. 1939 — Holotype: Mexico. Chiapas: northern slope of Mt. Ovando, 1,000 m, Feb 1939, *E. Matuda 2645* (MICH!; isotypes: CAS!, MEXU!, US!).

Description. Leaves descending, 80–155 cm long, green at emergence with brown trichomes; petiole and rachis linear, green in adult leaves, unarmed to armed with prickles. Leaflets 23–53 pairs, lanceolate, papyraceous, flat, green with base yellow, articulations yellow. Pollen strobilus 9.5–16 cm long, 3.5–5 cm in diameter; peduncle 8–14 cm long, 1.8–2.2 cm in diameter, pubescence, reddish-brown to brown; microsporophylls 0.78–1.4 × 0.77–1.1 cm, yellowish cream with reddish-brown trichomes at maturity, discoid, fertile portion deeply lobate, apical infertile portion 0.37–0.56 cm long, orbicular, horn 0.16–0.35 cm. Ovulate strobilus 8–15 cm long, 5–8 cm in diameter with aristate apex; peduncle 11–17 cm long, 1–2 cm in diameter, with scarce trichomes, blackish, pendulous and erect; megasporophylls 16–25, dark green with blackish trichomes at maturity, 4–5 orthostichies (rows), 4–6 sporophylls per row, 2.5–3.4 × 4.5–5.3 cm wide, horns 0.59–1.50 cm long, recurved, 0.92–1.56 cm distance between horns. Seeds 2.9–4.0 cm long, 1.9–4.0 cm in diameter, globose.

Specimens examined. Mexico. Chiapas: Acacoyagua, 1400 m, *A.P. Vovides 1069* (XAL); 1,000 m, Feb 1939, *E. Matuda 2646* (MEXU); 1420 m, 24 Jun 2018, *F. Nicolalde-Morejón & al. 2832–2840* (CIB); 1420 m, 24 Jun 2018, *L. Martínez-Domínguez & al. 1404–1409, 1412, 1415, 1416, 1418, 1419* (CIB), *1405, 1407* (CIB, MEXU); 1480 m, 29 Dec 1993, *M.A. Pérez-Farrera 27* (CIB, CH, CHIP, MEXU, USCG); 22 Feb 1995, *M.A. Pérez-Farrera 142* (CHIP, MEXU); 1300 m, 9 Jan 1987, *D.W. Stevenson & al. 681* (MO). Escuintla, 8 Feb 2000, *O. Farrera S. 1875* (CHIP). Siltepec, 1500 m, 6 Nov 1945, *E.H. Xolocotzi & A. J. Sharp 367* (MEXU); 1500 m, Oct-Nov 1940, *E. Matuda 4032* (MEXU).

Ceratozamia mirandae Vovides, Pérez-Farr. & Iglesias. Bot. J. Linn. Soc. 137: 81. 2001 — Type: Mexico. Chiapas: Villa Flores, Ejido La Sombra de la Selva, 880 m, 20 Sep. 1997, *De La Cruz R. 66* (lectotype, designated by Martínez-Domínguez et al., 2022: MEXU!).

Description. Leaves ascending, brown at emergence with whitish trichomes; petiole and rachis linear, brown in mature leaves, with prickles. Leaflets 28–94 pairs, linear to lanceolate, papyraceous, slightly involute, articulations brown. Pollen strobilus 16–55 cm long, 4.0–7.7 cm in diameter; peduncle 5–11 cm long, 0.8–1.4 cm in diameter, pubescence, reddish-brown to brown; microsporophylls 1.23–1.80 × 0.62–0.86 cm, yellowish cream with blackish trichomes at maturity, elliptic, fertile portion lobate, apical infertile portion 0.62–0.81 cm long, linear, horn 0.17–0.39 cm, straight. Ovulate strobilus 13.5–35 cm long, 7–12.6 cm in diameter with acuminate apex; peduncle with scarce trichomes, brownish, 4.5–12 cm long, 1.0–2.2 cm in diameter, erect; megasporophylls 42–72, glaucous green with reddish brown to blackish trichomes at maturity, 6–8 orthostichies (rows), 7–10 sporophylls per row, 2.0–3.0 × 2.8–6.1 cm wide, horns 0.29–1.47 cm long, straight, 1.55–2.09 cm distance between horns. Seeds 1.9–2.9 cm long, 1.2–2.5 cm in diameter, spherical.

Specimens examined. Mexico. Chiapas: Jiquipilas, 1015 m, 8 Mar 1995, *J.J. Castillo-Hernández 548* (CHIP); 1170 m, 19 Jun 2018, *F. Nicolalde-Morejón & al. 2749–2759* (CIB); 1170 m, 19 Jun 2018, *L. Martínez-Domínguez & al. 1312–1316, 1318, 1320, 1321* (CIB), *1317* (CIB, MEXU), *1319* (CIB, MEXU); 1270 m, 25 May 1995, *M.A. Pérez-Farrera 465* (CHIP). Villa Corzo, 1320 m, 12 Jul 2004, *A. Reyes-García & al. 7134* (MEXU); 1500 m, 9 Feb 1972, *D.E. Breedlove 23,999* (MEXU, MO); 1170 m, 16 Mar 1989, *U. Bachem & R. Rojas C. 579* (CHIP, MEXU, SLPM). Villaflores, 960 m, 6 Apr 1995, *A.P. Vovides & M.A. Pérez-Farrera 1261* (XAL); 1157 m, 7 Jul 2004, *D. Álvarez 9809* (MEXU); 1277 m, 25 Jun 2018, *F. Nicolalde-Morejón & al. 2854–2863* (CIB); 1195 m, 20 Jun. 2019, *F. Nicolalde-Morejón & L. Martínez-Domínguez 3208–3213* (CIB); 1350 m, 21 Jun 2019, *F. Nicolalde-Morejón & L. Martínez-Domínguez 3215–3224* (CIB); 1015 m, 5 Aug 1994, *J.J. Castillo-Hernández 230* (CIB, MEXU, USCG); 1250 m, 5 Apr 1995, *J.J. Castillo-Hernández* 595 (CHIP); 1140 m, 6 Aug 2002, *L. Alvarado C. & al. 368* (MEXU); 1195 m, 20 Jun 2019, *L. Martínez-Domínguez & F. Nicolalde-Morejón 1808–1813* (CIB); 1350 m, 21 Jun 2019, *L. Martínez-Domínguez & F. Nicolalde-Morejón 1814–1825* (CIB); 1277 m, 25 Jun 2018, *L. Martínez-Domínguez & al. 1428, 1429, 1431–1438* (CIB), *1430* (CIB, MEXU); 940 m, 22 Dec 1993, *M.A. Pérez-Farrera s/n* (XAL), *26* (CHIP, XAL); 910 m, 28 Apr 1994, *M.A. Pérez-Farrera 37* (CHIP, XAL); 1460 m, 16 Sep 1994, *M.A. Pérez-Farrera 126* (CHIP); 950 m, 12 Apr 1996, *M.A. Pérez-Farrera 1480* (CHIP).

Ceratozamia norstogii D.W. Stev. Brittonia. 34: 181. 1982 — Holotype: Mexico. Chiapas, Mar-Apr 1925, *Purpus 6* (NY!; isotypes: F!, MO!, US!).

Description. Leaves ascending, copperish brown at emergence with whitish trichomes; petiole and rachis twisted, copperish with abundant grayish trichomes in young leaves, with prickles. Leaflets 33–91 pairs, linear, coriaceous, strongly involute, green with yellowish-green base, articulations yellowish. Pollen strobilus 13.2–25 cm long, 3.1–8 cm in diameter; peduncle 2.0–5.5 cm long, 1.3–2.3 cm in diameter, tomentose, brown; microsporophylls 0.92–1.16 × 0.86–0.1.1 cm, yellowish-green to cream at maturity, discoid, fertile portion lobate, apical infertile portion 0.36–0.45 cm long, orbicular, horn 0.07–0.13 cm, straight, 0.35–0.56 cm between horns. Ovulate strobilus 21–35 cm long, 8.5–10.5 cm in diameter with acuminate apex; peduncle 4.8–9 cm long, 1.8–2.2 cm in diameter, tomentose, brown, erect; megasporophylls 42–63, grayish green with abundant blackish trichomes at maturity, 6–7 orthostichies (rows), 7–9 sporophylls per row, 2.6–3.0 × 3.6–4.0 cm wide, horns 0.37–0.58 cm long, straight, 0.37–0.42 cm distance between horns. Seeds 2.0–3.5 cm long, 1.1–1.9 cm in diameter ovate.

Specimens examined. Mexico. Chiapas: Cintalapa, 1100 m, 19 Mar 1993, *A.P. Vovides al. 1230, 1231, 1233, 1235* (XAL); Mar-Apr 1925, *C.A. Purpus 6* (MO); 1600 m, 3 Nov 1971, *D.E. Breedlove 21,813* (MEXU, MO); 1600 m, 21 Apr 1972, *D.E. Breedlove 24,709* (MO); 1600 m, 12 Oct 1979, *D.E. Breedlove 44,431* (MEXU); 800 m, 4 May 1988, *E. Palacios E. 375* (CHIP, IBUG); 1240 m, 6 Jun 1993, *E. Palacios E. 2155* (CHIP); 1280 m, 22 May 2001, *J.M. Lázaro-Zermeño 251* (CHIP); 1038 m, 20 Jun 2018, *F. Nicolalde-Morejón & al. 2762–2770* (CIB); 1038 m, 20 Jun 2018, *L. Martínez-Domínguez & al. 1326–1334* (CIB), *1335* (CIB, MEXU); 1325 m, 20 Jun 2018, *F. Nicolalde-Morejón & al. 2771–2780* (CIB); 1,325 m, 20 Jun 2018, *L. Martínez-Domínguez & al. 1337–1349* (CIB); 1100 m, 5 Oct 1995, *M.A. Pérez-Farrera 775* (CH, CIB, XAL); *5 Dec 1996, M.A. Pérez-Farrera 1483* (HEM). Oaxaca: San Miguel Chimalapa, 1120 m, 1 Apr 1996, *S. Salas-Morales & al. 1173* (SERO, XAL). Santo Domingo Zanatepec, 800 m, 22 Jun 2018, *F. Nicolalde-Morejón & al. 2819–2828* (CIB); 800 m, 22 Jun 2018, *L. Martínez-Domínguez & al. 1380, 1381, 1383, 1386, 1389–1391, 1394, 1396, 1399* (CIB).

Ceratozamia vovidesii Pérez-Farr. & Iglesias. Bot. J. Linn. Soc. 153: 394. 2007. — Holotype: Mexico. Chiapas: Sierra Madre, Jul 2001, *M.A. Pérez-Farrera 2620*^{a} (HEM!; isotypes: CHIP, MEXU, MO, XAL).

Description. Leaves ascending, reddish-brown at emergence with whitish trichomes. Petiole and rachis linear, green in adult leaves, with prickles. *Leaflets* 30–85 pairs, lanceolate, papyraceous, flat, articulations green. Pollen strobilus 15–45 cm long, 3.5–5 cm in diameter, peduncle 6–9.5 cm long, 1.6–1.9 cm in diameter, tomentose, reddish-brown to brown; microsporophylls 1.2–1.5 × 0.8–1.04 cm, yellowish cream with reddish-brown trichomes at maturity, obconic, fertile portion lobate, apical infertile portion 0.37–0.50 cm long, rounded, horn 0.30–0.42 cm, straight, 0.52–0.84 cm between horns. *Ovulate strobilus* 26–40 cm long, 7.1–9.6 cm in diameter with acuminate apex; peduncle 7–15 cm long, 1.7–2.2 cm in diameter, pubescence, brown; megasporophylls 60–70, green with abundant blackish trichomes at maturity, 6–8 orthostichies (rows), 7–12 sporophylls per row, 4–5 × 3.8–4.5 cm wide, horns 0.60–0.80 cm long, straight, 0.99–1.40 cm between horns. Seeds 2.2–2.7 cm long, 1.3–1.7 cm in diameter, ovate.

Specimens examined. Guatemala. Huehuetenango: 900–1,300 m, 3 Sep. 1942, *J.A. Steyermark 51,818* (NY; US); 1,630 m, 9 Jul. 2006, *M. Véliz & V. Davila 17,042, 17,043, 17,044* (BIGU); 30 May. 1906, *O.F. Cook 51* (US); 1,629 m, 10 Jul. 2006, *V. Davila & M. Véliz 1050* (BIGU); 1,622 m, 10. Jul. 2006, *V. Davila & M. Véliz 1052, 1053* (BIGU). MEXICO. Chiapas: Ángel Albino Corzo, 730 m, 23 Jan 1968, *Alush Shilom Ton 3554* (ENCB); 800–1000 m, 8 Nov 1945, *E.H. Xolocotzi & A.J. Sharp 402* (CHAPA, ENCB, MEXU); 1000 m, 2 Jun 1987, *E.M. Martínez S. & al. 21,586* (MEXU); 1819 m, 13 Aug 2009, *H. Gómez-Domínguez 2316* (MEXU); 1650 m, 18 May 1982, *J.I. Calzada & al. 9131* (XAL); 5 Mar 1989, *U. Bachem C. & R. Rojas 405* (CHIP). Jaltenango de la Paz, Jun 1995, *E. Matuda s/n* (MEXU); 1500 m, 23 Jun 1990, *M. Heath & A. Long 1287* (CHIP); 25 Feb 1995, *Miranda 7042* (MEXU). La Concordia, 1000 m, 5 Jun 1988, *E. Palacios E. 1050* (CHIP); 1840 m, 26 Jun 2018, *F. Nicolalde-Morejón & al. 2864–2875* (CIB); 1,840 m, 26 Jun 2018, *L. Martínez-Domínguez & al. 1439–1451* (CIB); 1156 m, 19 Sep 2001, *M.A. Pérez-Farrera 2621* (XAL); 1600 m, *M.A. Pérez-Farrera s/n* (CHIP); 1120 m, 17 Jun 2014, *M.G. Díaz M. 961* (CHIP);

24 Mar 2001, *R. Martínez-Camilo 54* (CHIP); 1100 m, 11 Jun 1988, *T.G. Cabrera Cachón 74* (CHIP); 1700 m, 1 Jun 1989, *U. Bachem C. & R. Rojas 795* (CHIP). Mapastepec, 1750 m, 13 May 1982, *J.I. Calzada & al. 8874* (IBUG, MEXU, MO, XAL). Siltepec, 28 Feb 2000, *O. Farrera S. 1958* (CHIP).

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Author contribution LMD: designed and carried out the research, sample collection, data analysis, and writing of the manuscript. FNM: study design, sample collection, and writing of the manuscript. FVS: study design and writing of the manuscript. RG: data analysis and writing of the manuscript. DSG: interpretation of data and writing of manuscript. DWS: design of the research and writing of the manuscript. All authors read and approved the final manuscript.

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Data availability The authors declare the availability of data in the supplementary section of the manuscript. Further material is available in the MEXU and CIB herbaria.

Declarations

Competing interests The authors declare no competing interests.

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II.II. Capítulo II.

Una monografía botánica de *Ceratozamia*: sintetizando el conocimiento de un siglo de exploraciones

Artículo de requisito

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Monograph of *Ceratozamia* (Zamiaceae, Cycadales): an endangered genus

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Abstract

Ceratozamia (Zamiaceae, Cycadales), is a member one of the most endangered seed plant groups. Species of *Ceratozamia* grow from near sea level up to 2,100 meters in Mexico and Mesoamerica. We present a modern taxonomic treatment of *Ceratozamia*, based on fieldwork combined with detailed study of herbarium specimens in and from Mexico and Central America. This new revision is based on incorporation of morphological, molecular and biogeographic data that have been previously published in circumscriptions of species complexes by our group. Detailed morphological descriptions of the 36 species of *Ceratozamia* are provided and relevant characters for the genus are discussed and described. A majority are endemic to Mexico and are concentrated at high elevations in mountainous areas. Synonymies, lectotypifications, etymologies, taxonomic notes, distribution maps, illustrations and detailed species-level comparisons are included, as well as a dichotomous key for identification of all species. Data on distributional ranges and habitats of all species are summarized. *Ceratozamia osbornei* D.W.Stev., Mart.-Domínguez & Nic.-Mor., **sp. nov.** is described from evergreen tropical forests of Belize and we highlight new populations and distributional ranges for *C. subroseophylla* Mart.-Domínguez & Nic.-Mor. and *C. vovidesii* Pérez-Farr. & Iglesias in the Mesoamerican region.

Keywords

Circumscription, cryptic species, cycads, lectotypification, Mesoamerica, Mexico

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Introduction

Ceratozamia Brongn. (Zamiaceae, Cycadales) is characterized by being dioecious with both megasporophylls and microsporophylls having two distinct apical horns. The 36 species of *Ceratozamia* occur in the tropical region of Mega-Mexico II *sensu* Rzedowski (1991), which was established using floristic criteria and includes Mexico, Guatemala, Belize and northern Nicaragua. This genus inhabits in all these countries except Nicaragua and the greatest diversity and endemism in *Ceratozamia* are concentrated in the Sierra Madre Oriental and Southwest regions of Mexico (Nicolalde-Morejón et al. 2014; Martínez-Domínguez et al. 2018a). Species described as *Ceratozamia* from outside this region are properly recognized as other cycad genera (see Excluded names). The genus primarily occurs in oak forest, cloud forest and tropical rain forest on karstic rocks, at an elevational range of 19–2,050 m. *Ceratozamia* is morphologically distinguished from other genera of Zamiaceae by its two parallel adaxial canals along the rachis, entire leaflet margins, and two horns on the distal end of sporophylls in ovulate and pollen strobili.

Ceratozamia is monophyletic with a fossil record dating to the lower Oligocene (Kvaček 2014). Recent phylogenetic analyses of molecular data have supported *Stangeria* (Kunze) Baill. as sister to *Ceratozamia* (Nagalingum et al. 2011) while others have placed *Stangeria* as sister to *Microcycas* (Miq.) A.DC. + *Zamia* L. (Salas-Leiva et al. 2013; Condamine et al. 2015; Liu et al. 2022). The phylogenetic relationships within *Ceratozamia* have been explored using different molecular data sets. González and Vovides (2012) sampled half of the known species and used three genes (*nrITS*, *trn*L-F, *D-SCAR*). More recently, a phylogenetic analysis of this genus that included 28 species and a molecular data set composed of six genes (*nrITS*, rpoC1, matK, rbcL, *psbK/I*, *atpF/H*) produced a topology resolved with low support values (Medina-Villarreal et al. 2019); in turn, Martínez-Domínguez et al. (2020) sampled 32 species and only two genes (*nrITS* and matK) but produced a poorly resolved topology. Studies of diversification ages of Cycadales using phylogenomic analyses have included between 70 and 83% of currently accepted *Ceratozamia* species, which recovered better node support (Nagalingum et al. 2011; Condamine et al. 2015; Liu et al. 2022). As a result, species relationships within *Ceratozamia* are becoming clearer and more work will be needed with continued exploration that will undoubtedly lead to new discoveries.

Since the description of the genus by Brongniart (1846), there has been an explosion of interest in this group. Miquel (1848) described five new species using only vegetative characters. *Ceratozamia* poses a taxonomic challenge, however, due to its significant vegetative morphological variation, which was a fact realized by Miquel (1868, 1869a) when he lumped all of his previously described species into *C. mexicana* Brongn. These early taxonomic treatments varied greatly in the number of species recognized within the genus with contrasting findings in each treatment (Regel 1857a, b; Miquel 1861, 1868, 1869a, b; Regel 1876a, b; Thiselton-Dyer 1884; Schuster 1932). Particularly, the 'Miquel taxonomic names' have undergone a series of rearrangements in synonymy and recognition of infraspecific categories. Many of these names were clarified by Stevenson and Sabato (1986) when they focused on the typification of all described species in the genus at that time, but did not include a list of invalid names. More recently, a regional taxonomic review focused on the Mexican Sierra Madre Oriental provided new observations that have allowed detailed reassessment and designation of nomenclatural types for some of these names (Martínez-Domínguez et al. 2018a).

Explorations in Mexico during the past three decades have uncovered new species of *Ceratozamia*, thereby, rapidly increasing its known diversity. Nevertheless, the taxonomy of *Ceratozamia* has been mainly characterized by rearrangements of infrageneric taxa, treatments in floristic works and species-level treatments of species complexes (Vovides et al. 1983; Stevenson et al. 1986; Pérez-Farrera et al. 1999; Avendaño et al. 2003; Pérez-Farrera et al. 2009; Vovides et al. 2012; Martínez-Domínguez et al. 2016, 2017c). Recent taxonomic efforts based on analyses of quantitative and qualitative morphological variation, phenology as well as DNA sequences of nuclear, plastid and mitochondrial genomes have produced new insights on the circumscription of species complexes and the most comprehensive taxonomic reviews of *Ceratozamia* to date (Martínez-Domínguez et al. 2016, 2017a, b, 2018a, 2022a). In addition, work focusing on the morphometric variation of vegetative characters and anatomical features have contributed to the clarification of some species (Pérez-Farrera et al. 2014; Vovides et al. 2016; Medina-Villarreal et al. 2019; Gutiérrez-Ortega et al. 2021). These studies have greatly enhanced our understanding of morphological variation, particularly in vegetative characters. Recently, genomic data from 100 nuclear single-copy genes have provided evidence for future directions in the study of the ontogeny of reproductive structures and associated evolutionary processes (Liu et al. 2022).

Currently, quantitative vegetative morphological characters are considered as the most taxonomically informative characters within *Ceratozamia* (Vovides et al. 2004; Pérez-Farrera et al. 2009). Because these types of characters exhibit high intra- and interspecific variation, renewed morphological exploration into reproductive structures has contributed additional information. This is particularly the case for ovulate strobilus characters, such as the angle between the horns of the megasporophylls, the strobilus apex and the shape of the distal face of megasporophylls (Martínez-Domínguez et al. 2018a). Additional characters are also present in pollen strobili such as the shape of the microsporophylls, the size of the horns, and shape of the fertile and infertile part. However, the use of reproductive characters has been limited by the difficulty of collecting reproductive structures in the field and the lack of these in herbaria. Also, usually ovulate and/or pollen strobili are fragmented as a result of incorrect processing of these structures and damage during handling.

The reproductive phenology of *Ceratozamia* is annual (Stevenson 1981). To date, our knowledge of the phenological phases for ovulate and pollen strobili indicates four phases in each (Martínez-Domínguez et al. 2022a). In both pollen and ovulate strobili the cycle starts with emergence, which is characterized by emergence from among the cataphylls. In pollen strobili, the sequence goes from a closed strobilus, followed by an open strobilus with dehiscence of the sporangia and, finally, senescence. In ovulate strobili, opening cracks develop between sporophylls (indicating receptivity), then a late ovulate strobilus with seeds if pollinated and, finally, disintegration (Martínez-Domínguez et al. 2018b). The receptivity and open pollen phases are critical for pollination and subsequent fertilization of ovules because this genus is pollinated by "pleasing fungus beetles" of family Erotylidae (Norstog and Nicholls 1997).

In this paper, as part of our comprehensive systematic review of the genus, we describe in detail ovulate and pollen strobili, including internal structures and their character states. This was possible by broad sampling in wild populations. We also examine overlooked morphological characters, conduct a nomenclatural review of species, and offer a comprehensive identification key for the genus. Here, we synthesize biogeographic information to account for their distributional range. By default, *Ceratozamia* and all of its species are listed and covered by CITES Appendix 1, but only 21 of the 36 species have had asessments (Calonje et al. 2013–2022; IUCN 2021). Those data are included in species treatments when available. Thus, this monograph is particularly timely and valuable in this era of habitat destruction and illegal collecting. Having a complete taxonomic treatment will allow for appropriate identifications that will lead to informed conservation assessments and actions (Stevenson et al. 2003; Rutherford et al. 2013; Martínez-Domínguez et al. 2021).

Materials and methods

The species circumscriptions for *Ceratozamia* presented here are based on extensive fieldwork, as well as review of specimens from a substantial number of herbaria, including BIGU, BM, BRH, CAS, CH, CHAPA, CHIP, CIB, EAP, ENCB, F, FCME, FTG, GH, HEM, HNT, IBUG, IEB, K, L, LE, LSU, MICH, MEXU, MO, NAP, NY, P, SERO, SLPM, TEFH, U, US, XAL, XALU, UAT, USCG (acronyms according to Thiers 2022), either by examining the specimens physically or electronically through the Global Plants web portal (http://plants.jstor.org/). Because most cycad specimens are sterile, we have taken the opportunity to record the presence of cones when they are present in a collection. We also examined the protologues of all species described and exhaustively searched for type specimens in the registered herbaria. It should be noted, however, that the type specimens for *C. chimalapensis* Pérez-Farr. & Vovides, *C. dominguezii* Pérez-Farr. & Gut.Ortega, *C. sancheziae* Pérez-Farr., Gut.Ortega & Vovides, and *C. zoquorum* Pérez-Farr., Vovides & Iglesias could not be found. Decisions on lectotypes were taken only when we confirmed that there was no evidence of the holotype's existence at the herbaria cited in the protologue. The specimens collected by us were under scientific collection permit SGPA/DGVS/5506 from SEMARNAT (Mexico) and MP-0209-2021 from ICF (Honduras). The geographical coordinates of each specimen were compiled in a database in ArcMap 10.2 (Esri, Redlands, USA) to determine the distribution of *Ceratozamia* using the biogeographic regionalization of the Neotropical region scheme of Morrone et al. (2022). Because species are all CITES listed, the specimens examined only indicate Municipality and elevation.

Criteria to delimit species. The taxonomic treatment presented here is partly based on our previously published work on species complexes, considered from an integrative taxonomy perspective for corroboration or refutation of taxonomic hypotheses and employing the "taxonomic circle" *sensu* DeSalle et al. (2005), in which multiple data sources are analyzed independently for hypotheses testing (Martínez-Domínguez et al. 2016; 2017a, b, 2018a, 2020, 2022a, b). For taxa we have not treated previously, species were recognized by a unique combination of morphological characters evaluated at the population level, considering their biogeographic distribution patterns as an additional source of evidence. We have recognized neither subspecies nor varieties, but have rather described the variability when it was present.

Specialized characters and terminology. The morphological terminology used in the descriptions was standardized according to Moreno (1984) and Harris and Harris (2000). Most *Ceratozamia* reproductive structures and characters have been recently described (Martínez-Domínguez et al. 2020). We evaluated 20 qualitative and 23 quantitative reproductive characters in pollen and ovulate strobili (Figs 1, 2; Table 1; Suppl. material 1). Generally, the terminology for the orientation of ovulate strobilus is given using the terms "decumbent" or "erect". However, the fertile portion of a strobilus could be inclined at an angle of 180° or prostrate, but not curved. Usually, this condition is derived from weight at maturity. Only the peduncle is curved or erect; the definition for decumbent is reclined with tip ascending, but the peduncle in this genus is not ascending. Here, we have used "pendulous", which is a more appropriate term and is defined as drooping downward, rather than "decumbent", as often appears in previous literature.

In microsporophylls, seven qualitative characters have been described: 1) microsporophyll shape, 2) distal face of the microsporophyll, 3) microsporophyll horn type, 4) infertile apical portion shape, 5) fertile portion shape, 6) direction of microsporophyll horns, and 7) angle between the horns of the microsporophylls. The direction of horns was coded in relation to the base of the infertile portion in the microsporophyll.

In addition, six quantitative characters have been evaluated: 1) length of microsporophylls, 2) width of microsporophylls, 3) number of microsporangia per microsporophyll, 4) horn length of microsporophylls, 5) distance between the horns of a microsporophyll, and 6) length of the infertile apical portion of a microsporophyll (Figs 2, 3; see Suppl. material 1 for details).

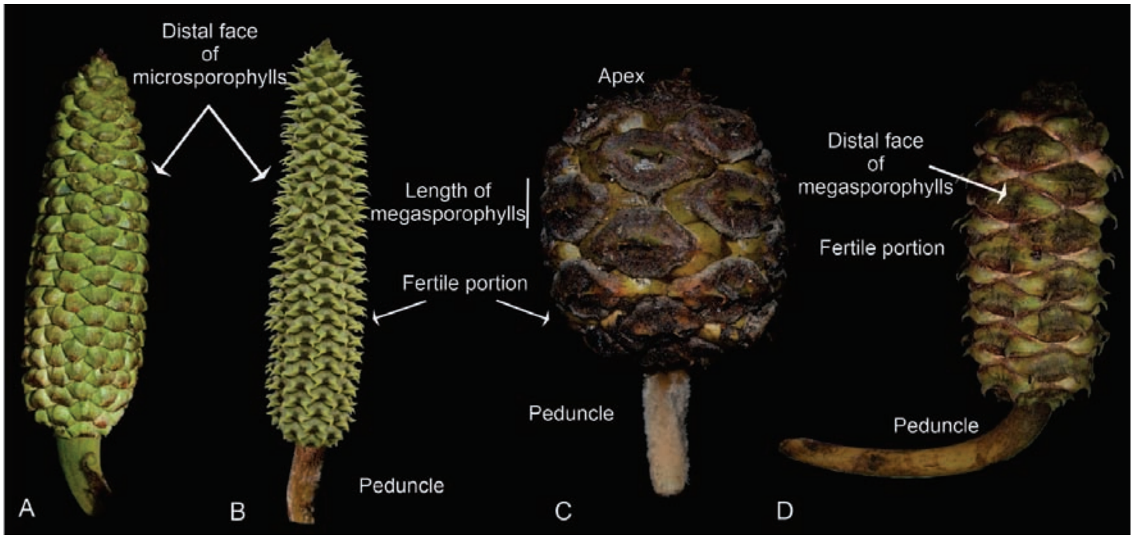

Figure 1. Characters evaluated in reproductive structures **A** pollen strobilus of *Ceratozamia sabatoi* **B** pollen strobilus of *C. santillanii* **C** ovulate strobilus of *C. aurantiaca* **D** ovulate strobilus of *C. zoquorum*.

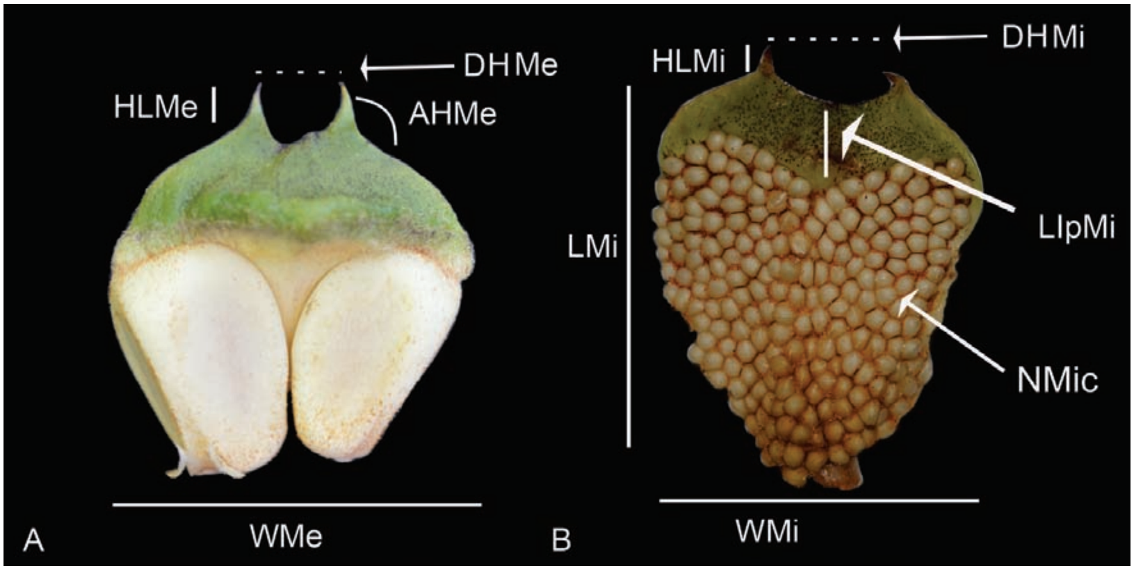

Figure 2. Characters evaluated in sporophylls **A** megasporophylls of *Ceratozamia delucana* **B** microsporophylls of *C. delucana*. HLMe = Horn length of megasporophylls; DHMe = Distance between horn of megasporophylls; AHMe = Angle between horns of megasporophylls; WMe = Width of megasporophylls; LMi = Length of microsporophylls; WMi = Width of microsporophylls; NMic = Number of microsporangia; LlpMi = Length infertile portion of microsporophylls; DHMi = Distance between horn of microsporophylls; HLMi = Horn length of microsporophylls.

Table 1. Qualitative and quantitative morphological characters evaluated in *Ceratozamia* reproductive structures.

Character	Character state
Pollen strobilus position	(0) erect; (1) pendulous
Pollen strobilus shape	(0) angulate; (1) cylindrical
Pollen strobilus color	(0) greenish yellow; (1) greenish brown (2) reddish brown; (3) yellowish brown
Distal face of microsporophylls	(0) non-recurved; (1) recurved
Microsporophylls shape	(0) discoid; (1) obconic; (2) elliptic
Microsporophylls horns type	(0) thin; (1) robust
Infertile portion shape	(0) orbicular; (1) rounded; (2) linear
Fertile portion shape	(0) lobate; (1) deeply lobate
Orientation of horns microsporophylls	(0) straight; (1) curved
Angle between horns of microsporophylls	(0) acute; (1) obtuse; (2) right
Ovulate strobilus position	(0) erect; (1) pendulous
Ovulate strobilus color (trichomes)	(0) light brown; (1) blackish to dark; (2) brown; (3) reddish brown; (4) greyish; (5) wine
Ovulate strobilus apex	(0) acuminate; (1) acute; (2) mucronate; (3) apiculate
Ovulate strobilus shape	(0) cylindrical; (1) globose
Angle between horns of megasporophylls	(0) acute; (1) right; (2) obtuse
Megasporophylls horns type	(0) thin; (1) robust
Direction of horns megasporophylls	(0) non-recurved; (1) recurved
Orientation of horn horns megasporophylls	(0) straight; (1) curved
Distal face of megasporophylls shape	(0) truncate; (1) prominent
Sarcotesta color	(0) whitish yellow; (1) whitish red
Seed shape	(0) ovate; (1) globose; (2) spherical

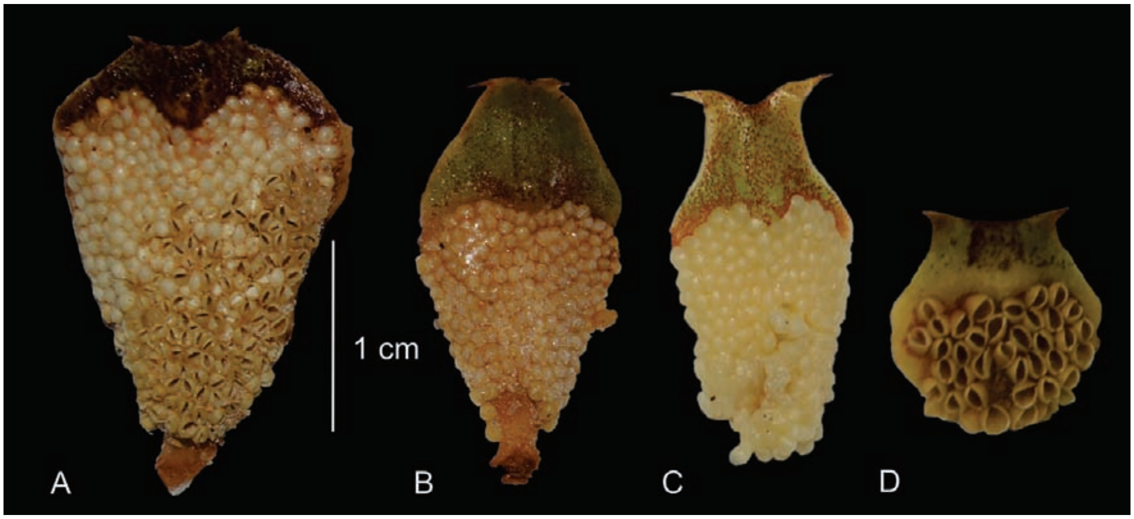

Figure 3. Comparison of qualitative characters of microsporophylls **A** *Ceratozamia fuscoviridis* **B** *C. brevifrons* **C** *C. chamberlainii* **D** *C. kuesteriana*.

The position of leaves is a character that refers to the arrangement of the leaves in relation to the stem apex. The leaves are ascending when they are oriented obliquely upward or with the upper half of the leaf curved apically. The prickles on the petioles of leaves can be robust when the prickle is abruptly tapered from the base to the apex or thin when the shape is homogenous or is gradually tapering. In several species, the prickles are grouped and can bifurcate. The number of prickles

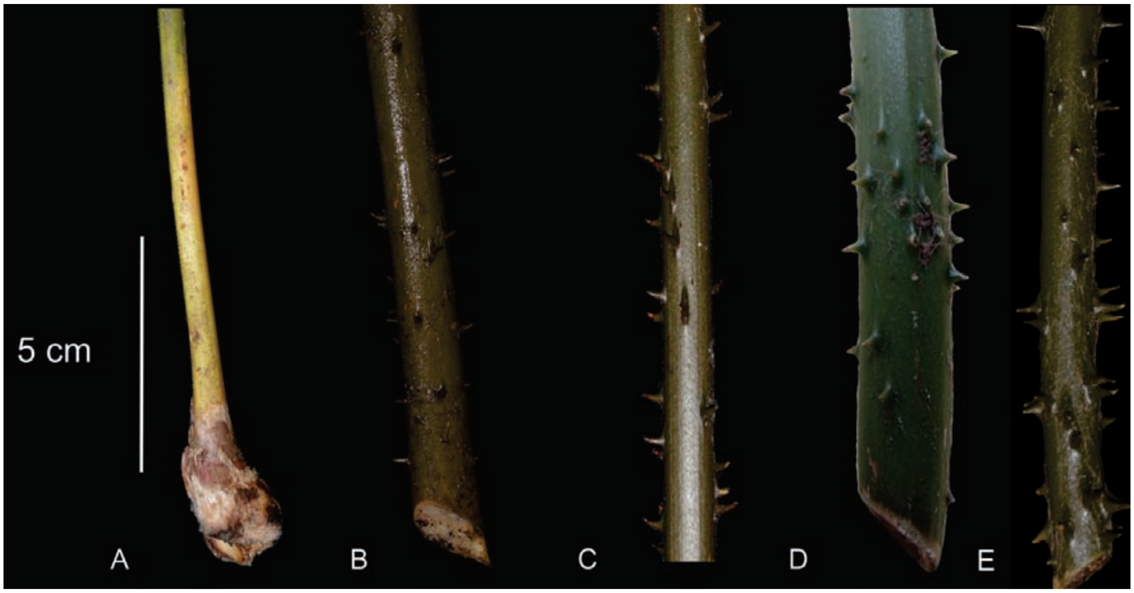

Figure 4. Type and number of prickles on the petiole **A** unarmed **B** thin prickles **C** robust and long prickles **D** robust and short prickles **E** bifurcate.

differs among species; this character is most conspicuous at the base of the petiole. Here, the prickles were counted along 10 cm from the base to distal end on the petiole (Fig. 4).

Results

Circumscription

We recognize 36 species in *Ceratozamia*, including one new species from Belize, characterized by megasporophylls covered by abundant purple to wine-colored trichomes present throughout the ontogeny of the strobilus, the acuminate apex of its ovulate strobili, and pollen strobili with a long infertile portion from 0.65 to 0.80 cm long (Figs 5, 6).

Habit

The species are terrestrial or rupicolous. *Ceratozamia brevifrons* Miq., *C. morettii* Vázq. Torres & Vovides and *C. tenuis* (Dyer) D.W.Stev. & Vovides are terrestrial, but all three species can occasionally be found as epiphytes (Fig. 7J). We found ovuliferous plants of *C. morettii* growing as epiphytes in Veracruz, Mexico. Adult individuals have been recorded growing up to 8 meters in height from the base of a host tree. All *Ceratozamia* species have a pachycaulous stem and are epigeous or semi-epigeous. Most of the species have erect stems, which may branch dichotomously with age. Only four species have an arborescent appearance, with stems of up to 2.5 meters long (*C. aurantiaca* Pérez-

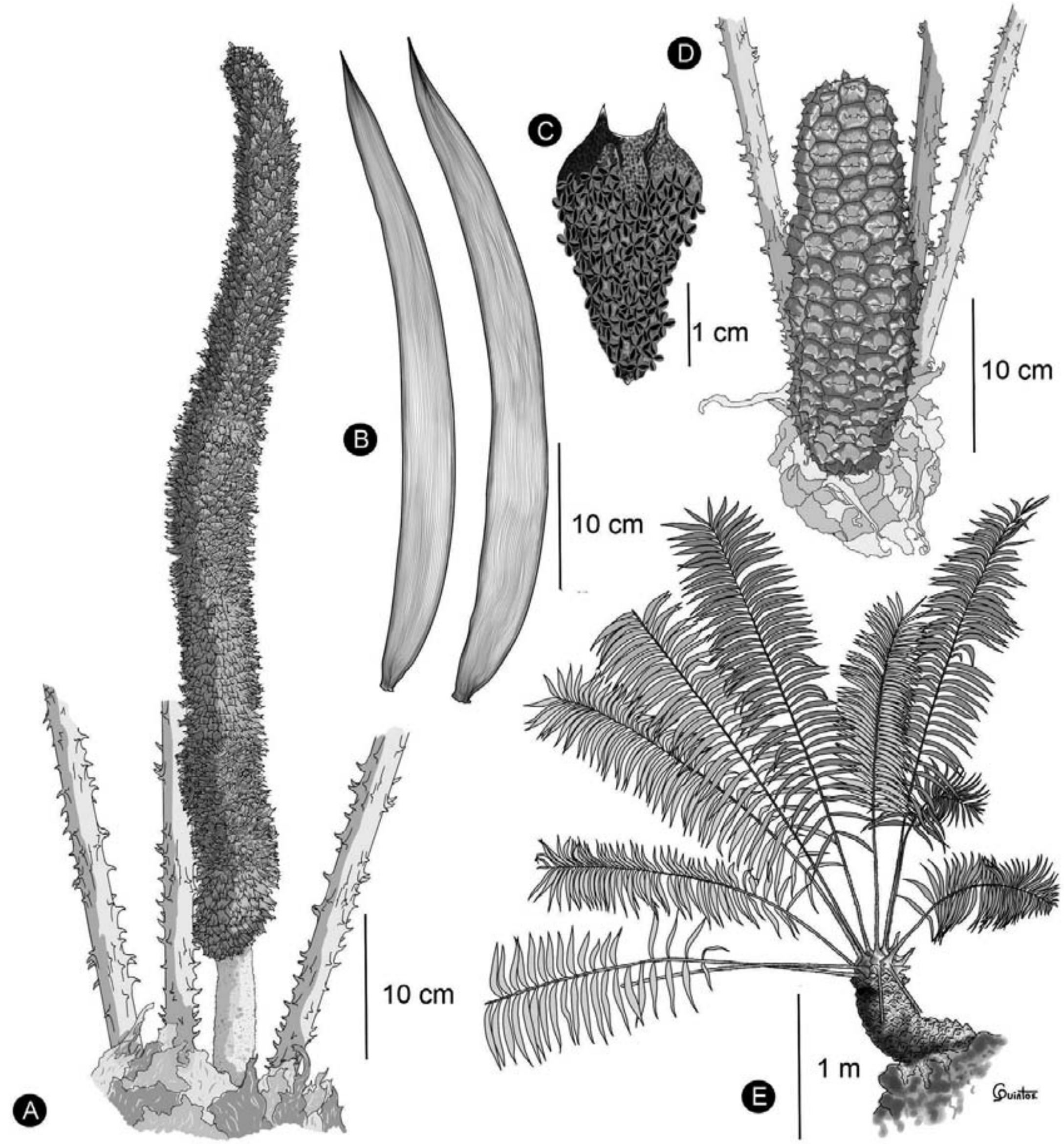

Figure 5. Illustration of *Ceratozamia osbornei* **A** pollen strobilus at maturity **B** variation of leaflets **C** microsporophyll **D** ovulate strobilus at maturity **E** adult plant **A–C** based on *B. K. Holst 4105* and **D, E** based on cultivated plant in Fairchild Tropical Botanic Garden.

Farr., Gut.Ortega, J.L.Haynes & Vovides, *C. osbornei* sp. nov., *C. robusta* Miq., and *C. subroseophylla* Mart.-Domínguez & Nic.-Mor.). Some plants of *C. subroseophylla* have a stem up to 5 meters long but decumbent.

Vegetative morphology

The ptyxis of leaves can be inflexed as in *C. matudae* Lundell or circinate as in *C. miqueliana* H.Wendl. (Fig. 8). The petiole of leaves elongates during leaf growth while the leaflets are progressively expanded. In the early stages of elongation, the apical end of the leaf becomes reflexed and subsequently straightens. During their

Figure 6. Ovulate strobilus of *Ceratozamia osbornei* **A** immature ovulate strobilus **B** mature ovulate strobilus. These photos were taken in Fairchild Tropical Botanic Garden.

development, the leaves have trichomes that are unbranched and brown or white in color. The trichomes are shed during elongation of the leaf. The color of new leaves can be green to yellowish or brown to reddish brown (Fig. 9). This leaf coloration disappears at maturity, except for a few species such as *C. chamberlainii* Mart.-Domínguez, Nic.-Mor. & D.W.Stev., in which the color remains at the base of the leaflets, margin and rachis. The emerging leaf color is generally homogeneous among individuals in a population and within a species. However, this character is polymorphic in *C. fuscoviridis* W.Bull. and *C. sancheziae* Pérez-Farr., Gut.Ortega & Vovides where different individual plants may have brown or green leaves at emergence.

Ceratozamia has cataphylls and well-developed stipules (Stevenson 1981, 1992). The stipules are positioned on the base of petioles as a winglike structure with each stipule having two free tips. These stipules, which encircle shoot apex, have a linear shape and are covered by light brown trichomes (Figs 10C, D, 11) and are usually between 1.5 to 5 cm long and 0.4 to 2 cm wide. Cataphylls are foliar organs that emerge from apex of stem associated with reproductive events. Their development occurs in a series of two or more cataphylls. These are brown to reddish brown and triangular to narrowly triangular and covered by scarce or abundant brown or dark brown trichomes (Figs 10A, B, 11). They become glabrous or partially glabrous at maturity (Fig. 10A).

Figure 7. Plants of *Ceratozamia* in habitat **A** *C. kuesteriana.* **B** *C. latifolia* **C** *C. leptoceras* **D** *C. matudae* **E** *C. mexicana* **F** *C. miqueliana* **G** *C. mirandae* **H** *C. mixeorum* **I** *C. morettii* **J** Epiphytic *C. morettii.*

Reproductive morphology

Pollen grains are sulcate, and exine surface and sexine nearly identical (Dehgan and Dehgan 1988). Some slight differences in measurements of grains have been found; however, the ornamentation and shape of pollen are similar in all species (Vovides et al. 2021).

The ovulate and pollen strobili have two horns at the distal end of each sporophyll. The ovulate strobili show minimal variation at the species level. In most species, the

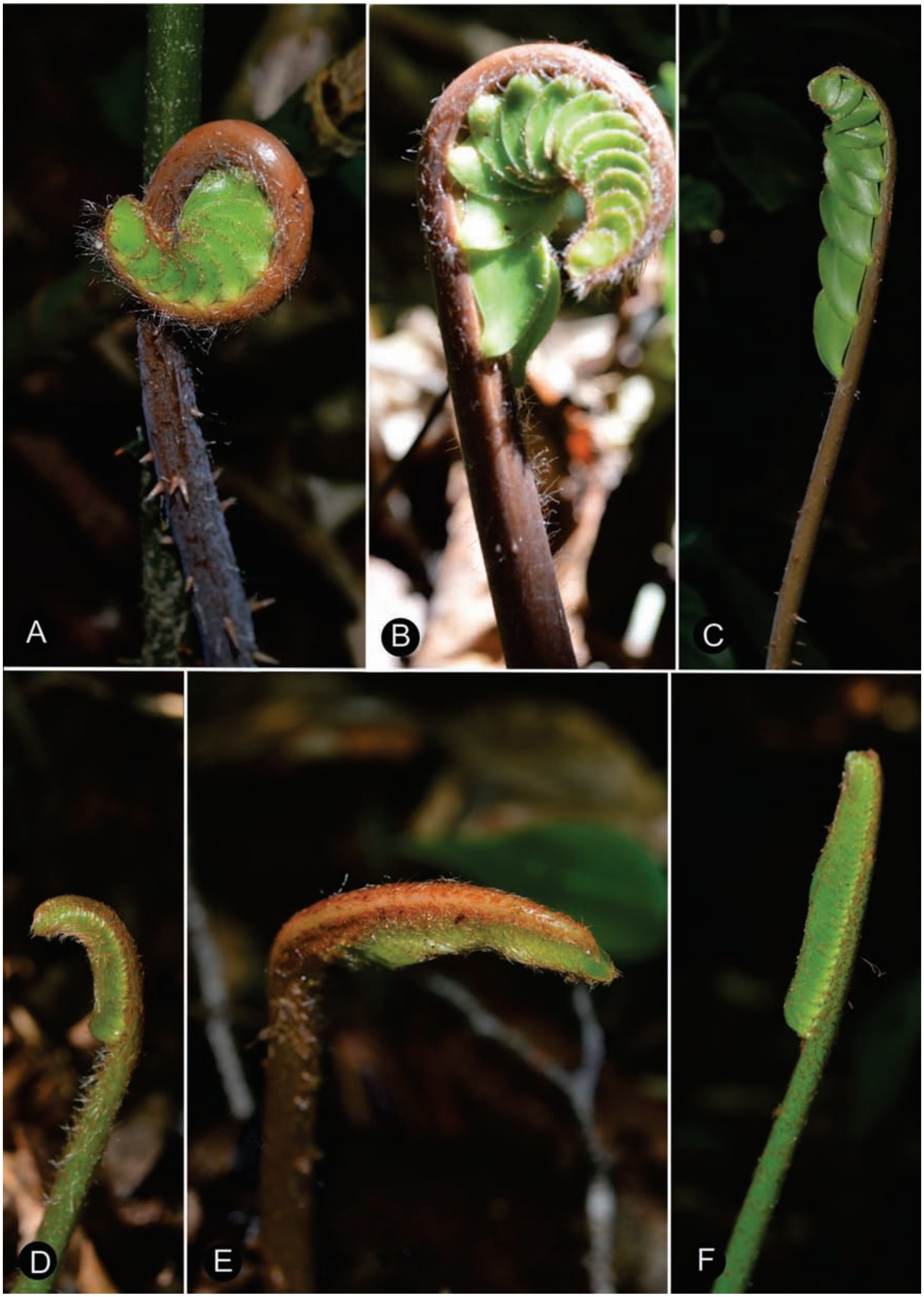

Figure 8. Ptyxis in *Ceratozamia* **A–C** *Ceratozamia miqueliana* **D–F** *C. matudae.*

Figure 9. Color of leaves at emergence **A** *Ceratozamia sancheziae* **B** *C. kuesteriana* **C** *C. fuscoviridis* **D** *C. miqueliana*.

ovulate strobilus shape is cylindrical, whereas in *Ceratozamia morettii* and *C. matudae* it is globose. However, the color of trichomes, apex shape, angle between horns and the distal face of megasporophylls are useful for discriminating between species (Table 1). In contrast, the pollen strobili possess distinctive characters for identifying several species including closely related taxa (Table 1). The microsporophyll horns can be thin or robust, and show different orientations (i.e., straight or recurved). *C. sabatoi* Vovides & Vázq.Torres is the only species with recurved microsporophylls with a downward distal face (Fig. 1A).

Generally, the characters of reproductive structures exhibit little variation within populations. However, these characters are polymorphic in some species such as *Ceratozamia delucana* Vázq.Torres, A.Moretti & Carv.-Hern., in which the infertile apical portion shape of microsporophylls can be orbicular or discoid in the same

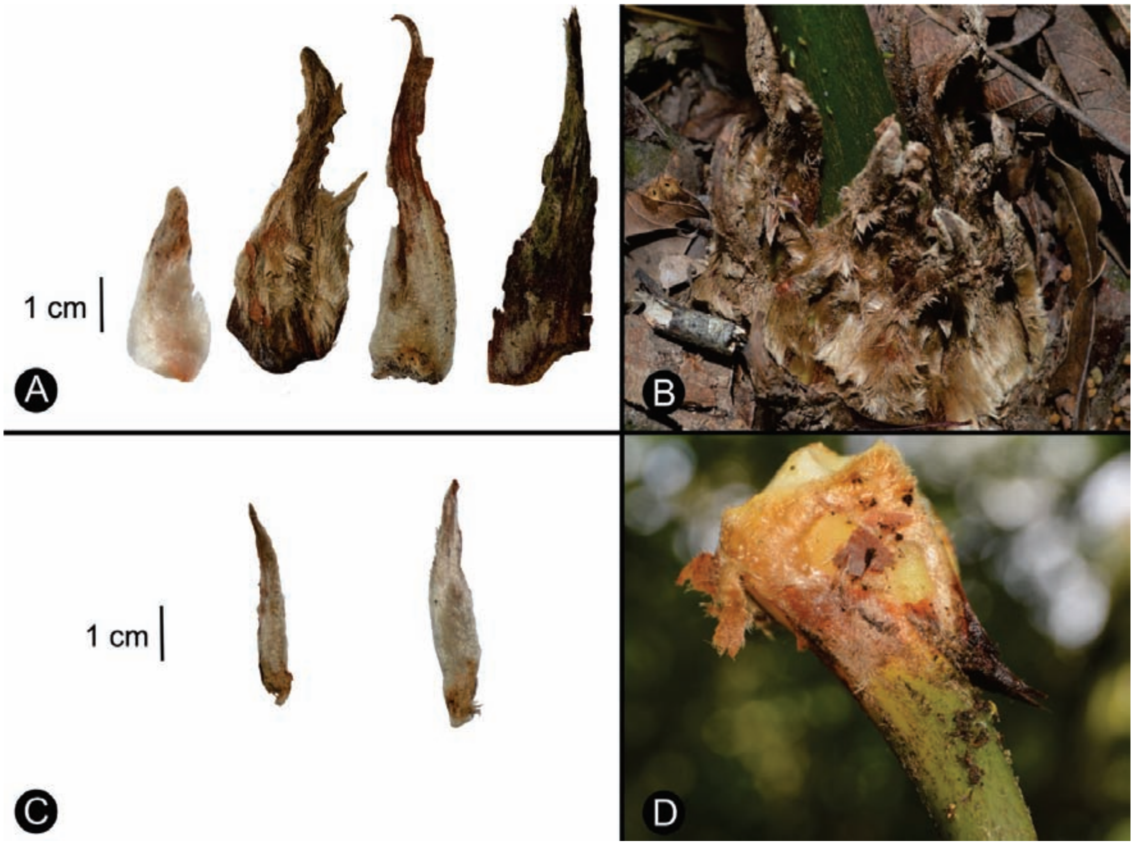

Figure 10. Shapes and pubescence of cataphylls and stipules **A** from left to right: *Ceratozamia latifolia*, *C. delucana*, *C. morettii* at emergence and *C. morettii* at maturity **B** cataphylls of *C. delucana* **C** stipules of *C. morettii* **D** stipules of *C. matudae*.

population. Other species with a similar variation pattern are *C. alvarezii* Pérez-Farr., Vovides & Iglesias and *C. mirandae* Vovides, Pérez-Farr. & Iglesias. Both of these species have an angle between the horns of microsporophylls that is either obtuse or acute.

The seeds are very similar in all species. The most common shape is ovate that can be somewhat irregular, whereas some species such as *Ceratozamia alvarezii* and *C. mirandae* have spherical seeds, and *C. matudae* and *C. sancheziae* have globose seeds (Fig. 12). Seeds are brown to light brown and are between 1.5 and 2.5 cm long, although some species have seeds up to 3.8 cm long (e.g., *C. subroseophylla*).

Phenology

Plants produce a new flush of leaves annually or biannually in a low spiral. The leaves emerge in flushes of from 2, as *C. latifolia* Miq., to 20 as in adult plants of *C. osbornei* and *C. subroseophylla*. However, the plants of most species produce between six and 12 leaves. Generally, the leaves emerge during January to March. Some plants at population level may produce leaves during August to September.

The ovulate and pollen strobili emerge from among the cataphylls. Polliniferous plants produce between 1 to 2 strobili per apex, whereas ovulate plants produce only 1 strobilus per apex and very rarely two in vigorous large plants such as

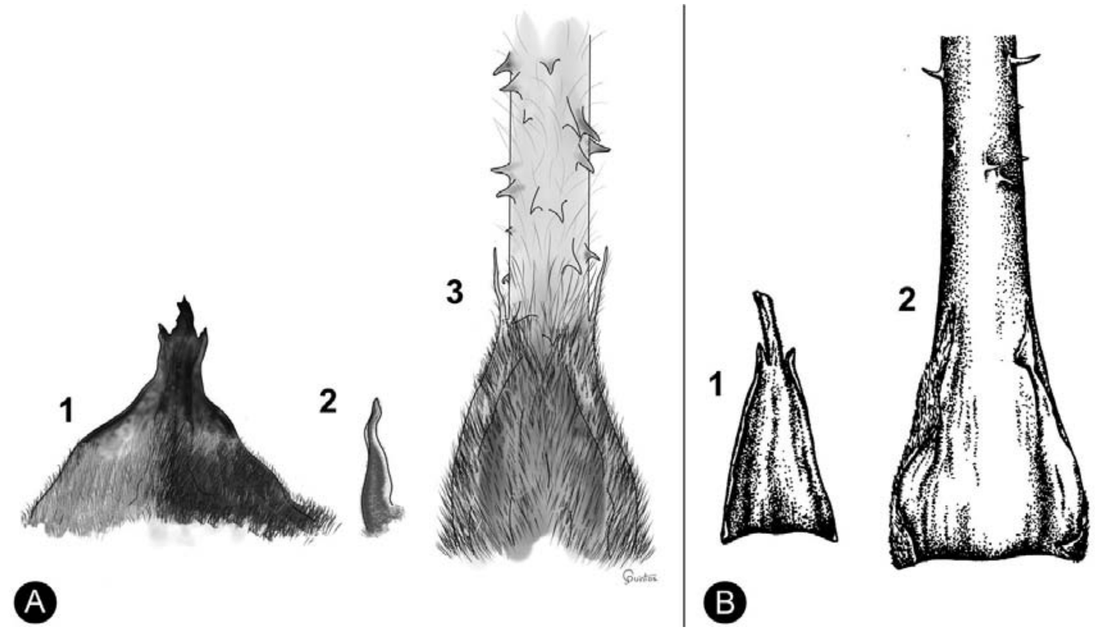

Figure 11. Shape and position of cataphylls and stipules of *Ceratozamia* **A** *C. miqueliana* (1) Cataphylls (2–3) Stipules **B** *C. mexicana* from Stevenson (1981). (1) Cataphylls. (2) Stipules.

Ceratozamia osbornei and *C. mexicana*, particularly in cultivation. We found up to 5 pollen strobili in the same plant during a reproductive season; generally, these pollen strobili emerge within 1 to 2 months of each other. We have recorded very few ovulate plants with more than one strobilus. The phenology shows a slightly different pattern between species. Generally, the strobili emerge between July and September. However, in some species the emergence can occur two months later (e.g., *C. miqueliana*).

Distribution, endemism and habitat

Ceratozamia occurs from Mexico to Guatemala, Belize and Honduras in Central America. According to the biogeographic regionalization of the Neotropical region (Morrone et al. 2022) *Ceratozamia* occurs in Mexican Transition Zone and Mesoamerican dominion of Brazilian subregion (Fig. 13). In the first, there are 69% (25 of 36 species) of the species, which are distributed in Sierra Madre Oriental (SMO), Sierra Madre del Sur (SMS), Transmexican Volcanic Belt Province (TVBP), and Chiapas Highlands (CH). The SMO and CH are the provinces with the highest concentration of species richness in the genus (8 species in each province). The SMS and TVBP have 5 (*C. aurantiaca*, *C. leptoceras* Mart.-Domínguez, Nic.-Mor., D.W.Stev. & Lorea-Hern., *C. mixeorum* Chemnick, T.J.Greg. & Salas-Mor., *C. oliversacksii* D.W.Stev., Mart.-Domínguez & Nic.-Mor., and *C. whitelockiana* Chemnick & T.J.Greg.) and 4 species, respectively (*C. delucana*, *C. mexicana*, *C. morettii* and *C. tenuis*).

In the Mesoamerican dominion of the Brazilian subregion, species occur in two provinces: Veracruzan province and Mosquito province. In Veracruzan province 14 species are present (*C. becerrae* Pérez-Farr., Vovides & Schutzman, *C. brevifrons*, *C. chimalapensis*, *C. decumbens* Vovides, Avendaño, Pérez-Farr. & Gonz.-Astorga,

Figure 12. Seeds of *Ceratozamia* **A** *C. sancheziae* **B** *C. alvarezii* **C** *C. brevifrons.* **D** *C. subroseophylla.*

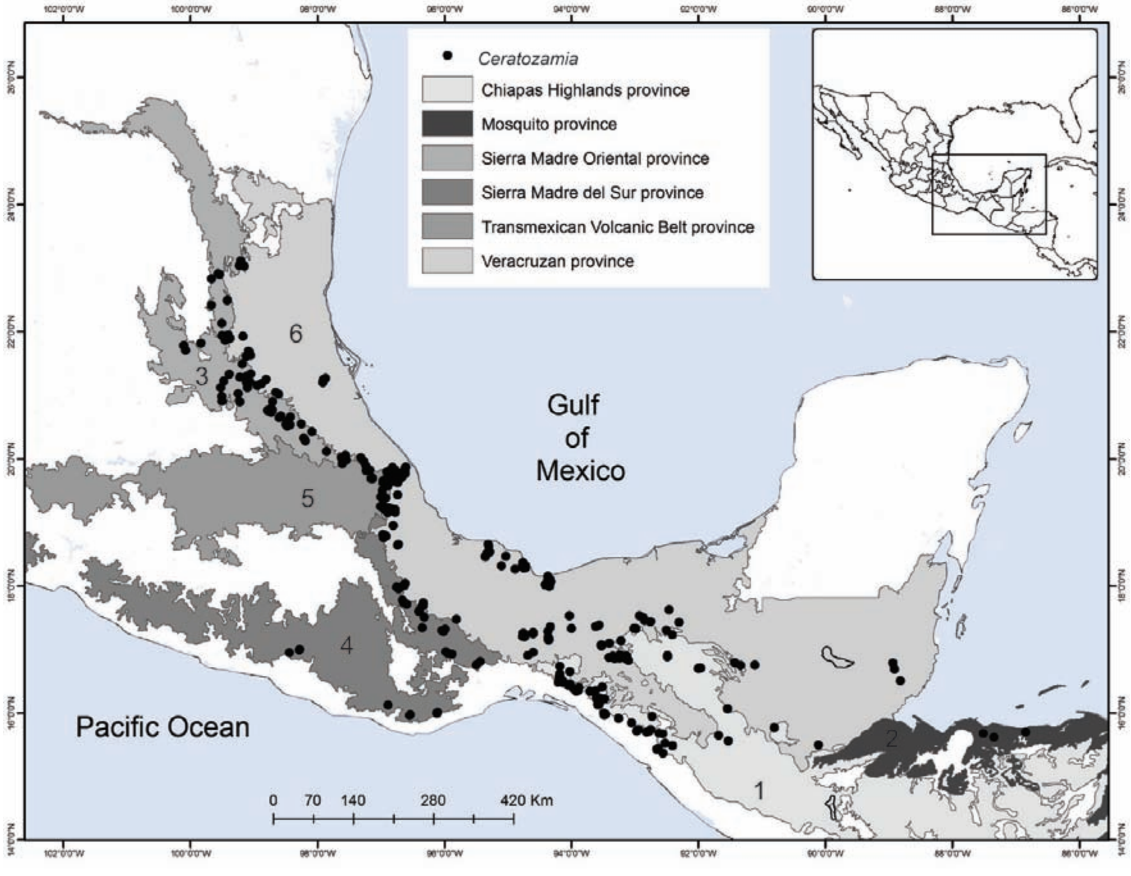

Figure 13. Distribution of Ceratozamia genus. Biogeographic regionalization of the Neotropical region according to Morrone et al. (2022).

C. delucana, *C. euryphyllidia* Vázq.Torres, Sabato & D.W.Stev., *C. huastecorum* Avendaño, Vovides & Cast.-Campos, *C. mexicana*, *C. miqueliana*, *C. robusta*, *C. santillanii* Pérez-Farr. & Vovides, *C. subroseophylla*, *C. osbornei*, and *C. zoquorum* Pérez-Farr., Vovides & Iglesias), whereas in Mosquito province only a single species (*C. hondurensis* J.L.Haynes, Whitelock, Schutzman & R.S.Adams) occurs. In particular, *C. mexicana* and *C. delucana* also occur in TVBP and *C. robusta* in CH and Veracruzan provinces. *Ceratozamia* has not been reported from the Yucatán Peninsula province (Fig. 13); we believe this is due to lack of collections in the southwest of Yucatán (Mexico). The apparent disjunct pattern from El Petén Department (Guatemala) may be explained by this collection deficiency.

With 32 endemic species of *Ceratozamia*, Mexico is the center of endemism of the genus. Only four species are occur in Central American countries: *C. hondurensis* from Honduras; *C. osbornei* from Belize; and *C. robusta* and *C. vovidesii* Pérez-Farr. & Iglesias from Guatemala. Some species have a broad geographic range, while others occur in a very limited area. Most of the species that have narrower distributions can be considered micro-endemics, such as *C. alvarezii* and *C. morettii* in Chiapas States and Central Veracruz from Mexico, respectively. In contrast, *C. robusta* has a broad distribution, with a range from northwest Chiapas (Mexico) to Guatemala. Other species of the genus with broad distributions are *C. miqueliana* and *C. subroseophylla*. The latter species has been considered endemic to the montane region of Los Tuxtlas (State of Veracruz, Mexico). However, we found populations at southwest of Veracruz in the Uxpanapa region and Tabasco State, which represent an extension of its geographical range. In particular, the population of *C. subroseophylla* from Tabasco represents a new record for this Mexican state.

Montane regions show the highest diversity of *Ceratozamia* species. The distributional pattern is congruent with the existence of the main mountain systems of Mexico. The mean elevation for *Ceratozamia* species is 1,100 m (range 19–2,000 m), with the majority of the species occurring between 800 to 1,200 m. *Ceratozamia miqueliana*, occurs at 19 meters of elevation in lowland vegetation of Veracruz, while *C. mixeorum* and *C. zaragozae* Medellín are the species that occur at higher elevations above 1,200 m of Oaxaca and San Luis Potosí, respectively.

Species richness in *Ceratozamia* tends to be correlated with moister habitats, principally in limestone rock areas. The genus is found in four vegetation types: cloud forest, evergreen tropical forest, oak forest and oak-pine forest. Most species inhabit cloud forest, whereas in oak-pine forest there are only a few species. No species is known from dry forest. Some species do grow near to rivers or lagoons, e.g., *C. aurantiaca* and *C. miqueliana* near the Santo Domingo River and Majahual lagoons, respectively.

Discussion

The cycad genus *Ceratozamia* as here circumscribed includes 36 species, 34 of which are found in Mexico with 32 of those endemic to Mexico. *C. robusta* is the species with the widest distribution in the genus, occurring continuously from the central region of Chiapas State in Mexico to Guatemala. Unfortunately, herbarium specimens from Guatemala are infertile and there are few sterile specimens in herbaria in general, which has led to ambiguous and/or conflicting taxonomic identifications. Comparative vegetative morphology with these specimens suggests that *C. robusta* also has a wide elevational range between 400 meters and up to 1,300 meters of elevation. However, recently collected reproductive material has been observed for plants from Belize, which have revealed features that demonstrate that the Belize plants can be distinguished from those in Mexico and Guatemala resulting in our description of *C. osbornei*. Further population level studies of this species are needed to evaluate genetic differentiation and gene flow throughout the entire ranges of both *C. osbornei* and *C. robusta*. *C. matudae* was reported for Guatemala from the Sierra de Cuchumatanes in the 1940s, but no past or recently collected specimens have been located. Considering this paucity of information and the exceptionally rapid loss of forest cover in the region, a thorough exploration and study of these localities is essential.

Recently, Vovides et al. (2020) recircumscribed the *Ceratozamia miqueliana* complex using leaflet anatomy and other macromorphological characters. As a result, *C. zoquorum* was recircumscribed with a much narrower taxonomic concept. They considered the presence or absence of girder sclerenchyma and a lignified hypodermis to be a significant trait to distinguish between *C. zoquorum* and *C. becerra*e, and the latter was removed from synonymy under *C. zoquorum*. However, we found that the corresponding taxonomic key failed to properly identify these closely related species with anatomical characters that are contradictory to the descriptions. Thus, there is a lack of correspondence between the key entries and homologous character states in both supposed species (Vovides et al. 2020; p. 11).

Therefore, the only two remaining anatomical characters relevant in this case are: 1) girder sclerenchyma present in *Ceratozamia zoquorum* and mostly absent in *C. becerrae*, and 2) a lignified adaxial hypodermis in *C. zoquorum* that is also absent in *C. becerrae*. According to the descriptions provided by the authors, the hypodermis in *C. becerrae* is absent only in the revolute leaflet margin with up to three layers of fibres, whereas in *C. zoquorum* such margin is discontinuous with 1–7 lignified isodiametric fibres.

In their anatomical evaluation, Vovides et al. (2020: 2) studied two cultivated individuals for evaluating phenotypic variation. The anatomical characters should therefore be characterized and reassessed under a broader approach by testing the morphological variation through different populations at the intra- and inter-population level: most leaflet macromorphological characters —including qualitative ones— often exhibit polymorphisms, as well as wide variation at the population level. Generally, leaflets exhibit high variation mainly in quantitative characters (Pérez-Farrera et al. 2009; Martínez-Domínguez et al. 2017a, c). Considering then that leaflets in *Ceratozamia* species show contrasting shapes between geographically close species, but similar shapes even when the biogeographic pattern is disjunct, we suggest that the leaflet anatomy should be re-assessed through broader sampling.

Based on our research, the taxonomic value of some traditional morphological characters —such as leaflet size— should only be secondarily important, and the focus should be put on defining qualitative characters in both leaves and leaflets, and characters derived from reproductive structures as proposed in Table 1. Regarding the latter, we observed that the position of ovulate strobilus was not consistent throughout the different ontogenetic states. Ovulate strobili can recline due to the weight of the fertile portion of ovulate strobilus and because of the position of the plant growing on a rocky wall. This is mainly because the ovulate strobilus position is correlated with the length of the peduncle of strobili and the fact that the peduncle curves at maturity. Our data support that other characters such as those of the microsporophylls and the shape of their horns can be coded into discrete character states and, therefore, provide more taxonomic utility than previously understood.

This modern taxonomic treatment is drawn from wide sampling, thorough review of specimens in collections and molecular data (Martínez-Domínguez et al. 2016, 2017c, 2020), thus establishing a framework for future research and providing a resource for disentangling confusing species. Ultimately, this work contributes to the conservation of this threatened genus. Despite advances in the taxonomy and systematics of this genus, taxon-level data are required for a complete systematic evaluation. We suggest that the use of phenological information as well as a more nuanced understanding of phenotypic evolution —including, for example, instances of plasticity and other ontogenetic development-related phenomena— that might impact diversification in species complexes or other groups of closest related species in this gymnosperm genus, will be increasingly relevant for the task.

Taxonomic treatment

Ceratozamia Brongn., Ann. Sci. Nat., Bot. ser. 3, 5: 7, t. 1. 1846.

Type species. *Ceratozamia mexicana* Brongn.

Description. ***Stem*** 10–250 cm long, 8–40 cm in diameter, epigeous or semi-hypogeous, erect or decumbent. ***Cataphylls*** persistent, triangular to narrowly triangular, reddish brown, tomentose to densely tomentose at emergence, partially tomentose at maturity, apex acuminate. ***Leaves*** stipulate, ascending to descending, light green or reddish brown at emergence with whitish gray or brown trichomes, generally glabrous at maturity; stipulate 2–6 cm long, linear, tomentose at maturity. ***Petiole*** straight or twisted, sometimes brown in mature leaves, without prickles or heavily to lightly armed with prickles; prickles can be bifurcate. ***Rachis*** straight or twisted, without prickles or armed with prickles up to half the length of the leaves. ***Leaflets*** articulate, sessile, membranaceous to coriaceous, linear to obovate, opposite to subopposite or clustered, not imbricate, generally acuminate at apex, attenuate at base, margins entire; articulations green to brown. ***Pollen strobili*** 1–2, with sterile tip, erect, cylindrical, green to cream with blackish to reddish brown trichomes at maturity; pollen sporangiophores deltoid to cuneate, basally stalked, distal face bicornate, fertile abaxial surface with 24–280 sporangia in clusters of (2)3(4–5), dehiscent by longitudinal slit; peduncle pubescent to tomentose. ***Ovulate strobili*** usually solitary, globose to cylindrical; green with pale pink to blackish trichomes at maturity, acute to apiculate apex; ovulate sporangiophores peltate with a narrow basal stalk and transversely hexagonal tips, bicornate at distal end; peduncle pubescent to tomentose, erect to pendulous. ***Seeds*** (ovules) 2 per megasporophyll projecting inward toward the strobilus axis, spherical, sarcotesta pink to yellowish when immature, light brown at maturity, sclerotesta smooth with several furrows longitudinal from micropylar end.

Distribution and habitats. The 36 species of *Ceratozamia* are only found from Mexico to Central America, usually in montane habitats on limestone soils at elevations from 19 to 2,000 m. Most of the species are narrowly endemic, and all are on CITES Appendix I.

Artificial key for *Ceratozamia*

1	Petiole and rachis twisted	**2**
–	Petiole and rachis straight	**3**
2	Petiole and rachis with prickles	***C. norstogii***
–	Petiole and rachis unarmed	***C. zaragozae***
3	Leaflets fasciculate	***C. hildae***
–	Leaflets opposite to subopposite	**4**
4	Leaflets obovate; veins prominent	**5**
–	Leaflets oblong to linear; veins not prominent	**6**
5	Leaflets with sinuate margin at the distal end and an asymmetrical apex	***C. euryphyllidia***
–	Leaflets with entire margin at the distal end and a symmetrical apex	***C. hondurensis***
6	Leaflets oblong to oblanceolate	**7**
–	Leaflets linear to lanceolate	**19**
7	Leaflets papyraceous	**8**
–	Leaflets coriaceous	**12**
8	Petiole and rachis unarmed	***C. latifolia***
–	Petiole and rachis with prickles	**9**
9	Leaflets with a symmetric apex	***C. totonacorum***
–	Leaflets with an asymmetric or abruptly symmetrical apex	**10**
10	New leaves reddish brown at emergence; ovulate strobilus green with brown trichomes at maturity; microsporophylls with the infertile apical portion partially covered with trichomes	***C. aurantiaca***
–	New leaves green at emergence; ovulate strobilus green with black trichomes at maturity; microsporophylls with the infertile apical portion completely covered with trichomes	**11**
11	Petiole armed with robust prickles; microsporophylls rounded; ovulate strobilus with an acuminate apex	***C. miqueliana***
–	Petiole armed with thin prickles; microsporophylls orbicular; ovulate strobilus with an acute apex	***C. delucana***
12	New leaves reddish brown at emergence; ovulate strobilus with abundant wine-colored to reddish brown trichomes	**13**
–	New leaves green at emergence; ovulate strobilus with blackish brown trichomes	**14**
13	Petiole armed with robust prickles; rachis armed with prickles; ovulate strobilus more than 20 cm long with an acuminate apex	***C. chamberlainii***
–	Petiole armed with thin prickles; rachis unarmed to armed with few prickles; ovulate strobilus up to 15 cm long with an acute apex	***C. decumbens***

14 Petiole prickles less than or equal to 0.20 cm long **15**

– Petiole prickles more than 0.20 cm long **18**

15 Apical leaflets less than or equal to 3.5 cm wide; brown trichomes of leaves at emergence; ovulate strobilus with an apiculate apex .... ***C. morettii***

– Apical leaflets more than 3.5 cm wide; whitish gray trichomes of leaves at emergence; ovulate strobilus with an acute apex....................................... **16**

16 Peduncle of ovulate strobilus 3 cm long or shorter .... ***C. santillanii***

– Peduncle of ovulate strobilus more than 3 cm long **17**

17 Plants from Sierra Madrigal at the border of Tabasco and Chiapas States (Mexico) .... ***C. becerrae***

– Plants from northern mountains of Chiapas State (Mexico)..... ***C. zoquorum***

18 Leaves with 20–43 pairs of leaflets; microsporophylls 1.3 cm long or longer, 1.3 cm wide or wider; trichomes of leaves at emergence brown; 10 or more leaves per leaf crown .... ***C. delucana***

– Leaves with 8–18 pairs of leaflets; microsporophylls less than 1.3 cm long, less than 1.3 cm wide; trichomes of leaves at emergence whitish gray; less than 10 leaves per leaf crown .... ***C. huastecorum***

19 Leaflets coriaceous **20**

– Leaflets papyraceous to membranaceous **22**

20 Leaflets keeled; petiole armed with robust prickles .... ***C. brevifrons***

– Leaflets plane; petiole armed with thin prickles.. **21**

21 Petiole sparsely prickly (30 prickles or fewer); microsporophylls with infertile apical portion more than 0.45 cm long, horns more than 0.25 cm long........ .... ***C. mexicana***

– Petiole abundantly prickly (more than 35 prickles); microsporophylls with infertile apical portion less than 0.45 cm, horns less than or equal to 0.25 cm . ... ***C. delucana***

22 Leaflets involute to lightly involute or canaliculate.................................... **23**

– Leaflets flat **26**

23 New leaves at emergence dark green with brown trichomes; basal leaflet more than or equal to 1 cm wide .... ***C. tenuis***

– New leaves at emergence brown to reddish brown with whitish gray trichomes; basal leaflet less than 1 cm wide.. **24**

24 Petiole sparsely prickly (less than or equal to 10 prickles); prickles short (less than or equal to 0.15 cm); microsporophylls discoid, up to 1.1 cm long....... .... ***C. kuesteriana***

– Petiole abundant prickly (more than 10 prickles); prickles long (more than 0.15 cm); microsporophylls elliptic, more than 1.2 cm long **25**

25 Leaves with rachis the same length or shorter than the petiole; plants from Cintalapa and Juquipilas municipalities in Chiapas State (Mexico) ..... ***C. alvarezii***

– Leaves with rachis longer than petiole; plants from Sierra Morena and Tres Picos in Chiapas State (Mexico) .... ***C. mirandae***

26 Petiole armed with robust prickles **27**

– Petiole armed with thin prickles **29**

27 Leaflets abaxially curved in both median and apical portions; ovulate strobilus dark green with scarce blackish trichomes or glabrous at maturity; microsporophylls with curved horns .... ***C. robusta***

– Leaflets abaxially curved in the median and mostly planar in the apical portion; ovulate strobilus green with abundant trichomes reddish brown to purple at maturity; microsporophylls with straight horns **28**

28 Ovulate strobilus with base pale pink megasporophylls and dark brown to reddish brown trichomes at maturity; microsporophylls with a lobate fertile portion and infertile portion up to 0.65 cm long .... ***C. subroseophylla***

– Ovulate strobilus with green megasporophylls base and dark purple to wine trichomes at maturity; microsporophylls with a deeply lobate fertile portion and infertile portion more than 0.65 cm long .... ***C. osbornei***

29 Leaflets linear **30**

– Leaflets lanceolate **31**

30 Leaflets membranaceous .... ***C. leptoceras***

– Leaflets papyraceous **32**

31 Base of leaflets and articulations yellow .... ***C. matudae***

– Base of leaflets and articulations green to brown **34**

32 Prickles on the petiole more than or equal to 0.50 cm long; horns of microsporophylls thin .... ***C. oliversacksii***

– Prickles on the petiole less than or equal 0.34 cm long; horns of microsporophylls robust **33**

33 Leaflets less than or equal 1.5 cm wide; recurved downward distal face of microsporophylls .... ***C. sabatoi***

– Leaflets more than 1.5 cm wide; non-recurved distal face of microsporophylls .... ***C. sancheziae***

34 New leaves green to yellowish green **35**

– New leaves brown to reddish brown **36**

35 Rachis unarmed .... ***C. whitelockiana***

– Rachis with prickles **37**

36 Microsporophylls with an orbicular infertile portion; megasporophylls with a truncate distal face .... ***C. fuscoviridis***

– Microsporophylls with a rounded to linear infertile portion; megasporophylls with a prominent distal face **39**

37 Leaves descending .... ***C. mixeorum***

– Leaves ascending **38**

38 Leaflets more than or equal 2.3 cm wide .... ***C. delucana***

– Leaflets less than 2.3 cm wide .... ***C. fuscoviridis***

39 Microsporophylls elliptic with curved horns .... ***C. chimalapensis***

– Microsporophylls obconic with straight horns .... ***C. vovidesii***

1. *Ceratozamia alvarezii* Pérez-Farr., Vovides & Iglesias, Novon 9: 410. 1999

Figs 12B, 15A

Type. Mexico. Chiapas: Mun. Cintalapa, Rancho El Cafetal, 950 m, 4 Mar 1996, *M.A. Pérez-Farrera 889* (holotype: CHIP! [acc. # 14306]; isotypes: F! [acc. # 2193633], HEM! [acc. # HEM004830], MEXU! [MEXU00827362], MO! [acc. # 04882667]).

Description. ***Stem*** 20–60 cm long, 15–30 cm in diameter, epigeous, erect and decumbent. ***Cataphylls*** 2.0–5.0 × 1.5–3.0 cm wide at the base, persistent, triangular, reddish brown, densely brownish tomentose at emergence, partially tomentose at maturity, apex acuminate. ***Leaves*** 2–30 (40), 36–125 cm long, ascending, brown at emergence with whitis gray trichomes, glabrous at maturity. ***Petiole*** 10–60 cm long, terete, linear, brown in mature leaves; with 11–40 thin prickles, 0.19–0.37 cm long. ***Rachis*** 25–87 cm long, terete, linear, brown to greenish brown in mature leaves, with prickles. ***Leaflets*** 25–68 pairs, opposite to subopposite, insertion in one plane, linear to lanceolate, generally longitudinally planar, not basally falcate, papyraceous, slightly involute, green with adaxial and abaxial sides glabrous, distal end with entire margins, acuminate and symmetric at the apex, attenuate at base, with conspicuous and light-green veins; median leaflets 12–38 × 0.3–0.9 cm, 0.14–1.30 cm between leaflets; articulations 0.14–0.75 cm wide, brown. ***Pollen strobili*** 15–40 cm long, 3.5–5.1 cm in diameter, solitary, cylindrical, erect, yellowish green with brown trichomes at emergence, yellowish cream with blackish brown trichomes at maturity; peduncle 4–10 cm long, 1.5–2.1 cm in diameter, reddish brown to brown pubescent; microsporophylls 1.24–1.56 × 0.59–0.83 cm, elliptic with a non-recurved distal face and a lobate fertile portion, infertile portion 0.50–0.81 cm long and linear with straight horns 0.14–0.30 cm long, 0.45–0.90 cm and an obtuse to acute angle between the horns. ***Ovulate strobili*** 17–27 cm long, 7.2–12.3 cm in diameter, solitary, cylindrical, erect, yellowish green with abundant blackish trichomes at emergence, glaucous green with reddish brown to blackish trichomes at maturity, acuminate apex; peduncle 4.0–10 cm long, 1.1–2.2 cm in diameter, erect, with scarce brownish tan trichomes; megasporophylls 20–80, 4–8 orthostichies with 5–10 sporophylls per orthostichy, 2.15–2.80 × 3.90–6.30 cm, with a prominent distal face, horns straight and thin and 0.32–0.51 cm long, 0.69–1.80 cm between horns with an obtuse angle between the horns. ***Seeds*** 2.5–3.0 cm long, 2.3–2.9 cm in diameter, spherical, sarcotesta whitish pink when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia alvarezii* is endemic to Mexico and only known from Cintalapa and Jiquipilas municipalities in Chiapas State, at the transition zone between pine and oak forest and oak forest; plants occur on karstic rocks between 900 and 1,450 m elevation (Fig. 14A).

Etymology. The specific epithet honors Miguel Álvarez del Toro in recognition of its outstanding contributions for conservation in Chiapas and the establishment of its first reserves (Pérez-Farrera et al. 1999).

Common names. Mexico. Chiapas: Espadaña cimarrona (*L. Martínez-Domínguez et al. 1370*); palma, palmita (*M.A. Pérez-Farrera 889*).

Uses. The seeds are used as food (*M.A. Pérez-Farrera 889*).

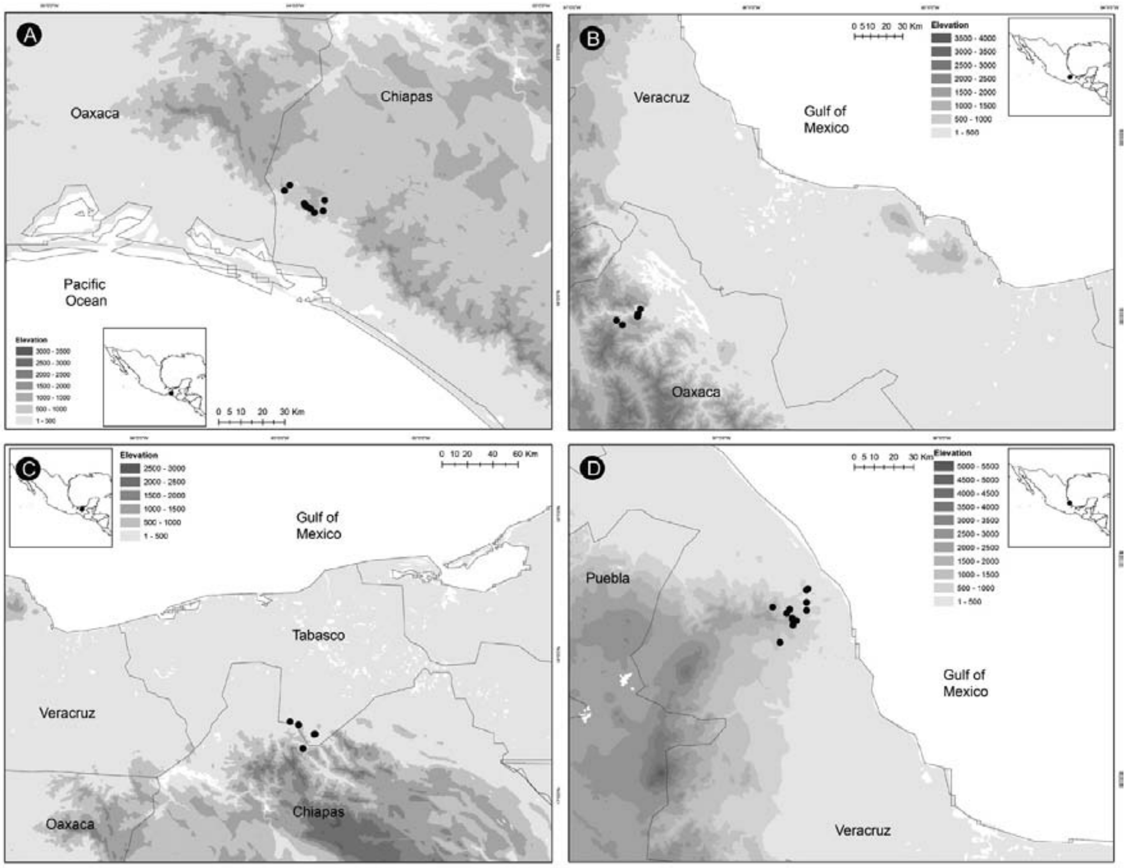

Figure 14. Distribution of *Ceratozamia* species **A** *C. alvarezii* **B** *C. aurantiaca* **C** *C. becerrae* **D** *C. brevifrons*.

Conservation status. (IUCN 2021). Endangered; A2ac; B1ab(I,iii)+2ab(i,iii); C1.

Discussion. The morphology of *Ceratozamia alvarezii* is not very different from *C. mirandae*. At population level, this species differs from *C. mirandae* by having generally shorter leaves, smaller ovulate strobilus (up to 27 cm long and 5 to 10 sporophylls per orthostichy), and longer seeds.

Specimens examined. Mexico. **Chiapas: Mun. Cintalapa,** 1,100 m, 10 Mar 1993, *A.P. Vovides 1234* (XAL); 920 m, 21 Jun 2018, *F. Nicolalde-Morejón et al. 2791–2799* (CIB); 980 m, 22 Jun 2018, *F. Nicolalde-Morejón et al. 2830* (CIB); 1,107 m, 18 Jun 2019, *F. Nicolalde-Morejón & L. Martínez-Domínguez 3177–3183* (CIB); 1,350 m, 18 Jun 2019, *F. Nicolalde-Morejón & L. Martínez-Domínguez 3186–3196* (CIB); 1,450 m, 11 Oct 1994, *J. Castillo et al. 445* (CHIP); 920 m, 21 Jun 2018, *L. Martínez-Domínguez et al. 1359–1369* (CIB), *1370* (CIB, MEXU); 980 m, 22 Jun 2018, *L. Martínez-Domínguez et al. 1402* (CIB, MEXU); 925 m, 18 Jun 2019, *L. Martínez-Domínguez & F. Nicolalde-Morejón 1770* (CIB); 1,107 m, 18 Jun 2019, *L. Martínez-Domínguez & F. Nicolalde-Morejón 1771,1772* (CIB, MEXU), *1773* (CIB), *1774* (CIB, MEXU), *1775–1777* (CIB); 1,350 m, 18 Jun 2019, *L. Martínez-Domínguez & F. Nicolalde-Morejón 1784–1794* (CIB); 900 m, 10 Jul 1994, *M.A. Pérez-Farrera 71* (CIB, CHIP, MEXU); 950 m, 5 Sep 1995, *M.A. Pérez-Farrera 776* (CIB, XAL);

Figure 15. Plants of *Ceratozamia* in habitat **A** *C. alvarezii* **B** *C. aurantiaca* **C** *C. becerrae* **D** *C. brevifrons* **E** *C. chamberlainii* **F** *C. chimalapensis* **G** *C. decumbens* **H** *C. delucana* **I** *C. euryphyllidia* **J** *C. fuscoviridis* **K** *C. hondurensis* **L** *C. huastecorum*.

1,000 m, 27 Mar 2000, *O. Farrera S. 2068* (CHIP). **Mun. Jiquipilas,** 1,200 m, 6 Jun 2002, *A. Reyes-García 5017* (MEXU); 1,380 m, 17 Feb 2000, *E. Palacios E. 2469* (CHIP); 1,170 m, 7 Jul 1994, *M.A. Pérez-Farrera 68* (CIB).

2. *Ceratozamia aurantiaca* Pérez-Farr., Gut.Ortega, J.L.Haynes & Vovides, Taxonomy 1: 249. 2021

Figs 1C, 15B

Ceratozamia martinezii Mart.-Domínguez, Nic.-Mor. & D.W.Stev., Nordic J. Bot. 1: 2. 2021 [2022]. Type. Mexico. Oaxaca: Mun. San Pedro Teutila, El Faro, 615 m, 28 Sep 2020, *L. Martínez-Domínguez et al. 2049* ♀ (holotype: CIB! [acc. # 22845UV]; isotypes: MEXU!, NY!).

Type. Mexico. Oaxaca: Mun. San Pedro Teutila, Sierra Norte, 30 May 2021, *M.A. Pérez-Farrera & P. Díaz-Jiménez 4014* (holotype: HEM [n.v.]; isotype: XAL [n.v.]).

Description. ***Stem*** 30–250 cm long, 10–15 cm in diameter, epigeous, erect or sometimes decumbent. ***Cataphylls*** 3.6–6.5 × 2–3.3 cm wide at the base, persistent, triangular, reddish brown, densely brown tomentose at emergence, glabrous at maturity, apex acuminate. ***Leaves*** 7–30 (50), 117–240 cm long, ascending, reddish brown at emergence, with whitish gray trichomes, glabrous at maturity. ***Petiole*** 45–85 cm long, terete, linear, reddish brown or greenish brown at emergence, dark green in mature leaves; with 16–47 thin prickles, 0.30–0.66 cm long. ***Rachis*** 70–160 cm long, terete, linear, dark green in mature leaves, with prickles in lower third. ***Leaflets*** 12–38 pairs, opposite to subopposite, insertion in one plane, oblong, generally longitudinally planar, not basally falcate, papyraceous, flat, green with adaxial and abaxial sides glabrous, distal end with entire margins, abruptly acuminate and symmetrical at the apex, attenuate at base, veins conspicuous and light-green; median leaflets 21–42.5 × 3.3–4.8 cm, 3.2–6.0 cm between leaflets; articulations 0.9–1.5 cm wide, green. ***Pollen strobili*** 20–35 cm long, 3.1–4.6 cm in diameter, solitary, cylindrical, erect, greenish with reddish trichomes at emergence, greenish yellow with reddish brown trichomes at maturity; peduncle 7.0–15 cm long, 1.5–2.1 cm in diameter, pubescent, reddish brown; microsporophylls 1.50–2.2 × 0.98–1.30 cm, obconic with a non-recurved distal face and a lobate fertile portion, infertile portion rounded and 0.47–0.84 cm long with horns straight and 0.16–0.38 cm long, 0.50–0.80 cm between horns with an acute angle between the horns. ***Ovulate strobili*** 11–36 cm long, 9.4–12.5 cm in diameter, solitary, cylindrical, erect, greenish yellow with an abundant reddish trichomes at emergence, green with brown trichomes at maturity, with an acute apex; peduncle 5.0–19.5 cm long, 1.3–2.3 cm in diameter, erect, light brown pubescent; megasporophylls 20–200, 5–11 orthostichies with 4–19 sporophylls per orthostichy, 2.25–2.72 × 3.6–4.3 cm, with a truncate distal face, horns curved and 0.54–0.90 cm long, 1.05–1.77 cm between horns with an acute angle between the horns. ***Seeds*** 2.4–3.0 cm long, 1.4–1.7 cm in diameter, ovate, sarcotesta whitish yellow to yellow when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia aurantiaca* occurs in highlands south of Río Santo Domingo and north of Sierra Norte in Oaxaca State, Mexico (Fig. 14B), where it occurs in the evergreen tropical forest on karstic rocks at 458–800 m.

Etymology. The specific epithet alludes to the leaf color at emergence. This is derived from Latin word aurantiacus for the orange color of emerging leaves.

Common names. None recorded.

Uses. People use the leaves of *Ceratozamia aurantiaca* to make flower arrangements in wreaths (Martínez-Domínguez et al. 2022a).

Preliminary conservation status. We visited three populations for *Ceratozamia aurantiaca* in which we recorded between 100 to 300 adult plants. We observed seedlings, juveniles and reproductive individuals. However, one of these populations is in a risk area due to anthropogenic land-use changes and was affected by fire in 2018. In addition, it is not found within protected areas. Based upon this information, *C. aurantiaca* should be considered "Endangered (EN)" in accordance with IUCN criteria.

Discussion. *Ceratozamia aurantiaca* is most similar to *C. whitelockiana*, however, differs from it in its oblong leaflets abruptly acuminate with a symmetric apex, its petiole with abundant (16–24) and long prickles (0.32–0.55 cm), and its rachis that has prickles in the proximal third of the leaf. In addition, the ovulate strobili have an acute apex, and the pollen strobili have obconic microsporophylls with a rounded infertile portion. The leaves are reddish brown at emergence, whereas in *C. whitelockiana* they are green.

Specimens examined. Mexico. **Oaxaca: Mun. Chiquihuitlán de Benito Juárez**, 861 m, 26 Nov 2004, *C.A. Cruz-Espinosa & G. Juárez-García 1945* (MEXU). **Mun. San Felipe Jalapa de Díaz**, 500 m, 28 Jun 2008, *J.A. Pérez de la Rosa et al. 1966* (IBUG). **Mun. San Pedro Teutila**, 458 m, 23 Sep 2020, *F. Nicolalde-Morejón et al. 3323–3333* (CIB); 500 m, 23 Sep 2020, *F. Nicolalde-Morejón et al. 3334–3337* (CIB); 615 m, 28 Sep 2020, *F. Nicolalde-Morejón et al. 3415–3419* (CIB); 708 m, 10 Jun 2004, *G. Juárez-García 425* (MEXU); 458 m, 23 Sep 2020, *L. Martínez-Domínguez et al. 1946–1950* (CIB), *1951* (CIB, MEXU), *1952–1957* (CIB); 500 m, 23 Sep 2020, *L. Martínez-Domínguez et al. 1958–1960* (CIB, MEXU), *1961* (CIB); 615 m, 28 Sep 2020, *L. Martínez-Domínguez et al. 2050, 2052* (CIB, MEXU), *2051, 2053* (CIB); 539 m, 6 Apr 2021, *L. Martínez-Domínguez et al. 2141, 2142, 2144* (CIB), *2143* (CIB, MEXU, NY). **Mun. Santa María Tlalixtac**, 675 m, 25 Nov 2004, *G. Juárez-García & C.A. Cruz-Espinosa 868* (MEXU).

3. *Ceratozamia becerrae* Pérez-Farr., Vovides & Schutzman, Bot. J. Linn. Soc. 146: 124. 2004

Fig. 15C

Type. Mexico. Tabasco: Mun. Teapa, hill Madrigal, Aug 2003, *A.P. Vovides 1458* (holotype: XAL [XAL0148355]).

Description. ***Stem*** 13–38 cm long, 9–15 cm in diameter, epigeous, semi-hypogeous, erect. ***Cataphylls*** 2.6–5.7 × 1.3–4.5 cm wide at the base, persistent, triangular, reddish brown, densely brownish tomentose at emergence, glabrous at apex when mature, apex acuminate. ***Leaves*** 2–12 (17), 49–210 cm long, descending, light green, glaucous at emergence with whitish gray trichomes, glabrous at maturity. ***Petiole*** 35–111.5 cm long, terete, linear, pink at emergence, yellowish green when mature; unarmed to armed with 3–27 thin prickles, 0.02–0.13 cm long. ***Rachis*** 30–105 cm long, terete, linear, pink at emergence, yellowish green at mature leaves, generally unarmed. ***Leaflets*** 6–15 pairs, opposite to subopposite, insertion in one plane, oblong to oblanceolate, longitudinally curved abaxially to planar, generally basally falcate, coriaceous, flat, green with adaxial and abaxial sides glaucous or glabrous, distal end with entire margins, acuminate to acute, and asymmetrical (rarely symmetrical in apical leaflets) at the apex, attenuate at base, with conspicuous and light-green veins; median leaflets 16–30 × 4.5–8.8 cm, 5.5–11.1 cm between the leaflets; articulations 0.5–1.5 cm wide, green and yellowish. ***Pollen strobili*** solitary, 10.8–20 cm long, 2.8–4.0 cm in diameter, cylindrical, erect, green with blackish trichomes at emergence, yellow-cream with blackish trichomes at maturity; peduncle 5–10 cm long, 1.5–2.0 cm in diameter, tomentose, light brown; microsporophylls 0.9–1.37 × 0.7–0.9 cm, discoid with a non-recurved distal face and a deeply lobate fertile portion, infertile portion 0.35–0.39 cm long and linear with straight horns and 0.30–0.45 cm long, 0.70–0.80 cm and an acute angle between the horns. ***Ovulate strobili*** 15–23 cm long, 7.5–8.5 cm in diameter, solitary, cylindrical, erect, green with reddish brown trichomes at emergence, green with brown to blackish trichomes at maturity, acute apex; peduncle 5–12 cm long, 1.6–2 cm in diameter, pendulous and erect, tomentose, light brown; megasporophylls 43–56, 6–8 orthostichies with 5–7 sporophylls per orthostichy, 1.7–2.0 × 3.5–4.0 cm, with a prominent distal face, horns straight and 0.70–0.87 cm long, 0.92–1.50 cm between horns with a right angle between the horns. ***Seeds*** 1.5–2.4 cm long, 1.2–2.0 cm in diameter, ovate, sarcotesta whitish pink at emergence, light brown at maturity.

Distribution and habitat. *Ceratozamia becerrae* is endemic to the mountains of Sierra Madrigal in southern Tabasco and Chiapas States, Mexico (Fig. 14C), where it occurs on karstic outcrops in evergreen tropical forest and oak forest from 100–800 m.

Etymology. The specific epithet was established in honor of Professor Marco E. Becerra for his relevant contributions in ethnology, archaeology and floristic research in Tabasco (Vovides et al. 2004).

Common names. None recorded.

Uses. None recorded.

Conservation status. (IUCN 2021). Endangered; A2ac+4ac; B2ab(i,ii,iv); C1.

Discussion. *Ceratozamia becerrae* has oblong and coriaceous leaflets and leaves that have a few thin and short prickles. This species belongs to a cryptic taxonomic group with *C. zoquorum* and *C. santillanii* (c.f. Martínez-Domínguez et al. 2017c; Vovides et al. 2020) and is morphologically similar in both vegetative and reproductive characters to *C. zoquorum*. This species differs from *C. santillanii* by its peduncle of ovulate strobili more than 3 cm long, but lacks morphological diagnoses with *C. zoquorum*.

Specimens examined. Mexico. **Chiapas: Mun. Amatán**, 300 m, *J.M. Lázaro Z. 376* (CHIP). **Tabasco: Mun. Tacotalpa**, 260 m, 12 Apr 2014, *F. Nicolalde-Morejón et al. 1999, 2000* (CIB); 260 m, 12 Apr 2014, *L. Martínez-Domínguez et al. 108–127* (CIB); 19 Jan 2001, *S. Avendaño R. 5214b* (XAL). **Mun. Teapa**, 100 m, 29 Jan 1985, *B.M. Schutzman 645, 648–650* (XAL); 204 m, 22 Feb 2014, *F. Nicolalde-Morejón et* *al. 1968, 1969* (CIB); 800 m, 11 Jun 1989, *J.A. Alejandre Rosas 494* (CIB); 204 m, 22 Feb 2014, *L. Martínez-Domínguez et al. 35–40* (CIB); 400 m, 16 Apr 1996, *M.A. Pérez-Farrera 901* (CHIP, HEM, MEXU); 800 m, 7 Apr 1914, *M.E. Becerra s/n* (MEXU).

4. *Ceratozamia brevifrons* Miq., Tijdschr. Wis-en natuurk Wet. 1: 41. 1847

Figs 3B, 12C, 14D, 15D

Type. Mexico. Veracruz: Mun. Alto Lucero de Gutiérrez Barrios, Apr 2005, *S. Avendaño R. 5699* (neotype, designated by Vovides et al. 2012, pg. 38: XAL! [XAL0132508]).

Description. ***Stem*** 20–70 cm long, 15–40 cm in diameter, epigeous, erect. ***Cataphylls*** 2–5 × 1.5–4 cm wide at the base, persistent, triangular, reddish brown, densely brownish tomentose at emergence, glabrous at maturity with an acuminate apex. ***Leaves*** 6–36, 58–173.5 cm long, descending, yellowish green at emergence with brown trichomes, glabrous at maturity. ***Petiole*** 20–56 cm long, terete, linear, green in mature leaves; with 40–90 robust prickles, 0.24–50 cm long. ***Rachis*** 35–125.5 cm long, terete, linear, green in mature leaves, with prickles. ***Leaflets*** 13–38 pairs, opposite to subopposite, insertion keeled, sometimes imbricate, lanceolate, abaxially curved, basally falcate, coriaceous, flat, light green with adaxial and abaxial sides glabrous, distal end with entire margins, acuminate and symmetrical to asymmetrical at the apex, attenuate at base, with conspicuous and green-light veins; median leaflets 15.5–41 × 2–4.1 cm, 0.5–3.2 cm between leaflets; articulations 0.6–1.7 cm wide, yellow. ***Pollen strobili*** 18–31 cm long, 4–7 cm in diameter, solitary, cylindrical, erect, greenish yellow at emergence, greenish yellow with brown to blackish trichomes at maturity; peduncle 4–10.3 cm long, 1.7–2.2 cm in diameter, reddish brown to light-brown pubescent; microsporophylls 1.55–2.3 × 0.80–1.5 cm, obconic with a non-recurved distal and a lobate fertile portion, infertile portion 0.50–0.70 cm long and rounded with straight horns 0.24–0.40 cm long, 0.50–1.02 cm and an acute angle between the horns. ***Ovulate strobili*** 25–33 cm long, 9.8–12.5 cm in diameter, solitary, cylindrical, erect, green with blackish trichomes at emergence, greenish yellow with brown to blackish trichomes at maturity and with an acuminate and apiculate apex; peduncle 6–14 cm long, 2.0–2.4 cm in diameter, pendulous and erect, brown to reddish brown pubescent; megasporophylls 80–224, 8–16 orthostichies with 10–15 sporophylls per orthostichy, 1.5–2.8 × 2.5–3.2 cm, with a prominent distal face, horns straight and robust and 0.75–0.95 cm long, 1.15–1.70 cm between horns with an acute angle between the horns. ***Seeds*** 2.0–3.0 cm long, 1.3–2.0 cm in diameter, ovate, sarcotesta whitish yellow to yellow when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia brevifrons* is known only from Sierra de Chiconquiaco in Veracruz State, Mexico (Fig. 14D) where it occurs in the transition zone between cloud forest and oak forest at 450 to 1,370 m.

Etymology. The epithet is derived from its relatively short leaves.

Common names. Mexico. Veracruz: Palma (J. Rees 1636); palmilla (A.P. Vovides et al. 682).

Uses. None recorded.

Conservation status. The area of distribution of *Ceratozamia brevifrons* is small and populations are close. Martínez-Domínguez et al. (2021) using ecological niche model estimated approximately 817 km2 of potential distribution with 558 km2 of transformed habitat. In addition, this species is not in a protected area. All data suggest that *C. brevifrons* could be assigned as "Endangered (EN)".

Discussion. *Ceratozamia brevifrons* is easily distinguished from its congeners by having adaxially keeled and coriaceous leaflets, petioles armed with abundant short and robust prickles and greenish yellow ovulate strobili with brown to blackish brown trichomes at maturity.

Specimens examined. Mexico. **Veracruz**: **Mun. Alto Lucero de Gutiérrez Barrios**, 24 Aug 1976, *A.P. Vovides 119* (XAL); 8 Jan 2009, *D. Jimeno-Sevilla 694* (XAL); 1,052 m, 12 Jan 2013, *F. Nicolalde-Morejón et al. 1711–1731* (CIB); 1,052 m, 22 Aug 2014, *F. Nicolalde-Morejón & L. Martínez-Domínguez 2027–2046* (CIB); 1,250 m, 6 Apr 1981, *G. Castillo-Campos 1297* (XAL); 700 m, 3 Dec 1974, *J. Rees 1636* (MO, XAL), *1641, 1642,* 850 m, 21 Sep 1976, *1675* (XAL); 1,052 m, 21 Jun 2014, *L. Martínez-Domínguez & F. Nicolalde-Morejón 130–133* (CIB); 842 m, 6 Feb 2015, *L. Martínez-Domínguez & F. Nicolalde-Morejón 216*–226 (CIB); 1,052 m, 22 Mar 2015, *L. Martínez-Domínguez & F. Nicolalde-Morejón 298–309* (CIB); 450 m, 14 Jul 1995, *M. Vázquez-Torres 4790* (CIB); 24 Jun 2010, *M. Vázquez-Torres et al. 9186* (CIB); 850 m, 10 Jan 2001, *T.W. Walters 2001-02-A, B* (XAL). **Mun. Chiconquiaco**, 1,268 m, 10 April. 2015, *F. Nicolalde-Morejón & L. Martínez-Domínguez 2237–2241* (CIB); 1,340 m, 2 May 2019, *F. Nicolalde-Morejón et al. 3138–3147* (CIB); 1,268 m, 10 Apr 2015, *L. Martínez-Domíguez & F. Nicolalde-Morejón 556–560* (CIB); 1,340 m, 2 May 2019, *L. Martínez-Domíguez et al. 1729–1738* (CIB). **Mun. Colipa**, *Marts 1841* (MO). **Mun. Juchique de Ferrer**, 850 m, 30 Aug 1981, *A.P Vovides 682* (XAL); 1,250 m, 6 May 1981, *G. Castillo-Campos 1710, 1763, 1768* (XAL); 1,300 m, 7 May 1981, *G. Castillo-Campos 1815, 1824, 1981* (XAL); 1,370 m, 24 Jul 2008, *M. Vazquez-Torres 8633* (CIB). **Mun. Vega de Alatorre**, 650 m, 21 Jul 1981, *B. Guerrero & J.I. Calzada 1826* (XAL); 550 m, 21 Jul 1981, *G. Castillo-Campos 2033* (XAL).

5. *Ceratozamia chamberlainii* Mart.-Domínguez, Nic.-Mor. & D.W.Stev., Phytotaxa 317(1): 22. 2017

Figs 3C, 15E

Type. Mexico. San Luis Potosí: Mun. Xilitla, 1,044 m, 20 Mar 2016, *L. Martínez-Domínguez et al. 933* ♀ (holotype: CIB! [acc. # 17766UV]; isotypes: MEXU! [MEXU1492226, MEXU1492227, MEXU14922278], NY!).

Description. ***Stem*** 20–60 cm long, 15–30 cm in diameter, epigeous, erect and decumbent. ***Cataphylls*** 2.5–5.5 × 1.5–3.5 cm wide at the base, persistent, narrowly triangular, reddish brown, densely brownish tomentose at emergence, glabrous at maturity, apex acuminate. ***Leaves*** (5)10–40, 100–207 cm long, descending, reddish brown at emergence with whitish gray trichomes, glabrous at maturity. ***Petiole*** 30–69 cm long, terete, linear, blackish brown in mature leaves; with 9–30 robust prickles, 0.03–0.30 cm long. ***Rachis*** 65–144 cm long, terete, linear, reddish brown in mature leaves, with prickles. ***Leaflets*** 20–42 pairs, opposite to subopposite, insertion in one plane, oblong, longitudinally curved abaxially to planar, not basally falcate, coriaceous, flat, dark green with adaxial and abaxial sides glabrous, distal end with entire margins, acuminate and asymmetric at the apex, attenuate at base, with conspicuous and reddish brown veins; median leaflets 20–37 × 2.3–4 cm, 1.6–3.5 cm between leaflets; articulations 0.5–1.3 cm wide, generally reddish brown. ***Pollen strobili*** 20–31 cm long, 4.5–6 cm in diameter, generally solitary (1–2), cylindrical, erect, greenish brown at emergence with reddish trichomes becoming greenish with reddish brown trichomes at maturity; peduncle 5.2–8.1 cm long, 1.6–2.3 cm in diameter, reddish brown pubescent; microsporophylls 1.6–2.3 × 0.9–1.5 cm, obconic with a non-recurved distal face and a deeply lobate fertile portion, infertile portion 0.44–0.55 cm long and linear with curved horns 0.25–0.40 cm long, 0.40–0.80 cm and an acute angle between the horns. ***Ovulate strobili*** 25.5–30 cm long, 7.5–10.5 cm in diameter, cylindrical, erect, greyish green with reddish brown trichomes at emergence and becoming light grayish brown with reddish brown trichomes at maturity with an acuminate apex; peduncle 4.5–11.5 cm long, 1.3–2.5 cm in diameter, erect, tomentose, brown to reddish brown; megasporophylls 49–180, 7–12 orthostichies with 7–15 sporophylls per orthostichy, 2.0–3.0 × 2.3–4.0 cm, with a prominent distal face, horns straight and 0.40–0.80 cm long, 2.0–2.70 cm between horns with an acute angle between the horns. ***Seeds*** 2.2–3.5 (4) cm long, 0.6–1.7 cm in diameter, ovate, sarcotesta whitish red to pink when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia chamberlainii* is distributed in the northern montane region of Carso Huasteco in San Luis Potosí, Querétaro and Hidalgo States, Mexico (Fig. 16A), where it occurs in cloud forest and pine-oak forest on rocky outcrops between 900–1,200 m.

Etymology. The specific epithet is in honor of Charles Joseph Chamberlain in recognition of his remarkable contributions to knowledge of the biology of the cycads and his fieldwork on Mexican Cycads (Martínez-Domínguez et al. 2017a).

Common names. Mexico. San Luis Potosí: Chamal (A.P. Vovides 1288), chamalillo (H. Puig 3979); Hidalgo: teocintle (A. Vite-Reyes et al. 23).

Uses. None recorded.

Preliminary conservation status. According to the IUCN criteria, the data suggest “Endangered (EN)”. *Ceratozamia chamberlainii* is included in Biosphere Reserve Sierra Gorda and have 9 populations recorded by us with several adult plants, juveniles and seedlings.

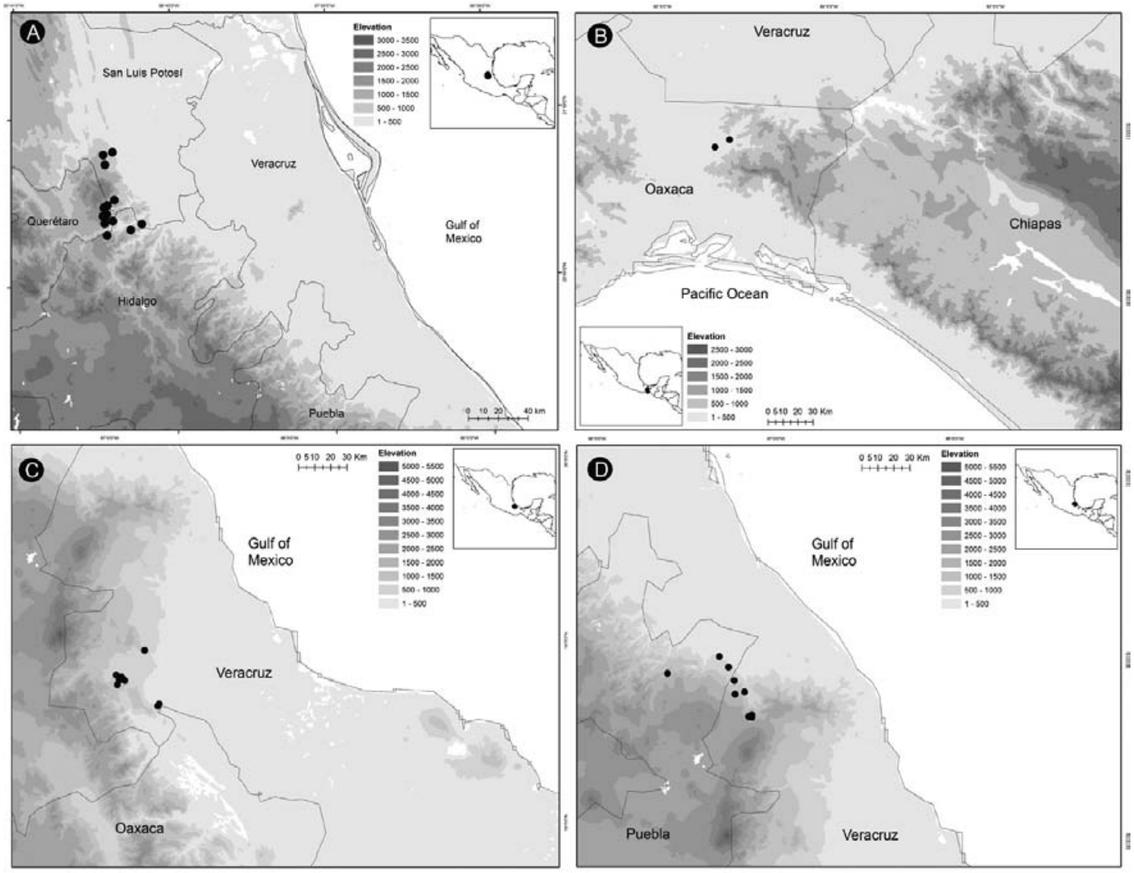

Figure 16. Distribution of *Ceratozamia* species **A** *C. chamberlainii* **B** *C. chimalapensis* **C** *C. decumbens* **D** *C. delucana*.

Discussion. *Ceratozamia chamberlainii* is close geographically and morphologically to *C. fuscoviridis* and *C. latifolia*; however, it differs by its petioles and rachis bearing abundant and short prickles, and its oblong and coriaceous leaflets with conspicuous, reddish brown veins. In addition, the leaves are reddish brown at emergence and at maturity have this color in the leaflet articulations and at the base of leaflets in contrast to the yellowish to greenish articulations in *C. fuscoviridis* and *C. latifolia*.

Specimens examined. Mexico. **Hidalgo: Mun. Chapulhuacán**, 1,500 m, 21 Feb 1998, *Alcántara-Ayala 3650* (FCME); 8 Jan 2009, *A. Vite-Reyes et al. 23* (XAL); 1,157 m, 30 Mar 2015, *F. Nicolalde-Morejón et al. 2200–2203* (CIB); 20 Sep 1964, *L. González-Quintero 4634* (ENCB); 1,157 m, 30 Mar 2015, *L. Martínez-Domínguez et al. 429–454* (CIB). **Mun. La Misión**, 1,120 a 1,400 m, 5 Oct 2007, *A. Castro-Castro et al. 1017* (IBUG, XAL); 7 Jan 2009, *A. Vite-Reyes et al. 20* (XAL). **Querétaro**: **Mun. Landa de Matamoros**, 1,050 m, 28 May 1999, *A.P. Vovides 1288, 1289, 1290, 2000* (XAL), *1291* (XAL, MEXU); 2 Apr 1991, *E. Carranza 6333* (IEB); 940 m, 2 Apr 1991, *3119* (MEXU); 1,335 m, 30 Mar 2015, *F. Nicolalde-Morejón et al. 2192–2197* (CIB); 1,145 m, 30 Mar 2015, *F. Nicolalde-Morejón et al. 2198, 2199* (CIB); 1,335 m, 30 Mar 2015, *L. Martínez-Domínguez et al. 379* (CIB, MEXU), 380–*407* (CIB); 1,145 m, 30 Mar 2015, *L. Martínez-Domínguez et al. 408–428* (CIB); 1,070 m, 9 May 1989, *R. Hiram 647* (XAL). **San Luis Potosí**: **Mun. Aquismón**, 600 m, 10 Feb 1969, *H. Puig 3979* (ENCB, P); 27 May 1979, *J.A. Alcorn 3093* (MEXU); 1,125 m, 9 Jun 2015, *T. Diego-Vargas & M. Bonta 35* (XAL). **Mun. Tamazunchale**, Jul 1952, *A. Wilson 273, 274* (US); Jul 1937, *C.L. Lundell & A.A. Lundell 7235* (CIB, IEB); 600–900 m, 29 Jun 1959, *J. Rzedowski 11087* (ENCB). **Mun. Xilitla**, 1,044 m, 20 Mar 2016, *F. Nicolalde-Morejón et al. 2407–2420* (CIB); 1,044 m, 20 Mar 2016, *L. Martínez-Domínguez et al. 924–932, 934–937* (CIB), *938* (CIB, MEXU), *939* (CIB); 1,948 m, 12 Jan 2001, *T.W. Walters, TW-2001-04-A,B* (XAL), *TW-.2001-04-C* (MEXU).

6. *Ceratozamia chimalapensis* Pérez-Farr. & Vovides, Bot. J. Linn. Soc. 157: 169. 2008

Fig. 15F

Type. Mexico. Oaxaca: Chimalapa, 21 Jan 2002, *M.A. Pérez-Farrera 2622* ♀ (holotype: HEM [n.v.]; isotypes: XAL [XAL0146074], MEXU [n.v.], MO [n.v.]).

Description. ***Stem*** 20–60 cm long, 15–30 cm in diameter, epigeous, erect and decumbent. ***Cataphylls*** 4–7.5 × 2.5–4.3 cm wide at the base, persistent, triangular, reddish brown, scarce brown tomentose at emergence, glabrous at maturity, apex acuminate. ***Leaves*** 7–24, 66–250 cm long, ascending, brown at emergence, glabrous at maturity. ***Petiole*** 40–70 cm long, terete, linear, green in mature leaves; with 20–30 thin prickles, 0.05–0.30 cm long. ***Rachis*** 60–150 cm long, terete, linear, green in mature leaves, with prickles. ***Leaflets*** 36–70 pairs, opposite to subopposite, insertion in one plane, lanceolate, longitudinally curved abaxially to planar, not basally falcate, papyraceous, flat, light green with adaxial and abaxial sides glabrous, distal end with entire margins, acuminate and symmetrical at the apex, attenuate at base, with conspicuous and green-light veins; median leaflets 27–46 × 1.2–1.8 cm, 0.8–1.3 cm between leaflets; articulations 0.5–0.9 cm wide, brown to yellowish brown. ***Pollen strobili*** 25–40 cm long, 3–5 cm in diameter, solitary, cylindrical, erect, greenish yellow at emergence with few brown trichomes, greenish to cream at maturity; peduncle 5–11 cm long, 1.5–3 cm in diameter, reddish brown pubescent; microsporophylls 1.3–2 × 0.6–1 cm, elliptic with a non-recurved distal face and a lobate to slightly lobate fertile portion, infertile portion 0.7–1.1 cm long and linear with curved horns 0.30–0.43 cm long, 0.8–1.1 cm and an acute angle between the horns. ***Ovulate strobili*** 35–40 cm long, 7.3–10.6 cm in diameter, solitary, cylindrical, erect, green with blackish trichomes at emergence, green with blackish trichomes at maturity, acuminate apex; peduncle 7–11 cm long, 1.7–2 cm in diameter, erect, brown to reddish brown pubescent; megasporophylls 99–192, 11–12 orthostichies with 9–16 sporophylls per orthostichy, 4–5.9 × 1.8–2.5 cm, with a prominent distal face, horns straight and thin and 0.78–0.98 cm long, 0.98–2.0 cm between horns with an acute angle between the horns. ***Seeds*** 2.0–3.0 cm long, 1.4–

1.9 cm in diameter, ovate, sarcotesta whitish yellow to yellow when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia chimalapensis* is endemic to Oaxaca State (Mexico) in the Sierra Atravesada mountain range at 290–1,000 m (Fig. 16B) in oak forest on clay soils.

Etymology. The specific epithet is in honor of the Chimalapa region renowned for its biological richness, particularly its floristic diversity.

Common names. Mexico. Oaxaca: Mazacopa (Vovides et al. 2008).

Uses. The sarcotesta of seeds is used as food; the ground seed is used as a rodenticide and with honey added, used as an insecticide (Vovides et al. 2008).

Preliminary conservation status. *Ceratozamia chimalapensis* is only known from a narrow area with several individuals. Data are insufficient to propose a conservation status at this time.

Discussion. *Ceratozamia chimalapensis* is similar to *C. mirandae* and *C. alvarezii* in leaf morphology; however, there are differences in reproductive structures; the ovulate strobilus of *C. chimalapensis* is longer than in these species. The ovulate strobili have between 11–12 orthostichies with 9–16 sporophylls per orthostichy, and more than 90 seeds per strobilus. The seeds are ovate, whereas in the other two species, they are spherical.

Specimens examined. Mexico. **Oaxaca: Mun. Santa María Chimalapa**, 290 m, 11 Jun 1995, *R. García S. 319* (SERO, XAL). **Mun. Santiago Niltepec**, 1000 m, 3 Apr 1946, *E. Hernández Xolocotzi & A.J. Sharp 1277* (MEXU).

7. ***Ceratozamia decumbens*** Vovides, Avendaño, Pérez-Farr. & Gonz.-Astorga, Novon 18 (1): 110. 2008

Fig. 15G

Type. Mexico. Veracruz: Naranjal, Near Cueva de Los Muñecos, 700 m, 8 Apr 2005, *S. Avendaño & G. Alducin 5706* (holotype: XAL! [XAL0005416, XAL0005418]; isotypes: HEM [n.v.], MO!).

Description. ***Stem*** 10–40 cm long, 10–25 cm in diameter, epigeous, erect and decumbent. ***Cataphylls*** 1.5–3 × 2–4.2 cm wide at the base, persistent, triangular, densely tomentose at emergence, reddish brown and partially tomentose at maturity, apex acuminate. ***Leaves*** 2–7, 80–190 cm long, descending, reddish brown at emergence with whitish gray trichomes, glabrous at maturity. ***Petiole*** 40–100 cm long, terete, linear, greenish brown in mature leaves; with 2–22 (28) thin prickles, 0.02–0.23 cm long. ***Rachis*** 40–123 cm long, terete, linear, brown and green in mature leaves, with prickles and occasionally unarmed. ***Leaflets*** 8–24 pairs, opposite to subopposite, insertion in one plane, oblong, in general longitudinally planar, not basally falcate, coriaceous, flat, green, adaxial and abaxial sides glabrous, distal end with entire margins, acuminate and symmetrical to asymmetrical at the apex, attenuate at base, with conspicuous and light-green veins; median leaflets 23–47.5 × 2.8–5 cm, 2.6–6.5 cm between leaflets; with articulations 0.7–1.2 cm wide, brown. ***Pollen strobili*** 20–23 cm long, 3.8–4.5 cm in diameter, solitary, cylindrical, erect, greenish yellow at emergence with reddish brown trichomes at maturity; peduncle 8–10.8 cm long, 1.2–1.6 cm in diameter, reddish brown to brown pubescent; microsporophylls 1–1.9 × 1–1.4 cm, obconic with a non-recurved distal face and lobate fertile portion, infertile portion 0.59–0.65 cm long and linear with straight horns 0.27–0.33 cm long, 0.67–0.80 cm and an acute angle between the horns. ***Ovulate strobili*** 9–11 cm long, 7–8 cm in diameter, solitary, cylindrical, erect, wine at emergence, wine with blackish brown trichomes at maturity, with an acute apex; peduncle 3–4 cm long, 1–1.2 cm in diameter, erect, brown pubescent; megasporophylls 18–49, 6–7 orthostichies with 3–7 sporophylls per orthostichy, 2.3–2.5 × 2–3 cm, with truncate distal face, horns straight and thin and 0.45–0.58 cm long, 0.99–1.48 cm between horns with a right angle between the horns. ***Seeds*** 1.2–2 cm long, 1.2–1.5 cm in diameter, ovate, sarcotesta whitish red when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia decumbens* is endemic to a small central mountain range in Veracruz State, Mexico (Fig. 16C) growing on karstic rocks in mountain tropical forest and cloud forest at 450–1,100 m.

Etymology. The epithet alludes to the decumbent nature of trunks in older mature plants.

Common names. None recorded.

Uses. None recorded.

Preliminary conservation status. *Ceratozamia decumbens* has not been listed in the IUCN Red List of Threatened Species (https://www.iucnredlist.org/). Its distribution area has been severely affected by anthropogenic land-use changes (Martínez-Domínguez et al. 2021). However, known populations have between 100 to 150 adult plants with juvenile and seedlings. All data suggest that the conservation status should be "Endangered (EN)".

Discussion. *Ceratozamia decumbens* is distinguished from its most similar species (*C. mexicana* and *C. moretti*) by the ovulate strobili which are are wine red with blackish brown trichomes at maturity and an acute apex, whereas in *C. mexicana* they are green with blackish brown and gray trichomes with an acuminate apex, and *C. morettii* are green with with blackish brown trichomes with an apiculate apex. In terms of vegetative morphology, it differs from these species by its reddish brown leaves with whitish gray trichomes at emergence.

Specimens examined. Mexico. **Veracruz: Mun. Atoyac**, 900 m, 28 Jan 1986, *R. Acevedo R. 728* (XAL). **Mun. Coetzala**, 650 m, 30 Nov 2001, *A. Rincón G. 2798* (MEXU, XAL); 870 m, 15 Jul 2015, *L. Martínez-Domínguez et al. 655–683* (CIB). **Mun. Córdoba**, 1,100 m, 10 Jun 1985, *A. Espíritu & J.L. Martínez 94* (XAL). **Mun. Ixtaczoquitlán**, 1,090 m, 25 May 1985, *A. Pérez P. 282* (XAL**). Mun. Naranjal**, 11 Sep 1982, *A.P. Vovides 751* (XAL); 10 Oct 1993, *Brigada T. Walters s/n* (XAL); 11 Sep 1982, *J. Rees 1690* (XAL); 10 Oct 1993, *T.W. Walters 41277, 41308, 41397* (XAL). **Mun. Tequila**, 445 m, 28 Oct 2007, *J.E. Rivera Hdez. & A. Vergara*

V. 4195 (MEXU, XAL); 959 m, 15 Jul 2015, *F. Nicolalde-Morejón et al. 2259, 2260* (CIB); 959 m, 15 Jul 2015, *L. Martínez-Domínguez et al. 684–703* (CIB). **Mun. Tezonapa**, 1 Dec 1995, *M.A. García B. 980* (XAL); 475 m, 24 Jun 1986, *R. Robles G. 882* (XAL). **Mun. Zongolica**, 11 Mar 2011, *L. Hermann Bojórquez G. et al. 2337* (CIB).

8. ***Ceratozamia delucana*** Vázq.Torres, A.Moretti & Carv.-Hern., Delpinoa 50–51: 129. 2013 ("2008-2009")

Figs 2A, B, 10A, B, 15H

Type. Mexico. Veracruz: Mun. Atzalan, road Atzalan- Tlapacoyan, 3 km NE from Atzalan, 20 Jan 2012, *M. Vázquez-Torres & C. Carvajal-Hernández 10200* ♀ (holotype: CIB! [acc. # 13915UV]; isotypes: XAL [n.v.], XALU!).

Description. ***Stem*** 20–90 cm long, 25–40 cm in diameter, epigeous, erect and decumbent. ***Cataphylls*** 2–5.5 × 2.5–4.5 cm wide at the base, persistent, narrowly triangular, reddish brown, densely tomentose at emergence, partially tomentose at maturity, apex acuminate. ***Leaves*** 10–100, 106–223 cm long, ascending, yellowish green at emergence with brown trichomes, glabrous at maturity. ***Petiole*** 30–87 cm long, terete, linear, light green at mature leaves; with 35–76 thin prickles, 0.21–0.76 cm long. ***Rachis*** 60–150 cm long, terete, linear, green in mature leaves, with prickles. ***Leaflets*** 20–43 pairs, opposite to subopposite, insertion in one plane, lanceolate and oblong, longitudinally curved abaxially to planar, basally falcate to non-basally falcate, papyraceous to coriaceous, flat, green, adaxial side glaucous and glabrous and abaxial side glaucous, distal end with entire margins, acuminate and symmetrical to asymmetrical at the apex, attenuate at base, with conspicuous and light-green veins; median leaflets 22–45 × 2.3–4.6 cm, 1.5–5 cm between leaflets; articulations 0.6–1.6 cm wide, green. ***Pollen strobili*** 24–32 cm long, 5.5–7.6 cm in diameter, solitary, cylindrical, erect, greenish yellow at emergence, greenish yellow with blackish trichomes at maturity; peduncle 3.5–12.5 cm long, 1.3–2 cm in diameter, tomentose, reddish brown to light-brown; microsporophylls 1.5–2.5 × 1.3–2 cm, discoid and obconic with a non-recurved distal face and a lobate fertile portion, infertile portion 0.30–0.45 cm long and orbicular with straight horns 0.15–0.25 cm long, 0.40–0.60 cm and a right angle between the horns. ***Ovulate strobili*** 17–40 cm long, 10–13.5 cm in diameter, solitary, cylindrical and globose, erect, dark green with blackish trichomes at emergence, green, generally glabrous at maturity, acute apex; peduncle 5.2–15 cm long, 1.8–2.2 cm in diameter, erect and pendulous, tomentose, brown to reddish brown; megasporophylls 48–266, 7–14 orthostichies with 6–19 sporophylls per orthostichy, 2.2–4.2 × 3.4–4.5 cm, with a truncate distal face, horns straight and 0.45–0.72 cm long, 1.05–1.67 cm between horns with a right angle between the horns. ***Seeds*** 2.0–3 cm long, 1.35–2.1 cm in diameter, ovate, sarcotesta whitish yellow to yellow when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia delucana* occurs in Veracruz and Puebla States, Mexico (Fig. 16D) and grows on karstic rocks in evergreen tropical forest at 500–1,650 m.

Etymology. The epithet is in honor of Dr. Paolo De Luca, Professor at University of Naples Federico II and a researcher into the biology of Mexican cycads (Vázquez-Torres et al. 2013).

Common names. None recorded.

Uses. None recorded.

Conservation status. During the last 6 years, we have monitored the 6 populations for *Ceratozamia delucana*, in which no loss of individuals and regeneration has been observed. These populations are periodically producing ovulate and pollen strobili. The main problem is the loss of habitat in the central area of Veracruz, where there are no protected natural areas (Martínez-Domínguez et al. 2021). These data suggest the category "Endangered (EN)" under (B1ab(iii)) criteria.

Discussion. *Ceratozamia delucana* is highly variable and in vegetative morphology similar to *C. morettii*. However, there are clear differences in their ovulate strobili. In *C. delucana*, ovulate strobili are green and generally without trichomes at maturity and have an acute apex, whereas in *C. morettii* they are green with blackish trichomes at maturity and have an apiculate apex. Additionally, *C. delucana* is a larger plant than *C. morettii*, with *C. delucana* having leaves up to 223 cm long with up to 43 pairs of leaflets and ovulate strobili 17–40 cm long.

Specimens examined. Mexico. **Puebla: Mun. Hueytamalco**, 520 m, 25 Feb 2008, *G. Ibarra Manríquez et al. 5485* (MEXU, MO). **Mun. Xochitlán de Vicente Suárez**, 1,644 m, 9 Jun 2015, *F. Nicolalde-Morejón & L. Martínez-Domínguez 2244, 2245* (CIB); 850 m, 25 Apr 1991, *G. Villalobos & E. Guerrero C. 325* (MEXU); 1,644 m, 9 Jun 2015, *L. Martínez-Domínguez & F. Nicolalde-Morejón 587–616* (CIB). **Veracruz: Mun. Atzalan**, 1,400 m, 20 Feb 2013, *F. Nicolalde-Morejón et al. 1740–1742* (CIB); 1,400 m, 16 Aug 2014, *F. Nicolalde-Morejón et al. 2125–2144* (CIB); 1,400 m, 13 Mar 2015, *F. Nicolalde-Morejón et al. 2168* (CIB); 1,400 m, 27 Oct 2008, *L. Lagunes-Galindo et al. 153* (CIB); 1396 m, 28 Oct 2008, *L. Lagunes-Galindo et al. 155* (CIB); 1,400 m, 16 Aug 2014, *L. Martínez-Domínguez et al. 163* (CIB); 863 m, 13 Mar 2015, *L. Martínez-Domínguez et al. 228–238* (CIB); 1,400 m, 13 Mar 2015, *L. Martínez-Domínguez et al. 239–248* (CIB). **Mun. Las Minas**, 2 Jun 1979, *A.P. Vovides 427* (XAL); 1,500 m, 22 Sep 1988, *C. Durán et al. 658* (XAL), *660* (MEXU, XAL); 1,500 m, 22 Sep 1988, *C. Durán E. 659* (MEXU, XAL); 1,586 m, 20 Feb 2013, *F. Nicolalde-Morejón et al. 1735–1739* (CIB); 1,621 m, 16 Aug 2014, *F. Nicolalde-Morejón & L. Martínez-Domínguez 2107–2124* (CIB); 1,621 m, 16 Aug 2014, *L. Martínez-Domínguez et al. 162* (CIB); 1,621 m, 18 Mar 2015, *L. Martínez-Domínguez et al. 249–260* (CIB); 1 Mar 2005, 1,430 m, *L.H. Bojórquez-Galván 1349* (CIB); 1,420 m, 16 Mar 2005, *L.H. Bojórquez-Galván 1374* (CIB); 1,470 m, 28 Apr 2009, *M. Vázquez-Torres et al. 8972* (CIB). **Mun. Tlapacoyan**, 900 m, 10 Jun 1970, *Nevling & A. Gómez-Pompa 1083* (MEXU).

9. *Ceratozamia euryphyllidia* Vázq.Torres, Sabato & D.W.Stev., Brittonia 38(1): 17. 1986

Fig. 15I

Type. Mexico. Veracruz: Mun. Minatitlán, 21 Jun 1984, *M. Vázquez-Torres 2842* ♀ (holotype: NY! [acc. # 1157–1166]; isotypes: CHAPA [n.v.], NY! [♂ acc. # 00001167–00001173], XALU [n.v.]).

Description. ***Stem*** 25–50 cm long, 8–15.3 cm in diameter, semi-hypogeous, erect and decumbent. ***Cataphylls*** 4.5–7 × 4.2–7.5 cm wide at the base, persistent, triangular, reddish brown, densely tomentose at emergence, glabrous at maturity, apex acute. ***Leaves*** 2–22, 95–337.5 cm long, ascending, light green, glaucous at emergence, with whitish gray trichomes, glabrous at maturity. ***Petiole*** 40–193 cm long, terete, linear, green to yellowish (pink in new leaves); with 8–35 thin prickles, 0.13–0.50 cm long. ***Rachis*** 53–218.5 cm long, terete, linear, green to yellowish in mature leaves, with prickles. ***Leaflets*** 6–17 pairs, opposite to subopposite, insertion in one plane, broadly obovate, in general longitudinally planar, not basally falcate, membranaceous, flat, green with adaxial and abaxial sides glabrous, distal end with sinuate margins, acuminate and asymmetrical at the apex, attenuate at base, with prominent and light-green veins; median leaflets 19.9–35.7 × 8.5–17.6 cm, 9–22 cm between leaflets; articulations 0.9–1.4 cm wide, yellow and green. ***Pollen strobili*** 27–35 cm long, 3–4.3 cm in diameter, solitary, cylindrical, erect, greenish yellow with reddish trichomes at emergence, greenish with black trichomes at maturity; peduncle 6–12 cm long, 1.5–2 cm in diameter, tomentose, reddish brown to brown; microsporophylls 1.0–1.5 × 0.5–1.3 cm, discoid with a non-recurved distal face and a lobate fertile portion, infertile portion 0.37–0.45 cm long and orbicular and rounded with straight horns 0.35–0.50 cm long, 0.60–0.72 cm and an acute angle between the horns. ***Ovulate strobili*** 15–20 cm long, 5–6 cm in diameter, solitary, cylindrical, erect, yellowish green to green with abundant, deep red trichomes at emergence, greenish brown with abundant, dark reddish brown trichomes at maturity, acuminate apex; peduncle 5–11.5 cm long, 1–2.5 cm in diameter, erect, tomentose, light brown; megasporophylls 35–64, 1.5–3 × 1.2–1.8 cm, 7–8 orthostichies with 5–8 sporophylls per orthostichy, 2.0–4.0 × 2.8–3.4 cm wide, with a truncate distal face, horns straight and 0.60–0.80 cm long, 0.99–1.40 cm between horns with an acute angle between the horns. ***Seeds*** 2.3–2.5 cm long, 1.5–1.7 cm in diameter, ovate, sarcotesta whitish red when immature, light brown at maturity.

Distribution and habit. *Ceratozamia euryphyllidia* is endemic to the forest of Uxpanapa-Chimalapas in Oaxaca and Veracruz States, Mexico (Fig. 17A) where it inhabits evergreen tropical rain forest on clay soils at the top and sides of hills between 100 and 630 m elevation.

Etymology. The specific epithet represents the very wide, diagnostic leaflets of this species. It comes from the Greek euryphyllos, which means, "wide leaflet".

Common names. None recorded.

Uses. None recorded.

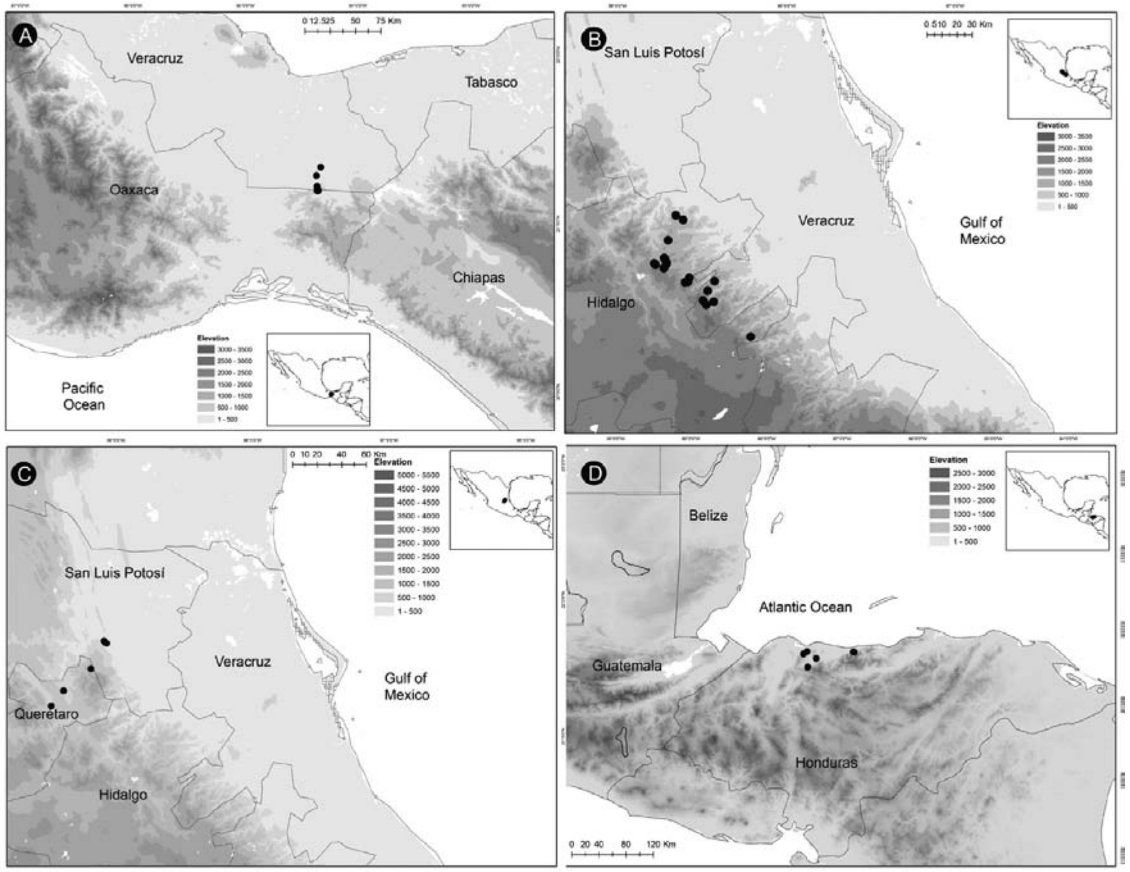

Figure 17. Distribution of *Ceratozamia* species **A** *C. euryphyllidia* **B** *C. fuscoviridis* **C** *C. hildae* **D** *C. hondurensis.*

Conservation status. (IUCN 2021). *Ceratozamia euryphyllidia* is listed as "Critically Endangered (CR)" under criteria B2ab(i,ii,iii,iv,v). We carried out a census in two populations for this species, in which we found between 20 to 60 adult plants. The population in Veracruz has reproductive plants, juvenile and seedlings; however, it is a small population and the surrounding area has been transformed to anthropogenic landscapes. We suggest that this status should be maintained.

Discussion. *Ceratozamia euryphyllidia* is easily diagnosable from its congeners in Mexico by its broadly obovate, lustrous and membranaceous leaflets asymmetrical apex of leaflets. Also, the ovulate strobili are greenish brown with an acuminate apex, and its megasporophylls are green with abundant dark brown trichomes. This species is most similar to *C. hondurensis*, but it can be distinguished by its sinuate margins at distal end of leaflets, whereas in *C. hondurensis* they are entire.

Specimens examined. Mexico. **Veracruz**: **Mun. Minatitlán**, 100 m, 24 Jul 1986, *A.P. Vovides & E.R. Acosta 1108* (MO, XAL); 21 Jun 1984, *M. Vázquez-Torres 1157* (NY). **Mun. Jesús Carranza**, 24 May 1982, *M. Vázquez-Torres 2532* (CIB, MEXU); 26 Mar 1982, *M. Vázquez-Torres et al. 2374* (CHAPA, MEXU, NY); 100 m, 5 Jun 1989, *M. Vázquez-Torres & J.P. Sclavo* 4037 (CIB); 18 Apr 1982, *M. Vázquez-Torres 2451* (CHAPA, MEXU, MO, NY); 120 m, 28 Jul 1983, *M. Vázquez-Torres 2614*

(CIB, NY); 21 Jun 1984, *M. Vázquez-Torres 4126* (NY); 120 m, 28 Dec 1984, *T. Wenth et al. 4732* (CHAPA). **Mun. Uxpanapa**, 398 m, 18 Mar 2014, *F. Nicolalde-Morejón & L. Martínez-Domínguez 1984* (CIB); 19 Mar 2014, *L. Martínez-Domínguez & F. Nicolalde-Morejón* 71–77 (CIB). **Mun. Hidalgotitlán** (plant cultivated at JBC-INECOL), *V.E. Luna M. 1520* (XAL). **Oaxaca: Mun. Santa María Chimalapa**, 232 m, 13 May 1995, *E. Torres B. 687* (SERO, XAL); 398 m, 18 Mar 2014, *F. Nicolalde-Morejón & L. Martínez-Domínguez 1982, 1983* (CIB); 335 m, 5 May 1996, *J.E. Rivera H. & S. Escobedo 102* (XAL); 630 m, 16 May 1998, *J.E. Rivera H. et al. 700* (MEXU); 475 m, 21 Mar 1999, *J.E. Rivera H. et al. 1195* (SERO); 398 m, 18 Mar 2014, *L. Martínez-Domínguez & F. Nicolalde-Morejón 51–70* (CIB).

10. *Ceratozamia fuscoviridis* W.Bull., Retail List. 154: 4. 1879

Figs 3A, 9C, 15J

Ceratozamia mexicana Brongn. f. *fuscoviridis* (W.Bull.) J.Schust., Pflanzenr. (Engler) Heft 99, 4 fam 1: 132. 1932, as *C. mexicana* var. *longifolia* f. *fuscoviridis*. Type: Based on *Ceratozamia fuscoviridis* W.Bull.

Type. Cultivated at Glasnevin, Ireland "Hort. Bot. Glasnevin", 21 Mar 1878 (accessioned 1881), *D. Moore s.n.* (neotype, designated by Calonje and Sennikov 2017, pg. 161: K! [K000501714, K000501712, K000501713]). Mexico. Hidalgo: Mun. Molango, km 3 carretera Molango-Xochicoatlán, 1,860 m, 31 Mar 2015, *L. Martínez-Domínguez et al. 493* ♀ (epitype, designated by Martínez-Domínguez et al. 2018a: 105: CIB! [acc. # 17465UV]; isoepitype: MEXU! [acc. # 1520508, 1520282, 1520519]).

Description. ***Stem*** 20–90 cm long, 25–40 cm in diameter, epigeous, erect and decumbent. ***Cataphylls*** 2–4.5 × 2–3.5 cm wide at the base, persistent, narrowly triangular, reddish brown, densely brownish tomentose at emergence, tomentose at maturity, apex acuminate. ***Leaves*** 10–70, 92–215 cm long, ascending, light green and dark brown at emergence, with whitish gray trichomes, glabrous at maturity. ***Petiole*** 40–95 cm long, terete, linear, dark green in mature leaves; with 14–60 (66) thin prickles, 0.09–0.33 cm long. ***Rachis*** 65–150 cm long, terete, linear, green in mature leaves, with prickles. ***Leaflets*** 28–67 pairs, opposite to subopposite, insertion in one plane, lanceolate, abaxially curved longitudinally, basally falcate, papyraceous, flat, green, with adaxial and abaxial sides glabrous, distal end with entire margins, acuminate and symmetrical at the apex, attenuate at base, with conspicuous and green-light brown veins; median leaflets 16.6–42 × 1.3–2.1 cm, 0.6–2 cm between leaflets; articulations 0.6–1.3 cm wide, green and brown. ***Pollen strobili*** 22–30 cm long, 5–8 cm in diameter, solitary, cylindrical, erect, brownish yellow at emergence, greenish brown with reddish brown trichomes at maturity; peduncle 5–14.5 cm long, 1.6–2.3 cm in diameter, tomentose, reddish brown to brown; microsporophylls 1.6–2.4 × 1.2–1.6 cm, obconic with a non-recurved distal face and a deeply lobate fertile portion, infertile portion 0.45–0.56 cm long and orbicular with straight horns

0.16–0.29 cm long, 0.53–0.80 cm and a right angle between the horns. ***Ovulate strobili*** 24–35 cm long, 8.5–15 cm in diameter, solitary, cylindrical, erect, green with brown trichomes at emergence, brown-green with dark brown trichomes at maturity, acuminate apex; peduncle 4–15.5 cm long, 1.8–2.5 cm in diameter, erect or pendulous, tomentose, brown to reddish brown; megasporophylls 49–195, 7–15 orthostichies with 7–15 sporophylls per orthostichy, 2.3–3.5 × 3.0–4.0 cm, with a truncate distal face, horns straight and 0.70–0.99 cm long, 1.7–2.1 cm between horns and an obtuse angle between the horns. ***Seeds*** 2–2.6 cm long, 1.5–2 cm in diameter, ovate, sarcotesta whitish yellow to yellow when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia fuscoviridis* is endemic to south to central Carso Huasteco in Mexico from Hidalgo to northwest of Veracruz States (Fig. 17B) in cloud forests between 1,300–1,900 m elevation.

Etymology. The epithet refers to the dark-brown color of the leaf at emergence.

Common names. Mexico. Veracruz: teocintle, teocintli and tepecintli (Bonta et al. 2019).

Uses. This species has decorative uses associated with religious ceremonies and national holidays. The leaves are commonly used to make arches in the entrances of some Roman Catholic churches or decorate the walls during national holidays.

Conservation status. (IUCN 2021). *Ceratozamia fuscoviridis* is listed as “Critically Endangered” under criteria B1ab(i,iii,iv,v). This species requires a reassessment of its conservation status because new populations have recently been recorded, including its current circumscription that includes populations from Veracruz State. Based on the number of populations, modelled potential geographical distribution from ecological niche and its occurrence in a Natural Protected Areas (Los Mármoles National Park), *C. fuscoviridis* could be listed as “Endangered (EN)” (Martínez-Domínguez et al. 2021).

Discussion. *Ceratozamia fuscoviridis* is polymorphic within populations because individual plants may have either a light green or dark-brown leaf color at emergence. Individuals with dark-brown leaf at emergence also have a brownish abaxial side. This species is most geographically proximate to *C. chamberlainii*, but it can be distinguished by lanceolate and papyraceous leaflets, a petiole armed with long and thin prickles; and ovulate strobili that are brownish green with dark trichomes at maturity.

Osborne et al. (2006) intended to validate *Ceratozamia fuscoviridis* D.Moore, which was invalidly published in 1878. However, in 2017, Calonje & Sennikov published the correction to this name using a brief description by William Bull in horticultural catalogues. This work is the valid publication for the specie and the name “*C. fuscoviridis* D.Moore” is an isonym with no nomenclatural status.

Specimens examined. Mexico. **Hidalgo: Mun. Eloxochitlán**, 18 Mar 1995, *I. Luna-Vega 54716* (FCME). **Mun. Metztitlán**, 30 Dec 1992, *J.L. López-García 449* (ENCB, IBUG, MEXU). **Mun. Molango de Escamilla**, 1,380 m, 29 May 1999, *A.P. Vovides 1298* (XAL); 1,400 m, 29 May 1999, *A.P. Vovides 1301* (XAL); 1,500 m, 24 Jul 2008, *A. Vite-Reyes et al. 6* (XAL); 1,860 m, 31 Mar 2015, *F. Nicolalde-Morejón et al. 2209–2211* (CIB); 1,860 m, 31 Mar 2015, *L. Martínez-Domínguez et al. 485*–492, 494–*514* (CIB); *T.W. Walters 2001-03-A* (XAL). **Mun. Tenango de Doria**, 1,700 m, 12 Mar 1993, *I. Luna-*

Vega 914 (FCME). **Mun. Tlanchinol**, 1,450 m, 25 Aug 1992, *I. Luna-Vega s/n* (FCME); 1,420 m, 7 Oct 1992, *I. Luna-Vega 625* (FCME, XAL); 25 Aug 1992, *I. Luna-Vega 789* (XAL); 1,312 m, 31 Mar 2015, *F. Nicolalde-Morejón et al. 2204–2208* (CIB); 1,312 m, 31 Mar 2015, *L. Martínez-Domínguez et al. 455–484* (CIB). **Mun. Zacualtipán de Ángeles**, 23 Jan, 1983, *J. Rees 389* (CHAPA, FCME, MEXU); 1,360 m, 4 Dec 1974, *J. Rees 1611* (CHIP, XAL); 4 Dec 1974, *J. Rees 6339* (IEB). **Veracruz: Mun. Huayacocotla**, 1,913 m, 23 Feb 2005, *D. Saavedra Millán 64* (FCME); 1,850 m, 13 Mar 1980, *J. Palma G. 63* (XAL); 1,844 m, 1 Apr 2015, *F. Nicolalde-Morejón et al. 2212–2214* (CIB); 1,700 m, 24 Mar 1981, *L. Ballesteros & F. Ballesteros 460* (XAL); 1,550 m, 23 Apr 1981, *L.G. Juárez G. 47* (XAL); 1,844 m, 1 Apr 2015, *L. Martínez-Domínguez et al. 515–544* (CIB); 1,900 m, 11 Feb 1972, *R. Hernández M. 1507* (MEXU, XAL); 26 Feb 1975, *V. Sosa 59* (XAL).

11. *Ceratozamia hildae* G.P.Landry & M.C.Wilson, Brittonia 31(3): 422. 1979

Fig. 18

Type. Cultivated in Baton Rouge, Louisiana USA at 5988 South Pollard Parkway (plants originally from several km N of Xilitla, San Luis Potosí, Mexico), *G. Landry 76521*♂ (holotype: GH! [00003274]; isotypes: FTG!, LSU! [LSU00048484], MEXU! [MEXU00443083, MEXU00443084, MEXU00443085], MICH! [1050284A, 1050284B], NY! [00001153–00001156], US! [00011993]).

Description. ***Stem*** 10–20 cm long, 10–15 cm in diameter, semi-hypogeous, erect. ***Cataphylls*** 2.1–4.2 × 0.8–1.9 cm wide at the base, persistent, triangular, reddish brown, densely brown tomentose at emergence, partially tomentose at maturity, apex acuminate. ***Leaves*** 2–7, 95–202 cm long, ascending, reddish brown at emergence with whitish gray trichomes, glabrous at maturity. ***Petiole*** 43–89 cm long, terete, linear, greenish brown and green in adult leaves; with 2–12 thin prickles, 0.01–0.2 cm long. ***Rachis*** 60–130 cm long, terete, linear, greenish brown in mature leaves, with prickles and occasionally unarmed. ***Leaflets*** in 5–11 fascicles, 16–56 leaflets in total, clustered, insertion in one plane, oblong, in general longitudinally planar, basally falcate to non-basally falcate, membranaceous, flat, green with adaxial and abaxial sides glaucous, distal end with entire margins, acuminate and symmetrical to asymmetrical at the apex, attenuate at base, with conspicuous and green-light veins; median leaflets 14.5–24 × 2.4–5 cm, 6–15 cm between leaflets; articulations 0.2–0.5 cm wide, brown and green. ***Pollen strobili*** 8–12.5 cm long, 2–2.5 cm in diameter, solitary, cylindrical, erect, brown with reddish brown trichomes at emergence, reddish brown at maturity; peduncle 6–8 cm long, 0.8–1 cm in diameter, tomentose, reddish brown to brown; microsporophylls 0.8–1.2 × 0.6–1 cm, discoid with non-recurved distal face and a lobate fertile portion, infertile portion 0.23–0.29 cm long and rounded with curved horns, 0.20–0.25 cm long, 0.39–0.50 cm and an acute angle between the horns. ***Ovulate strobili*** 10–15 cm long, 6–9 cm in diameter, solitary, cylindrical, erect, green at emergence with brown trichomes, green with brown to blackish trichomes at maturity, acuminate apex; peduncle 7.5–16 cm long, 1.2–1.5 cm in diameter, erect, tomentose, brown; megasporophylls

20–56, 5–8 orthostichies with 4–7 sporophylls per orthostichy, 2–3.7 × 2–4 cm, with a prominent distal face, horns straight and 0.30–0.50 cm long, 1.70–1.90 cm between horns with a right angle between the horns. ***Seeds*** 1.3–2.3 cm long, 1.2–1.5 cm in diameter, ovate, sarcotesta whitish red when immature, light brown at maturity.

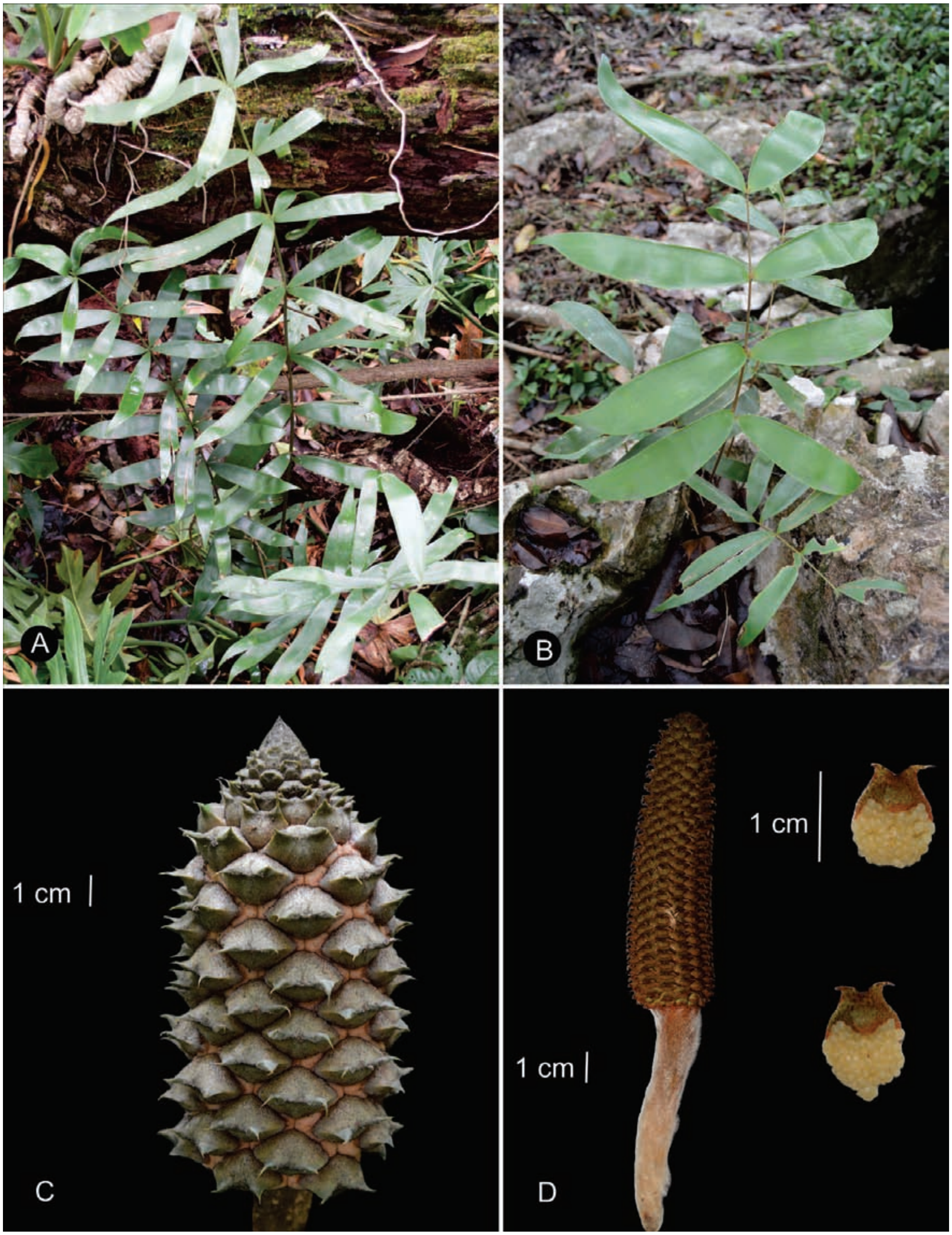

Figure 18. *Ceratozamia hildae* **A** leaves of an adult plant **B** leaves of a juvenile plant **C** mature ovulate strobilus **D** mature pollen strobilus and microsporophylls.

Distribution and habitat. *Ceratozamia hildae* is endemic to Sierra Gorda in Mexico, particularly in San Luis Potosí and Querétaro (Fig. 17C), where it occurs in the evergreen tropical forests on karstic rocks at 300–1,200 m.

Etymology. The epithet is in honor of Hilda Guerra Walker, daughter of the original collector (Luciano E. Guerra, plant collector from Mission Texas) (Landry and Wilson 1979).

Common names. Mexico. Querétaro: Chamalillo, pata de gallo (J. Rees 312).

Uses. None recorded.

Conservation status. (IUCN 2021). *Ceratozamia hildae* is listed as "Endangered" under criteria A2abcd; B1ab(ii,iii,iv).

Discussion. *Ceratozamia hildae* is easily distinguished from other members of the group by its clustered membranous oblong leaflets (Fig. 18A).

Specimens examined. Mexico. **Querétaro: Mun. Arroyo Seco**, 1,181 m, 9 Nov 2003, *J.A. Pérez de la Rosa & G. Vargas A. 1908* (IBUG). **Mun. Jalpan de Serra**, 850 m, 29 Dec 1977, *A.P. Vovides 337* (XAL); 29 Dec 1977, *A.P. Vovides s/n* (IEB); 1,200 m, 8 Mar1991, *B. Servin 870* (MEXU); 1,200 m, 20 Mar 1991, *B. Servin 986* (MEXU); 8 Mar 1991, *B. Servin 6328* (IEB); 20 Apr 1991, *B. Servin 6330* (IEB); Sep 1977, *J. Rees s/n* (IEB); *312* (XAL). **San Luis Potosí: Mun. Aquismón**, 300 m, 22 Sep 1977, *A.P. Vovides & J. Rees 312* (MEXU, XAL); 357 m, 20 Mar 2016, *F. Nicolalde-Morejón et al. 2391–2406* (CIB); 357 m, 20 Mar 2016, *L. Martínez-Domínguez et al. 910–921, 923, 940* (CIB), *922* (CIB, MEXU); 26 Nov 1970, *S. Longoria s/n* (MEXU; US); 617 m, 15 Jan 2001, *T.W. Walters TW-2001-14-A* (MEXU, XAL), *TW-2001-14-B* (XAL), *TW-2001-14-C* (MEXU).

12. *Ceratozamia hondurensis* J.L.Haynes, Whitelock, Schutzman & R.S.Adams, The Cycad Newsletter 31 (2/3): 16. 2008

Fig. 15K

Type. HONDURAS. Atlántida: Mun. Esparta, 0.5 km SE of Jilamo Nuevo along the Río Jilamito, 13 Apr 1994, *A.E. Brand & R. Zúniga 2830*, (lectotype, designated here: MO! [acc. # 5943287–5943289]; isolectotype: MEXU! [MEXU01347996–MEXU01347999]).

Description. ***Stems*** 20–50 cm long, 20 cm in diameter, semi-hypogeous, erect and decumbent. ***Cataphylls*** 4.5–7 × 4.5–7.5 cm wide at the base, persistent, triangular, reddish brown, densely brown tomentose at emergence, glabrous at maturity, apex acuminate. ***Leaves*** 3–25, 120–322 cm long, ascending, light green at emergence with whitish gray trichomes, glabrous at maturity. ***Petiole*** 56–150 cm long, terete, linear, green in mature leaves; with 8–34 thin prickles, 0.26–0.50 cm long. ***Rachis*** 80–200 cm long, terete, linear, with prickles, green in mature leaves. ***Leaflets*** 10–18 pairs, opposite to subopposite, insertion in one plane, obovate to broadly oblanceolate, in general longitudinally planar, generally not basally falcate, membranaceous, flat, green with adaxial side glabrous and abaxial side glaucous, distal end with entire margins, acuminate and symmetrical at the apex, attenuate at base, with prominent and light-green veins; median leaflets 23–

34.5 × 8–12.8 cm, 8.5–16 cm between leaflets; articulations 0.5–1.6 cm wide, green and yellow. ***Pollen strobili*** 30–35 cm long, 4–5 cm in diameter, solitary, cylindrical, erect, yellowish green at emergence, yellowish cream with blackish trichomes at maturity; peduncle 2–4 cm long, 2–3 cm in diameter, tomentose, brown; microsporophylls 0.9–1.2 × 0.7–1.2 cm, discoid with a non-recurved distal face and lobate fertile portion, infertile portion 0.52–0.60 cm long, and orbicular and rounded with straight horns 0.18–0.23 cm long, 0.50–0.62 cm and an acute angle between the horns. ***Ovulate strobili*** 18–22 cm long, 8–10 cm in diameter, solitary, cylindrical, erect, yellowish green to green with abundant, deep red trichomes at emergence, greenish brown with abundant, blackish trichomes at maturity, mucronate apex; peduncle 5–15 cm long, 1.0–3.0 cm in diameter, erect, tomentose, brown; megasporophylls 72–120, 9–11 orthostichies with 8–11 sporophylls per orthostichy, 1.5–2.5 × 4–5.5 cm, with a prominent distal face, horns curved and 0.60–0.80 cm long, 0.99–1.40 cm between the horns with an acute angle between horns. ***Seeds*** 2.1–2.6 cm long, 1.46–1.89 cm in diameter, ovate, sarcotesta whitish yellow to yellow when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia hondurensis* is endemic to Honduras in the Atlántida department (Fig. 17D), where it grows in evergreen tropical forest between 20–600 m elevation.

Etymology. The specific epithet alludes to the endemism of the species in Honduras.

Common names. Honduras. Atlántida: Camotillo (Haynes et al. 2008).

Uses. The stems are used to elaborate an infusion for poison animals or people. (L. Martínez-Domínguez et al. 2241). Also, the stems are processed for medicinal purposes (Bonta et al. 2019).

Preliminary conservation status. *Ceratozamia hondurensis* is not listed in The IUCN Red List of Threatened Species. There is insufficient data on the population's status of this species, however, it is known that populations have suffered illegal wildlife trade as shown by seizures made at Customs of Honduras.

Discussion. *Ceratozamia hondurensis* is similar in leaf morphology to *C. euryphyllidia*. It differs in its leaflets with a symmetric apex and an entire margin at distal end as compared to the asymmetric apex and sinuate margin at distal end of the leaflets in *C. euryphyllidia*. Additionally, *C. hondurensis* differs in reproductive structures; the ovulate strobilus has mucronate apex, whereas in *C. euryphyllidia* the ovulate strobilus has long (up to 5 cm) acuminate apex.

In protologue, two specimens from "*A.E. Brand & R. Zúniga 2830*" were cited as types: holotype in EAP and isotype in MO. The type specimen was not deposited in EAP (the herbarium has no record of this material nor was it found in unprocessed material). In addition, we consulted TEFH, the other herbarium in Honduras, with the same result. However, we found a duplicate specimen in MEXU. Thus, we are here designating the specimen in MO cited as isotype as the lectotype and the specimen at MEXU as the isolectotype.

Specimens examined. Honduras. **Atlántida**: **Mun. La Ceiba,** 225 m, 14 Apr 1996, *D.R. Hodel & Schleder 1485* (MO); 217 m, 29 Jul 2003, *J. Haynes et al. 47*

(TEFH); 151 m, 19 May 2022, *L. Martínez-Domínguez et al. 2440, 2443, 2444, 2446–2448* (TEFH). **Mun. Tela,** 0–500 m, 10 Feb 1994, *C. Nelson et al. 17586* (TEFH); 200–500 m, 9 Apr 1994, *D.L. Hazlett et al. 8036* (MO); 615 m, 13 Jul 2008, *G. Sandoval et al. 1312* (TEFH); 615 m, 31 Jul 2003, *J. Haynes et al. 40* (TEFH); Cultivated, 615 m, 16 Mar 2017, *L. Ferrufino et al. 777* (TEFH); 163 m, 17 May 2022, *L. Martínez-Domínguez et al. 2421, 2423, 2425, 2428–2430* (TEFH).

13. *Ceratozamia huastecorum* Avendaño, Vovides & Cast.-Campos, Bot. J. Linn. Soc. 141(3): 395. 2003

Fig. 15L

Type. Mexico. Veracruz: Mun. Tepetzintla, Sierra de San Juan Otontepec, 1,300 m, 16 Dec 1981, *G. Castillo-Campos et al. 2567* ♀ (holotype: XAL! [XAL0016937]).

Description. ***Stem*** 20–50 cm long, 14–20 cm in diameter, semi-hypogeous, erect and decumbent. ***Cataphylls*** 3.0–4.0 × 2.5–3.0 cm wide at the base, persistent, triangular, reddish brown, densely brown tomentose at emergence, glabrous at maturity, apex acuminate. ***Leaves*** 4–7, 50–80 cm long, ascending, light green at emergence with whitish gray trichomes, glabrous at maturity. ***Petiole*** 20–40 cm long, terete, linear, green in mature leaves; with 10–30 thin prickles 0.21–0.35 cm long. ***Rachis*** 40–70 cm long, terete, linear, green in mature leaves, with prickles. ***Leaflets*** 8–18 pairs, opposite to subopposite, insertion in one plane, oblong, in general longitudinally planar, not basally falcate, coriaceous, flat, green with adaxial side glabrous and abaxial side glaucous, distal end with entire margins, acuminate and asymmetrical at the apex, attenuate at base, with prominent and light-green veins; median leaflets 12–22 × 2.5–6 cm, 1.6–5 cm between leaflets; articulations 0.4–1.1 cm wide, green. ***Pollen strobili*** 15–18 cm long, 2.5–3.2 cm in diameter, solitary, cylindrical, erect, yellowish green at emergence, yellowish cream with blackish trichomes at maturity; peduncle 6–23 cm long, 1.6–2.2 cm in diameter, tomentose, brown; microsporophylls 0.8–1.2 × 0.72–1.0 cm, discoid with a non-recurved distal face and a lobate fertile portion, infertile portion 0.28–0.35 cm long and orbicular with straight horns 0.11–0.18 cm long, 0.50–0.68 cm and an acute angle between the horns. ***Ovulate strobili*** 13.5–18 cm long, 6–10 cm in diameter, solitary, cylindrical, erect, green at emergence, dark green with abundant blackish trichomes at maturity, acuminate apex; peduncle 3–8 cm long, 1.0–2.2 cm in diameter, erect, tomentose, brown; megasporophylls 54–99, 9–10 orthostichies with 6–9 sporophylls per orthostichy, 1.4–1.8 × 1.8–2.0 cm, with a truncate distal face, horns straight and 0.40–0.56 cm long, 1.0–1.60 cm between the horns with an acute angle between the horns. ***Seeds*** 1.1–1.5 cm long, 1.0–1.2 cm in diameter, ovate, sarcotesta light brown at maturity.

Distribution and habitat. *Ceratozamia huastecorum* is endemic to the Sierra de Otontepec in the northwest of Veracruz State, Mexico (Fig. 19A) where it occurs in cloud forest on clay soils with rocky outcrops at 800 to 1,300 m.

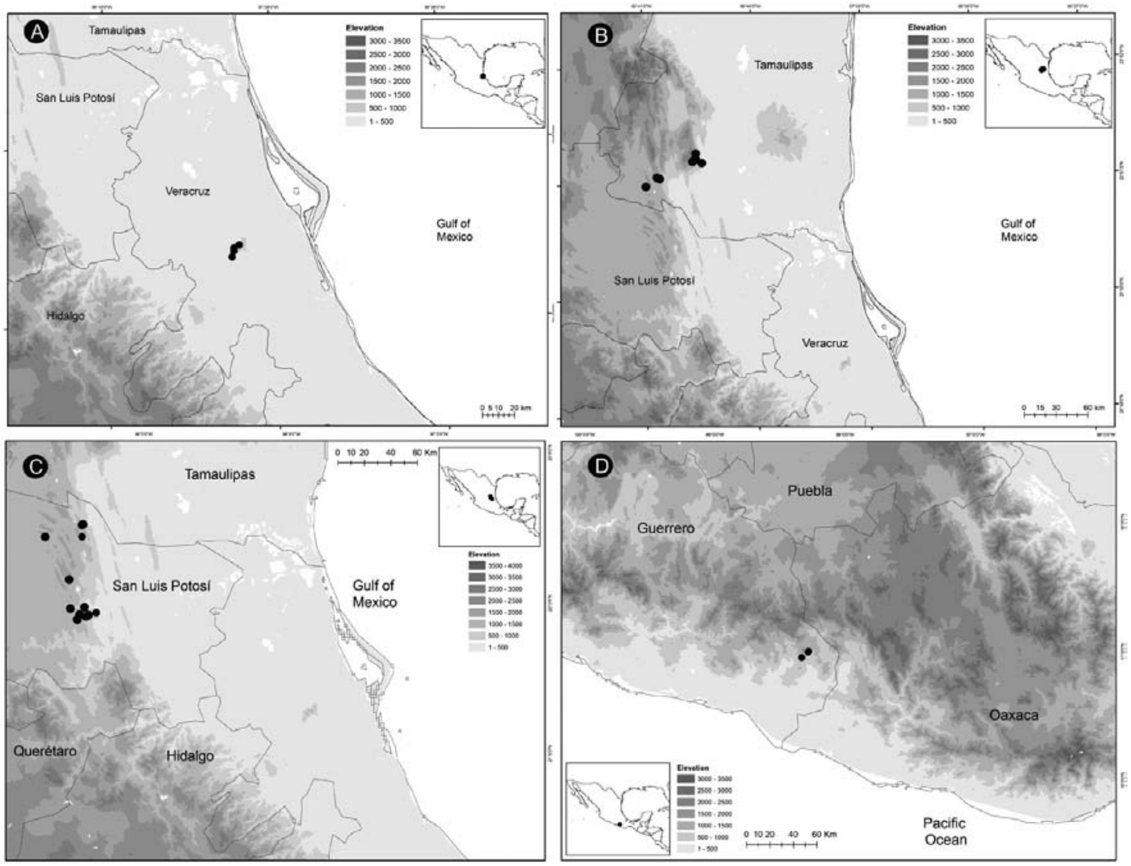

Figure 19. Distribution of *Ceratozamia* species **A** *C. huastecorum* **B** *C. kuesteriana* **C** *C. latifolia* **D** *C. leptoceras.*

Etymology. The specific epithet commemorates the Huasteca region, where this species is found; this region has cultural importance in Veracruz, Mexico.

Common names. Mexico. Veracruz: Palmilla (Bonta et al. 2019).

Uses. None recorded.

Conservation status. (IUCN 2021). *Ceratozamia huastecorum* is currently listed as "Critically Endangered" under criteria B2ab(iii,v).

Discussion. *Ceratozamia huastecorum* has a close morphological similarity to *C. latifolia*. However, *C. huastecorum* has light-green emergent leaves and coriaceous leaflets, whereas in *C. latifolia* the leaflets are reddish brown at emergence and papyraceous. In addition, the ovulate strobili are larger than *C. latifolia*, with 9 to 10 orthostichies as compared to *C. latifolia* with 4–7 orthostichies.

Specimens examined. Mexico. **Veracruz: Mun. Tepetzintla**, 1,250 m, 26 Aug 1981, *G. Castillo-Campos & A. Benavides M. 2215* (XAL); 1,200 m, 27 Aug 1981, *G. Castillo-Campos & A. Benavides M. 2232, 2244* (XAL); 820 m, 14 Dec 1981, *G. Castillo-Campos 2480, 2481, 2482* (XAL); 850 m, 10 Jan 1986, *G. Castillo-Campos 4472* (XAL); 20 Sep 1989, *P. Zamora C. et al. 1197* (CH, MEXU, XAL); 21 Sep 1989, *P. Zamora et al. 1249* (MEXU, XAL).

14. *Ceratozamia kuesteriana* Regel, Bull. Soc. Imp. Naturalistes Moscou 30: 187. t. 3. 1857

Figs 3D, 7A, 9B

Type. Cultivated in St. Petersburg, Russia "Ex Horto Petropolitano", 1856, *E. Regel s.n.* (holotype: LE! [LE00009046]; isotype: U! [U0007272]).

Description. ***Stem*** 10–30 cm long, 10–25 cm in diameter, semi-hypogeous, erect. ***Cataphylls*** 1.5–4 × 2–3.5 cm wide at the base, persistent, triangular, reddish brown, densely brown tomentose at emergence, partially tomentose at maturity, apex acuminate. ***Leaves*** 1–11, 80–133 cm long, ascending, reddish brown at emergence, with whitish gray trichomes, glabrous at maturity. ***Petiole*** 30–72 cm long, terete, linear, greenish brown in mature leaves; with 1–10 thin prickles, 0.01–0.15 cm long. ***Rachis*** 40–72 cm long, terete, linear, greenish brown in mature leaves, with prickles and rarely unarmed. ***Leaflets*** 22–50 pairs, opposite to subopposite, insertion in one plane, linear, adaxially curved, basally falcate, papyraceous, caniculate, green, adaxial and abaxial sides glabrous, distal end with entire margins, acuminate and symmetrical at the apex, attenuate at base, with conspicuous and light veins; median leaflets 17–32 × 0.6–1 cm, 0.3–2.5 cm between leaflets; articulations 0.2–0.8 cm wide, brown. ***Pollen strobili*** 11–15 cm long, 1.6–2.5 cm in diameter, solitary, cylindrical, erect, greenish yellow with brown trichomes at emergence, yellowish brown with reddish brown trichomes at maturity; peduncle 8–14 cm long, 0.7–1 cm in diameter, reddish brown to brown pubescent; microsporophylls 0.6–1.1 × 0.5–1 cm, discoid with a non-recurved distal face and a lobate fertile portion, infertile portion 0.28–0.32 cm long and linear with straight horns 0.05–0.25 cm long, 0.45–0.53 cm and an acute angle between the horns. ***Ovulate strobili*** 13–21 cm long, 7–9 cm in diameter, solitary, cylindrical, erect, green at emergence with brown trichomes, grayish light green with black trichomes at maturity, acuminate apex; peduncle 9–16 cm long, 1–2 cm in diameter, erect, tomentose, brown; megasporophylls 30–72, 6–8 orthostichies with 5–9 sporophylls per orthostichy, 2.5–4.0 × 2.3–3.5 cm, with a truncate distal face, horns straight and 0.30–0.52 cm long, 0.60–0.94 cm between horns with an obtuse angle between the horns. ***Seeds*** 1.5–2.3 cm long, 1.2–1.8 cm in diameter, ovate, sarcotesta whitish red when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia kuesteriana* is endemic to Tamaulipas State, Mexico (Fig. 19B), where it occurs on karstic rocks in pine-oak and cloud forests at 1,100–1,500 m.

Etymology. The specific epithet is in honor of Baron K. von Kuester.

Common names. None recorded.

Uses. None recorded.

Conservation status. (IUCN 2021). The status for *Ceratozamia kuesteriana* "Critically Endangered" under criteria A2cd; B2ab(ii,iii,iv).

Discussion. *Ceratozamia kuesteriana* is similar to *C. sabatoi*, but it is easily distinguished by its canaliculate leaflets and ascending leaves.

Specimens examined: Mexico. **Tamaulipas: Mun. Gómez Farías**, 1,260 m, 1 May 1967, *A. Gómez-Pompa 2029* (MEXU); 1,150 m, 12 Aug 1983, *A.P. Vovides 791, 800, 2056* (XAL); 13 Aug 1983, *A.P. Vovides & G. Pattison 771, 772, 801* (XAL); 27 Jun 2017, *C.I. Carvajal-Hernández et al. 1355* (CIB); 1,200–1400 m, 20 Nov 1970, *F. González-Medrano & E. Martínez 3288* (MEXU); 1,100 m, 21 Jan 1970, *F. González-Medrano et al. 3362* (MEXU, MO); 13 Aug 1985, *L. Trejo s/n* (UAT); 1,050 m, 30 Mar 1961, *P.S. Martin & C. Saravia 1170* (ENCB). **Mun. Ocampo**, 1,255 m, 14 Jan 2001, *T.W. Walters TW-201-11-A* (XAL), *TW-201-11-B* (MEXU). **Mun. Tula**, 1,450 m, 21 Jul 1983, *D.W. Stevenson 569H, 569G, 569K* (MEXU); 1,296 m, 19 Mar 2016, *F. Nicolalde-Morejón et al. 2357–2365* (CIB); 1,296 m, 19 Mar 2016, *L. Martínez-Domínguez et al. 854–867, 869* (CIB), *868* (CIB, MEXU); 1,140 m, 19 Mar 2016, *L. Martínez-Domínguez et al. 870–881* (CIB); 24 Apr 2001, *S. Avendaño 5328* (MEXU).

15. *Ceratozamia latifolia* Miq., Tijdschr. Wis-Natuurk. Wetensch. Eerste Kl. Kon. Ned. Inst. Wetensch. 1(4): 206. 1848

Figs 7B, 10A

Ceratozamia mexicana Brongn. var. *latifolia* (Miq.) J.Schust., Pflanzenr. 99: 131. 1932. Type: Based on *Ceratozamia latifolia* Miq.

Ceratozamia microstrobila Vovides & Rees, Madroño 30: 39. 1983. Type: Mexico. San Luis Potosí: Mun. Ciudad del Maíz, Ejido Las Abritas, km 47 Ciudad Mante-Ciudad del Maíz, 850 m, 7 Nov 1974, *J. Rees 1613* (holotype: XAL! [XAL0099666]; isotypes: FCME! [acc. # 132849], IBUG! [acc. # 155413], MO! [acc. # 5715707], XAL [n.v.]).

Type. Mexico. San Luis Potosí: Route 70, 46 km West of Ciudad Valles, 650 m, 20 Jul 1983, *D.E. Stevenson 565E* (neotype, designated by Stevenson and Sabato 1986, pg. 579: NY! [00001117]; isoneotypes: MEXU! [MEXU00469173, MEXU00469148]).

Description. ***Stem*** 5–15 cm long, 10–25 cm in diameter, semi-hypogeous, erect. ***Cataphylls*** 1.5–3 × 2–4 cm wide at the base, persistent, triangular, reddish brown, densely brown tomentose at emergence, partially tomentose at maturity, apex acuminate. ***Leaves*** 1–8, 53–163.5 cm long, descending, reddish brown at emergence, with whitish gray trichomes, glabrous at maturity. ***Petiole*** 25–80 cm long, terete, linear, greenish brown in mature leaves, generally unarmed, rarely up to 3 prickles, 0.08–0.10 cm long. ***Rachis*** 25–110 cm long, terete, linear, greenish brown in mature leaves, unarmed. ***Leaflets*** 7–22 pairs, opposite to subopposite, insertion in one plane, oblong, in general longitudinally planar, basally falcate, papyraceous, flat, green with adaxial and abaxial sides glabrous, distal end with entire margins, acuminate and asymmetrical at the apex, attenuate at base, with conspicuous and indistinct veins; median leaflets 12–28 × 2.3–5.1 cm, 1.7–12.5 cm between leaflets; articulations 0.4–1.1 cm wide, brown. ***Pollen strobili*** 10–20 cm long, 2.1–2.5 cm in diameter, solitary, cylindrical, erect, greenish yellow with reddish brown trichomes at emergence, reddish brown at maturity; peduncle 5–15 cm long, 0.79–1.5 cm in diameter, reddish brown to brown pubescent; microsporophylls 0.5–1.3 × 0.5–1.1 cm, discoid with a non-recurved distal face and a lobate fertile portion, infertile portion 0.20–0.30 cm long and rounded with straight horns 0.1–0.20 cm long, 0.40–0.55 cm and a right angle between the horns. ***Ovulate strobili*** 6.5–16 cm long, 5.5–8.0 cm in diameter, solitary, globose, erect, light green at emergence with brown trichomes, greyish light brown with brown trichomes at maturity, apiculate apex; peduncle 4–13.5 cm long, 1.3–1.8 cm in diameter, erect, brown pubescent; megasporophylls 24–63, 5–7 orthostichies with 4–9 sporophylls per orthostichy, 1.0–2.7 × 1.6–2.9 cm, with a prominent distal face, horns straight and 0.36–0.76 cm long, 0.54–0.63 cm between horns with an obtuse angle between the horns. ***Seeds*** 1.5–2.2 cm long, 1.2–1.6 cm in diameter, ovate, sarcotesta whitish red when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia latifolia* is distributed widely in the mountainous region of San Luis Potosí State, Mexico (Fig. 19C), where it occurs in pine-oak, oak and cloud forests between 600–1,100 m.

Etymology. The specific epithet is derived from the Latin word "latus" (wide) and "folium" (leaves).

Common names. Mexico. San Luis Potosí: Chamalillo, corosillo (S. Sabato et al. 2340); Konlif in the teenek/huasteco language (Bonta et al. 2019).

Uses. The seeds are used to obtain meal for tortillas (Bonta et al. 2019).

Conservation status. (IUCN 2021). The status listed for *Ceratozamia latifolia* is "Endangered" under criteria A2cd+4cd. However, the current circumscription and distribution data indicate that status should be changed to "Vulnerable (V)".

Discussion. *Ceratozamia latifolia* differs from *C. chamberlainii* by its unarmed petiole or if armed with no more than 3 prickles, its papyraceous leaflets, and discoid microsporophylls with a rounded distal face and horns up to 0.20 cm long. Additionally, this species is characterized by reddish brown leaves at emergence and ovulate strobilus is greyish light brown with brown trichomes at maturity and an apiculate apex.

Specimens examined. Mexico. **San Luis Potosí**: Cultivated, 24 Jan 1994, *F. García S. s/n* (SLPM); Dec 1977, *N.F. McCarten 2552* (ENCB). **Mun. Alaquines**, 1,428 m, 21 Dec 2008, *J. Fortanelli M. & H.A. Castillo 141* (SLPM). **Mun. Cárdenas**, 950 m, 12 to 15 Sep 1967, *J. Rzedowski 24746* (ENCB). **Mun. Ciudad del Maíz**, 750 m, 13 Oct 1968, *H. Puig 3420* (ENCB, P); 25 Apr 2001, *S. Avendaño 5320* (MEXU); 900–1,300 m, 5 Feb 1984, *S. Sabato et al. 2340* (ENCB, MEXU, MO). **Mun. El Naranjo**, 7 Nov 1951, *Clint 144* (US); 20 Jul 1997, *D.W. Stevenson 567* (XAL); 900 m, 19 Mar 2016, *F. Nicolalde-Morejón et al. 2375–2389* (CIB); 900 m, 19 Mar 2016, *L. Martínez-Domínguez et al. 894–909* (CIB); 895 m, 13 Jan 2001, *T.W. Walters TW-2001-08*; 895 M, 14 Jan 2001, *TW-2001-10A,B* (MEXU, XAL). **Mun. Rayón**, 812 m, 5 Aug 2003, *A.P. Vovides et al. 1466* (XAL); 20 Jul 1983, *D.W. Stevenson 1117* (NY); 650 m, 20 Jul 1983, *D.W. Stevenson 565A* (MEXU), *565B,C* (MEXU, XAL); 993 m, 18. Mar 2016, *F. Nicolalde-Morejón et al. 2320–2334* (CIB); 1,100 m, 30 Jun 1962, *F. Medellin L. 1330* (ENCB, MEXU, SLPM); 1,221 m, 6 Apr 2013, *H.A. Castillo-Gómez et al. 558* (SLPM); 600 m, 17 Jul 1988, *J.A. Pérez de la Rosa & L.M. González-Villarreal 1564* (IBUG); 993 m, 18 Mar 2016, *L. Martínez-Domínguez et al. 811–827* (CIB); 24 Apr 2001, *S. Avendaño 5282* (MEXU). **Mun. Tamasopo**, 700 m, 15 Aug 2003, *A.P. Vovides et al. 1465* (MEXU, XAL); 2 Jun 1968, *F. Medellin L. 27241* (ENCB); 2 Jun 1968, *F. Medellin L. s/n* (ENCB, IBUG, MEXU, SLPM); 716 m, 18. Mar 2016, *F. Nicolalde-Morejón et al. 2335–2348* (CIB); 716 m, 18 Mar 2016, *L. Martínez-Domínguez et al. 828–844* (CIB); 750 m, 11 Sep 1967, *J. Rzedowski 24571* (ENCB); 900 m, 24 May 1981, *P.A. Fryxell & W.R. Anderson 3586* (NY; US); 856 m, 19 Jan 2013, *U. Pineda M. 70* (SLPM).

16. *Ceratozamia leptoceras* Mart.-Domínguez, Nic.-Mor., D.W.Stev. & Lorea-Hern., PhytoKeys 156: 13. 2020

Fig. 7C

Type. Mexico. Guerrero: Mun. Tlacoachistlahuaca, 3 km NW de San Pedro Cuitlapan, 1,400 m, 26 Jun 2019, *L. Martínez-Domínguez & F. Nicolalde-Morejón 1867* ♀ (holotype: CIB! [acc. # 22405UV]; isotypes: MEXU!, NY!).

Description. ***Stem*** 30–150 cm long, 11–35 cm in diameter, epigeous, erect to decumbent. ***Cataphylls*** 9–11 × 2.5–3 cm wide at the base, persistent, triangular, reddish brown, densely brown tomentose abaxially at emergence, pubescent at maturity, apex acuminate. ***Leaves*** 7–37 (55), 93.5–281 cm long, descending, green at emergence with sparse reddish brown pubescent, glabrous at maturity. ***Petiole*** 45–85 cm long, terete, linear, copperish green in mature leaves; with 50–75 thin prickles, 0.48–0.68 cm long. ***Rachis*** 75–196 cm long, terete, linear, green in mature leaves, with prickles. ***Leaflets*** 22–61 pairs, opposite to subopposite, insertion in one plane, linear, abaxially curved, not basally falcate, membranaceous, flat, green with adaxial and abaxial sides glabrous, distal end with entire margins, acuminate and symmetrical at the apex, attenuate at base, with conspicuous and greenish veins; median leaflets 28–43.5 × 1.9–2.8 cm, 1.8–2.8 cm between leaflets; articulations 0.70–1.15 cm, generally copperish green. ***Pollen strobili*** 40–45 cm long 6.0–7.8 cm in diameter, generally solitary (rarely 2), cylindrical, erect, brownish yellow at emergence, yellowish green with brownish trichomes at maturity; peduncle 13–19 cm long, 1.5–2.0 cm in diameter tomentose, reddish brown to brown; microsporophylls 2.1–2.45 × 1.09–1.30 cm, obconic with a non-recurved distal face and a deeply lobate fertile portion, infertile portion 0.83–0.96 cm long and linear with straight and thin horns and 0.1–0.23 cm long, 0.44–0.56 cm and an acute angle between the horns. ***Ovulate strobili*** 23.5–28 cm long, 9.5–11 cm in diameter, solitary, cylindrical, erect, brownish green with greyish black trichomes at emergence, copperish green with greyish black pubescent at maturity with an acute apex; peduncle 11–16 cm long, 1.5–2.0 cm in diameter, erect, tomentose, brown; megasporophylls 56–81, 8–9 orthostichies with 7–9 sporophylls per orthostichy, 4.9–5.6 × 2.2–2.6 cm, with a prominent distal face, horns straight and robust and 0.63–0.81 cm long, 0.95–1.35 cm between horns and angle straight between the horns. ***Seeds*** 2.43–2.71 cm in long, 1.4–1.8 cm in diameter, ovoid, sarcotesta whitish pink when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia leptoceras* is endemic to the Sierra Madre del Sur in Guerrero State, Mexico (Fig. 19D), where it occurs on karstic rocks in cloud forest at 1,170–1,400 m.

Etymology. The specific epithet is derived from the Greek words that describe the shape of the horns on the sporophylls: "lepto" for thin or fine and "ceras" in reference to the horns.

Common names. Mexico. Guerrero: Shalukaá is the name used by the "Mixteco" ethnic group (Martínez-Domínguez et al. 2020).

Uses. None recorded.

Preliminary conservation status. Only three populations of *Ceratozamia leptoceras* are known. In particular, one of these populations has few adult plants (approximately 30 individuals). The cloud forests in this area are less affected by anthropogenic pressures; however, this vegetation type is one of the most threatened in Mexico (Williams-Linera 2002). Based upon this information, *C. leptoceras* should be considered "Endangered (EN)" in accordance with IUCN criteria.

Discussion. *Ceratozamia leptoceras* is distinguished from *C. oliversacksii* by its linear and membranaceous leaflets. The main differences are in reproductive structures, *C. leptoceras* has and linear infertile portion of microsporophylls and ovulate strobilus with abundant pubescence at the base of the megasporophylls.

Specimens examined. Mexico. **Guerrero: Mun. Cochoapa El Grande**, 1,170 m, 4 Feb 1984, *F. Lorea-Hernández 2928* (FCME). **Mun. Tlacoachistlahuaca**, 1,200 m, 29 May 219, *F. Nicolalde-Morejón et al. 3173* (XAL), *3174* (FCME), *3175* (CIB); 1,200 m, 29 May 2019, *L. Martínez-Domínguez et al. 1756* (CIB), *1757* (CIB, MEXU), *1758* (XAL), *1759* (CIB, MEXU); 1,400 m, 26 Jun 2019, *F. Nicolalde-Morejón & L. Martínez-Domínguez 3255–3261* (CIB); 1,400 m, 26 Jun 2019, *L. Martínez-Domínguez & F. Nicolalde-Morejón 1860, 1861* (MEXU), *1862–1866* (CIB).

17. *Ceratozamia matudae* Lundell, Lloydia 2: 75. 1939

Figs 7D, 8D–F, 10D

Type. Mexico. Chiapas: northern slope of Mt. Ovando, 1,000 m, Feb 1939, *E. Matuda 2645* ♂ (holotype: MICH! [1002583]; isotypes: CAS! [0001920], MEXU! [acc. # 86830], US! [00620111]).

Description. ***Stem*** 30–50 cm long, 20–30 cm in diameter, epigeous, erect and decumbent. ***Cataphylls*** 4–6.0 × 2.5–4.5 cm wide at the base, persistent, triangular, reddish brown, densely brown tomentose at emergence, partially tomentose at maturity, apex acuminate. ***Leaves*** 3–14, 80–155 cm long, descending, green at emergence with brown trichomes, glabrous at maturity. ***Petiole*** 30–79 cm long, terete, linear, green in adult leaves; unarmed to armed with 18–36 thin prickles, 0.1–0.28 cm long. ***Rachis*** 45–76 cm long, terete, linear, green with abundant brown trichomes in young leaves, yellow to yellowish green in mature leaves, unarmed to armed with prickles. ***Leaflets*** 23–53 pairs, opposite to subopposite, insertion in one plane, lanceolate, longitudinally curved abaxially to planar, not basally falcate, papyraceous, flat, green with base yellow and adaxial and abaxial sides glabrous, distal end with entire margins, acuminate and symmetrical at the apex, attenuate at base, with conspicuous and light-green veins; median leaflets 23–40 × 0.6–1.3 cm, 0.6–1.7 cm between leaflets; articulations 0.3–0.8 cm wide, yellow. ***Pollen strobili*** 9.5–16 cm long, 3.5–5 cm in diameter, solitary, cylindrical, erect, yellowish green with reddish brown trichomes at emergence, yellowish cream with reddish brown trichomes at maturity; peduncle 8–14 cm long, 1.8–2.2 cm in diameter, pubescent, reddish brown to brown; microsporophylls 0.78–1.4 × 0.77–1.1 cm, discoid with a non-recurved distal face and deeply lobate fertile portion, infertile portion 0.37–0.56 cm long and orbicular with straight horns 0.16–0.35 cm long, 0.50–0.81 cm and an acute angle between the horns. ***Ovulate strobili*** 8–15 cm long, 5–8 cm in diameter, solitary, globose, erect, yellowish green with abundant blackish trichomes at emergence, dark green with blackish trichomes at maturity, aristate apex; peduncle 11–17 cm long, 1–2 cm in diameter, pendulous, with trichomes scarce, blackish, pendulous and erect; megasporophylls 16–25, 4–5 orthostichies with 4–6 sporophylls per orthostichy, 2.5–3.4 × 4.5–5.3 cm, with a prominent distal face, horns recurved, straight and thin and 0.59–1.50 cm long, 0.92–1.56 cm between horns with an obtuse angle between the horns. ***Seeds*** 2.9–4.0 cm long, 1.9–4.0 cm in diameter, globose, sarcotesta whitish yellow to yellow when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia matudae* is endemic to Chiapas in Mexico (Fig. 20A), where it occurs on karstic rocks in evergreen tropical forest at 1,000–1,500 m.

Etymology. The specific epithet is in honor of professor Eizi Matuda, for his contributions to botany in Mexico.

Common names. None recorded.

Uses. None recorded.

Conservation status. (IUCN 2021). Currently, *Ceratozamia matudae* is listed as "Endangered" under criteria B1ab(ii,iii,v); C1.

Discussion. *Ceratozamia matudae* differs from other *Ceratozamia* species by its yellow rachis and petiole and leaflets with yellow base and articulations. In addition, the length of the peduncle is the same or longer than the fertile portion of ovulate strobili. The ovulate strobili have an aristate apex, and the megasporophylls have long recurved horns.

Specimens examined. Mexico. **Chiapas: Mun. Acacoyagua**, 1,400 m, *A.P. Vovides 1069* (XAL); 1,300 m, 9 Jan 1987, *D.W. Stevenson et al. 681* (FTG; MO; NY; US); 1,000 m, Feb 1939, *E. Matuda 2646* (MEXU); 1,420 m, 24 Jun 2018, *F. Nicolalde-Morejón et al. 2832–2840* (CIB); 1,420 m, 24 Jun 2018, *L. Martínez-Domínguez et al. 1404–1409, 1412, 1415, 1416, 1418, 1419* (CIB), *1405, 1407* (CIB, MEXU); 1,480 m, 29 Dec 1993, *M.A. Pérez-Farrera 27* (CIB, CH, CHIP, MEXU, USCG); 22 Feb 1995, *M.A. Pérez-Farrera 142* (CHIP, FTG, MEXU). **Mun. Escuintla**, 8 Feb 2000, *O. Farrera S. 1875* (CHIP). **Mun. Siltepec**, 1,500 m, 6 Nov 1945, *E.H. Xolocotzi & A.J. Sharp 367* (MEXU); 1,500 m, Oct-Nov 1940, *E. Matuda 4032* (MEXU; NY).

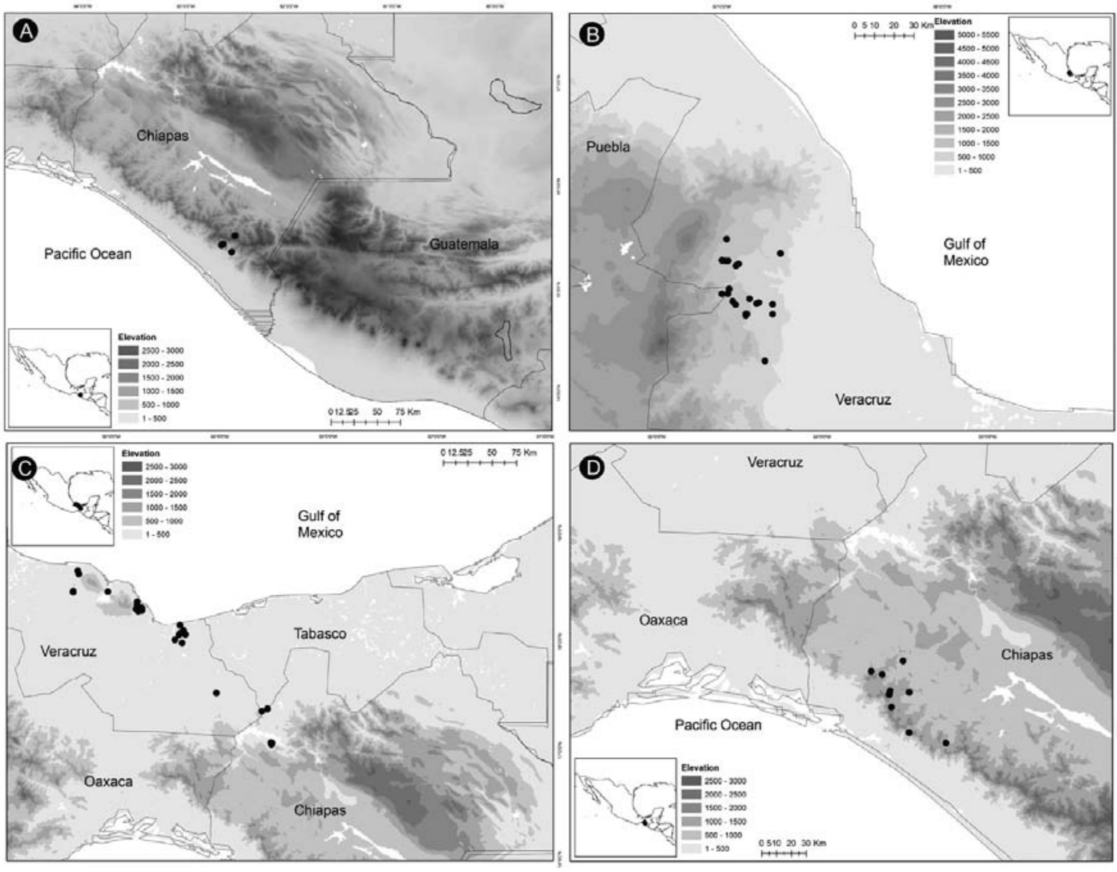

Figure 20. Distribution of *Ceratozamia* species **A** *C. matudae* **B** *C. mexicana* **C** *C. miqueliana* **D** *C. mirandae.*

18. ***Ceratozamia mexicana*** Brongn., Ann. Sci. Nat., Bot. sér. 3, 5: 8, t. 1. 1846.

Figs 7E, 11B

Ceratozamia intermedia Miq., Tijdschr. Wis-Natuurk. Wetensch. Eerste Kl. Kon. Ned. Inst. Wetensch. 1(4): 40. 1848. Type. Mexico. Veracruz: Mun. Puente Nacional, 1 km S of Palmillas, 600 m, 13 Mar 1985, *G. Castillo-Campo & Medina 4275* (neotype, designated by Martínez-Domínguez et al. 2018a, pg. 111: XAL! [acc. # 16924]).

Ceratozamia longifolia Miq., Tijdschr. Wis-Natuurk. Wetensch. Eerste Kl. Kon. Ned. Inst. Wetensch. 1(4): 40. 1848. Type. Mexico. Veracruz: Mun. Zacuapam, Apr 1913, *C. Purpus s.n.* [6362]! (neotype, designated by Martínez-Domínguez et al. 2018a, pg. 111: MO! [acc. # 741393]).

Ceratozamia longifolia Miq. var. *minor* Miq., Tijdschr. Wis-Natuurk. Wetensch. Eerste Kl. Kon. Ned. Inst. Wetensch. 2(4): 290. 1849. Type. Mexico. Veracruz: Mun. Teocelo, Barranca of Teocelo, 1,020 m, 23 Dec 1975, *M.G. Zola 146* (neotype, designated by Martínez-Domínguez et al. 2018a, pg. 111: XAL! [acc. # 16957]).

Ceratozamia mexicana Brongn. var. *longifolia* (Miq.) Dyer, Biol. Cent.-Amer., Bot. 3: 193. 1884. Type: Based on *Ceratozamia longifolia* Miq.

Type. Cultivated in Paris "Hort. Bot. Parisiensis", original material sent by A. Ghiesbrecht from Mexico, 1845, *Anon. s.n.* (holotype: P! [P02441737, P01637464–P01637466]). Mexico. Veracruz: Mun. Totutla, Barranca El Coyotito, 1 km road from El Mirador to Conejos, 900 m, 8 Sep 1982, *J. Rees & A.P. Vovides 1688* (epitype, designated by Vovides et al. 2016, pg. 427: XAL! [acc. # 17004]).

Description. ***Stem*** 20–80 cm long, 20–45 cm in diameter, epigeous, erect and decumbent. ***Cataphylls*** 2–6 × 2.5–6 cm wide at the base, persistent, triangular, reddish brown, densely brown tomentose at emergence, partially tomentose at maturity, apex acuminate. ***Leaves*** 5–55, 100–270 cm long, descending, green at emergence, with brown trichomes, glabrous at maturity. ***Petiole*** 30–93 cm long, terete, linear, dark green in mature leaves; with 8–30 thin prickles, 0.06–0.20 cm long. ***Rachis*** 56–154 cm long, terete, linear, green in mature leaves, with prickles and unarmed. ***Leaflets*** 25–42 pairs, opposite to subopposite, insertion in one plane, lanceolate, in general longitudinally planar, basally falcate, coriaceous, flat, green with adaxial and abaxial sides glabrous, distal end with entire margins, acuminate and symmetrical at the apex, attenuate at base, with conspicuous and green-light veins; median leaflets 29–51 × 2.3–3.7 cm, 1.8–4 cm between leaflets; articulations 0.6–1.5 cm wide, green. ***Pollen strobili*** 20–40 cm long, 4.5–7.6 cm in diameter, generally solitary (1–2), cylindrical, erect, greenish yellow at emergence, greenish yellow with blackish trichomes at maturity; peduncle 3.5–9 cm long, 1.5–2.3 cm in diameter, tomentose, reddish brown to light-brown; microsporophylls 1.5–2.7 × 0.9–2.1 cm, obconic with a non-recurved distal face and a lobate fertile portion, infertile portion 0.47–0.68 cm long and orbicular with straight horns 0.29–0.40 cm long, 0.51–1.05 cm and an acute angle between the horns. ***Ovulate strobili*** 23.5–40 cm long, 8–15 cm in diameter, generally solitary (1–2), cylindrical, erect, dark green with blackish trichomes at emergence, dark green with black and gray trichomes at maturity, acuminate apex; peduncle 7.6–11.5 cm long, 2.5–3 cm in diameter, tomentose, brown to reddish brown, erect or pendulous; megasporophylls 64–240, 8–16 orthostichies with 8–15 sporophylls per orthostichy, 2.0–2.7 × 3.2–5.3 cm, with a prominent distal face, horns straight and 0.53–1.2 cm long, 1–2.1 cm between horns and a right angle between the horns. ***Seeds*** 2.0–3.3 cm long, 1.4–2.5 cm in diameter, ovate, sarcotesta whitish yellow to yellow when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia mexicana* is endemic to Veracruz State, Mexico from the River La Antigua drainage system to the southern end of the Sierra Madre Oriental (Fig. 20B). It occurs in cloud forest on karstic rocks at 500–1,100 m.

Etymology. The specific epithet is derived from the country of origin of the material used for the description of this species. *Ceratozamia mexicana* was the first species described in the genus.

Common names. None recorded.

Uses. None recorded.

Conservation status. (IUCN 2021). *Ceratozamia mexicana* is listed as "Vulnerable" under criteria A2acd+4cd. According to the current circumscription, this species only occurs in a narrow area, which suffered dramatic decline in forest (Martínez-Domínguez et al. 2021). Besides, the populations visited by us have only between 30 and 50 adult plants. This data suggest *C. mexicana* should be listed as "Critically Endangered (CR)" under criteria B1ab(iiii, iv)

Discussion. *Ceratozamia mexicana* is similar to *C. tenuis*, *C. morettii* and *C. brevifrons*, but it can be easily distinguished from vegetative and reproductive chracters. This species has lanceolate leaflets, whereas *C. tenuis* and *C. morettii* have linear and oblong leaflets, respectively. In addition, *C. mexicana* has insertion in one plane for leaflets and thin prickles on petiole, whereas *C. brevifrons* has keeled leaflets and petioles armed with robust prickles.

Specimens examined. Mexico. **Veracruz: Mun. Coatepec**, 1,600 m, 26 Jun 1990, *P. Zamora C. 2450* (MEXU, XAL). **Mun. Comapa**, 1,003 m, 18 Feb 2013, *F. Nicolalde-Morejón et al. 1732–1734* (CIB); 1,003 m, 28 Sep 2014, *F. Nicolalde-Morejón et al. 2145–2156* (CIB); 970 m, 13 Nov 2015, *F. Nicolalde-Morejón et al. 2269–2272* (CIB); 1,003 m, 28 Sep 2014, *L. Martínez-Domínguez et al. 164* (CIB); 970 m, 13 Nov 2015, *L. Martínez-Domínguez & F. Nicolalde-Morejón 716–723, 725–730* (CIB), *724* (CIB, MEXU). **Mun. Puente Nacional**, 600 m, 13 Mar 1985, *G. Castillo-Campos & M. E. Medina 4299* (XAL). **Mun. Sochiapa**, 1,058 m, 9 Jul 2008, *M. Vázquez-Torres 8589* (CIB). **Mun. Teocelo**, 900 m, 16 Feb 1997, *L.H. Bojórquez-Galván et al. 531* (CIB); 1,070 m, 20 Nov 2015, *F. Nicolalde-Morejón et al. 2273–2278* (CIB); 1,065 m, 20 Nov 2015, *L. Martínez-Domínguez et al. 731* (CIB); 1,070 m, 20 Nov 2015, *L. Martínez-Domínguez et al. 732–733, 735–750* (CIB), *734* (CIB, MEXU); 1,070 m, 11 Mar 2016, *L. Martínez-Domínguez et al. 764*, 765, 767*–770* (CIB), *766* (CIB, MEXU); 740–800 m, 20 Jul 1995, *M. Vazquez-Torres* 4865 (CIB). **Mun. Tlaltetela**, 1,196 m, 5 Jun 2015, *F. Nicolalde-Morejón & L. Martínez-Domínguez 2242* (CIB); 1,084 m, 5 Jun 2015, *F. Nicolalde-Morejón & L. Martínez-Domínguez 2243* (CIB); 1,196 m, 10 Jun 2015, *F. Nicolalde-Morejón & L. Martínez-Domínguez 2246* (CIB)*;* 1,130 m, 21 Jun 2015, *F. Nicolalde-Morejón & L. Martínez-Domínguez 2253–2257* (CIB); 1,084 m, 5 Jun 2015, *L. Martínez-Domínguez & F. Nicolalde-Morejón 584–586* (CIB); 1,196 m, 10 Jun 2015, *L. Martínez-Domínguez & F. Nicolalde-Morejón 620* (CIB); 1,130 m, 21 Jun 2015, *L. Martínez-Domínguez & F. Nicolalde-Morejón 628–635* (CIB). **Mun. Totutla**, 900 m, 23 Feb 1982, *A.P. Vovides 730–733* (XAL); 900 m, 8 Sep 1982, *A.P. Vovides 748* (XAL); 900 m, 9 Oct 1993, *Brigada T. Walters s/n* (XAL); 875 m, 8 Aug 2015, *F. Nicolalde-Morejón et al. 2262* (CIB); 864 m, 27 Jan 2016, *F. Nicolalde-Morejón et al. 2279–2281* (CIB); *F. Vázquez B. 730* (XAL); 1,094 m, 21 Jun 2016, *J.M. Ramírez-Amezcua & A. Paizanni Guillén 715* (MEXU); 8 Sep 1982, *J. Rees 6344* (IEB); 800 m, 30 Dec 1975, *J. Rees & A.P. Vovides 1660, 1672* (XAL); 900 m, 8 Sep 1982, *J. Rees & A.P. Vovides 1689* (XAL); 875 m, 8 Aug 2015, *L. Martínez-Domínguez et al. 704–707* (CIB); 864 m, 27 Jan 2016, *L. Martínez-Domínguez et al. 752, 754–757* (CIB, MEXU), *758* (CIB). **Mun. Xico**, 1,195 m, 10 Jul 2015, *L. Martínez-Domínguez & F. Nicolalde-Morejón 640–646* (CIB); 1,159 m, 10 Jul 2015, *L. Martínez-Domínguez & F. Nicolalde-Morejón 647, 648* (CIB); 900 m, 13 Nov 1981, *M. Nee 23035* (NY).

19. *Ceratozamia miqueliana* H.Wendl., Index Palm. 68. 1854

Figs 7F, 8A–C, 9D, 11A

Ceratozamia mexicana Brongn. var. *miqueliana* (H.Wendl.) J. Schust., Pflanzenr. (Engler) Heft 99, 4 fam 1: 131. 1932. Type: Based on *Ceratozamia miqueliana* H. Wendl.

Type. Mexico. Veracruz: West of Santiago Tuxtla, Cerro el Vigía, 5 Jul 1983, *D.W. Stevenson 542 F* (neotype, designated by Stevenson and Sabato 1986, pg. 580: NY! [00001118]).

Description. ***Stem*** 45–75 cm long, 16–18 cm in diameter, epigeous, erect and decumbent. ***Cataphylls*** 3–7 × 1.5–4 cm wide at the base, persistent, triangular, reddish brown, densely brown tomentose at emergence, glabrous at maturity, apex acuminate. ***Leaves*** 12–37, 35–261 cm long, ascending, light green and glaucous at emergence with brown trichomes, glabrous at maturity. ***Petiole*** 31–97 cm long, terete, linear, pink at emergence, green in mature leaves; with 8–60 robust prickles, 0.22–0.59 cm long. ***Rachis*** 41–164 cm long, terete, linear, pink at emergence, yellowish green in mature leaves, with prickles. ***Leaflets*** 12–23 pairs, opposite to subopposite, insertion in one plane, oblong, in general longitudinally planar, not basally falcate, papyraceous, flat, green with abaxial and adaxial sides glabrous, acuminate and asymmetrical at the apex, distal end with entire margins, attenuate at base, with conspicuous and green-light veins; median leaflets 16–36 × 4.4–8.7 cm, 2.9–8.5 cm between leaflets; articulations 0.3–1.8 cm wide, greenish. ***Pollen strobili*** 15–30 cm long, 3.1–4.2 cm in diameter, usually solitary (1–2), cylindrical, erect, greenish yellow with red trichomes at emergence, greenish yellow to cream at maturity; peduncle 3.5–5.0 cm long, 1.9–2.2 cm in diameter, tomentose, light brown; microsporophylls 1–2 × 0.7–1.5 cm, obconical with a non-recurved distal face and deeply lobate fertile portion, infertile portion 0.35–0.50 cm long and rounded with straight horns 0.30–0.42 cm long, 0.52–0.70 cm and an acute angle between the horns. ***Ovulate strobili*** 22–30 cm long, 8.9–12 cm in diameter, solitary, cylindrical, erect, green with blackish trichomes, yellowish green with blackish trichomes at maturity, acuminate apex; peduncle 7–12 cm long, 2.7–3.3 cm in diameter, erect and pendulous, tomentose, light brown; megasporophylls 64–110, 8–11 orthostichies with 8–10 sporophylls per orthostichy, 2.0–3.0 × 3.5–4.2 cm, with a prominent distal face, horns straight and 0.35–0.43 cm long, 1.06–1.12 cm between horns with a right angle between the horns. ***Seeds*** 2.4–3 cm long, 1.3–1.8 cm in diameter, ovate, sarcotesta whitish yellow to yellow when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia miqueliana* is endemic to southwest Mexico in Chiapas, Tabasco and Veracruz States (Fig. 20C), where it occurs on shallow soils in evergreen tropical rain forest at 19–1,000 m.

Etymology. The specific epithet was assigned in honor of Friedrich Anton Wilhelm Miquel.

Common names. Mexico. Veracruz: Palmita (J. Rees 1657).

Uses. The leaves are used for decorations during festivities in Santiago Tuxtla (Veracruz).

Conservation status. (IUCN 2021). *Ceratozamia miqueliana* is listed as "Critically Endangered" under criteria A2acd. This species has a wide distribution; however, the evergreen tropical rain forest in Veracruz has declined dramatically in recent years (Guevara et al. 2004). In addition, most of the populations occur in areas of interest for the oil industry.

Discussion. *Ceratozamia miqueliana* differs from its congeners by its robust and long prickles, and papyraceous, oblong and glabrous (lustrous) leaflets. In addition, ovulate strobili are yellowish yellowish green with blackish trichomes and have an acuminate apex at maturity. This species is closely related to *C. zoquorum*, however, that species has descending leaves with coriaceous leaflets.

Specimens examined. Mexico. **Chiapas: Mun. Ocozocoautla de Espinosa**, 421 m, 23 Oct 1997, *R. Juárez-Galdamez 4* (CHIP, MO, XAL); 800 m, 21 Jan 2001, *S. Avendaño 5223* (MEXU); 765 m, 21 Jan 2001, *T.W. Walters 2001–2036-A* (MEXU); *2001–2036-C* (XAL). **Tabasco: Mun. Huimanguillo**, 405 m, 10 Aug 2013, *F. Nicolalde-Morejón et al. 1826–1846* (CIB); 23 Jun 1998, *G. Ortíz G. 5291* (MEXU). **Veracruz: Mun. Ángel R. Cabada**, 231 m, 29 Oct 2013, *F. Nicolalde-Morejón et al. 1868–1887* (CIB); 30 Oct 2013, *F. Nicolalde-Morejón et al. 1888–1909* (CIB). **Mun. Catemaco**, 31 Oct 2013, *F. Nicolalde-Morejón et al. 1910–1929* (CIB). **Mun. Coatzacoalcos**, 22 m, 2 Apr 2003, *C.H. Ramos 2266* (MEXU); Jun 1960, *E. Hernández X. et al. 162* (CHAPA); 26 Jun 1997, *G. Castillo C. & F. Morocini 16267* (XAL); 30 m, 7 Sep 2005, 30 m, *L.H. Bojórquez-Galván 1601* (CIB); 18 Jan 2001, *S. Avendaño R. 5214* (MEXU); 31 m, 18 Jan 2001, *T.W. Walters 2001–21-A, B* (XAL); *T.W. Walters 2001-21-D* (MEXU). **Mun. Ixhuatlán del Sureste**, 45 m, 13 Sep 2011, *J. Calónico-Soto & B. Gómez C. 27780* (MEXU). **Mun. Las Choapas**, 115 m, 26 Nov 2011, *A. Rincón G. 2894* (XAL); 115 m, 30 Jun 2003, *E. López P. 634* (XAL); 100 m, 25 Nov 2004, *F. Nicolalde-Morejón et al. 1434* (XAL). **Mun. Mecayapan**, 13 Jul 1994, *G. Castillo-Campos et al. 12,565* (ENCB, XAL); 9 Mar 1995, *G. Castillo-Campos et al. 13470, 13489* (XAL); 5 Aug 1985, *J.I. Calzada 11207* (CH, MEXU); 300 m, 16 Jul 1982, *M. Nee et al. 25066* (XAL), *25118* (MO, NY, XAL); 950 m, 26 Jan 1992, *M. Vázquez- Torres 4123* (CIB); 5 May 1995, *M. Vázquez- Torres 5017* (CIB). **Mun. Moloacán**, 60 m, 19 Dec 1974, *J. Rees 1657* (MEXU, XAL), *1658* (XAL), *6347, 6348* (IEB). **Mun. Nanchital**, 34 m, 12 Aug 2011, *J. Rivera H. et al. 4530* (XAL). **Mun. Santiago Tuxtla**, 900–1,000 m, 5 Jul 1983, *D.W. Stevenson et al. 541 A–C– F, I, K* (NY), *G, H, J, N* (MEXU, NY); 5 Jul 1983, *D.W. Stevenson et al. 542 A–E, H* (NY); 650 m, 28 Mar 2014, *F. Nicolalde-Morejón & L. Martínez-Domínguez 1988–1998* (CIB); 950 m, 24 Jan 1972, *J.H. Beaman 5507* (XAL); 650 m, 28 Mar 2014, *L. Martínez-Domínguez & F. Nicolalde-Morejón 88–106* (CIB), *107* (CIB, MEXU); 800 m, 28 May 1967, *L. Scheinvar 673* (MEXU); 29 Aug 1967, *M. Sousa 3201* (MEXU); 845 m, 17 Jan 2001, *S. Avendaño R. 5207* (MEXU); 845 m, 17 Jan 2001, *T.W. Walters et al. TW 2001–2019* (MEXU, XAL). **Mun. Pajapan**, 830–980 m, 15 Jul 1982, *M. Nee et al. 25066* (XAL). **Mun. Soteapan**, 250 m, 13 May 1986, *M. Vázquez-Torres 3360* (CIB, XAL). **Mun. Tatahuicapan de Juárez**, 849 m, *C.I. Carvajal & M.J. Fragoso 797* (CIB); 15 Mar 2008, *L H. Bojórquez-Galván et al. 1939, 1946* (CIB).

20. *Ceratozamia mirandae* Vovides, Pérez-Farr. & Iglesias, Bot. J. Linn. Soc. 137: 81. 2001

Fig. 7G

Type. Mexico. Chiapas: Mun. Villa Flores, Ejido La Sombra de la Selva, 880 m, 20 Sep 1997, *J. De La Cruz Rodríguez 66* (lectotype, designated here: MEXU! [MEXU00934924, MEXU00934907, MEXU00934905]).

Description. ***Stem*** 20–60 cm long, 20–30 cm in diameter, epigeous, erect and decumbent. ***Cataphylls*** 3–8.0 × 1.5–4.0 cm wide at the base, persistent, triangular, reddish brown, densely brown tomentose at emergence, partially tomentose at maturity, apex acuminate. ***Leaves*** 2–50 (150), 46–242.5 cm long, ascending, brown at emergence with whitish gray trichomes, glabrous at maturity. ***Petiole*** 16–98 cm long, terete, linear, brown in mature leaves; with 11–46 thin prickles, 0.30–0.40 cm long. ***Rachis*** 26–144.5 cm long, terete, linear, brown to greenish brown in mature leaves, lower 2/3rds with prickles. ***Leaflets*** 28–94 pairs, opposite to subopposite, insertion in one plane, linear to lanceolate, in general longitudinally planar, not basally falcate, papyraceous, lightly involute, green with adaxial and abaxial sides glabrous, distal end with entire margins, acuminate and symmetrical at the apex, attenuate at base, with conspicuous and light-green veins; median leaflets 10–40 × 0.4–0.9 cm, 0.2–1.6 cm between leaflets; articulations 0.12–1.20 cm wide, brown. ***Pollen strobili*** 16–55 cm long, 4.0–7.7 cm in diameter, solitary, cylindrical, erect, yellowish green with brown trichomes at emergence, yellowish cream with blackish trichomes at maturity; peduncle 5–11 cm long, 0.8–1.4 cm in diameter, pubescent, reddish brown to brown; microsporophylls 1.23–1.80 × 0.62–0.86 cm, elliptic with a non-recurved distal face and a lobate fertile portion, infertile portion 0.62–0.81 cm long and linear with straight horns 0.17–0.39 cm long, 0.56–0.95 cm and an obtuse to acute angle between the horns. ***Ovulate strobili*** 13.5–35 cm long, 7–12.6 cm in diameter, solitary, cylindrical, erect, yellowish green with abundant blackish trichomes at emergence, glaucous green with reddish brown to blackish trichomes at maturity, acuminate apex; peduncle with trichomes scarce, brownish, 4.5–12 cm long, 1.0–2.2 cm in diameter, erect; megasporophylls 42–72, 6–8 orthostichies with 7–10 sporophylls per orthostichy, 2.0–3.0 × 2.8–6.1 cm, with a prominent distal face, horns straight and 0.29–1.47 cm long, 1.55–2.09 cm between horns with an obtuse angle between the horns. ***Seeds*** 1.9–2.9 cm long, 1.2–2.5 cm in diameter, spherical, sarcotesta whitish pink when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia mirandae* is widely distributed in Chiapas State, Mexico from "Tres Picos" hill and Sierra Morena to a mountain range in Mun. Jiquipilas (Fig. 20D); it occurs on karstic rocks with abundant organic material in oak forest and transitional oak forest between pine oak forest and cloud forest between 850 and 1,500 m.

Etymology. This species was named in honor of Dr. Faustino Miranda for his untiring contributions to the flora of Chiapas (Vovides et al. 2001).

Common names. Mexico. Chiapas: Amenduai (L. Martínez-Domínguez et al. 1428; M.A. Pérez-Farrera 1261A), Espadaña de cochi (M.A. Pérez-Farrera 26A), Peinetilla (M.A. Pérez-Farrera 37).

Uses. The mature seeds are used as food (U. Bachem C. & R. Rojas 579).

Conservation status. (IUCN 2021). *Ceratozamia mirandae* is listed as "Endangered" under criteria C1.

Discussion. *Ceratozamia mirandae* is similar to *C. alvarezii*. However, in *C. mirandae*, the leaves and ovulate strobilus are larger at the population level than in *C. alvarezii*, which is a more diminutive species overall.

In the original publication of *C. mirandae*, there were no isotypes cited, only the holotype in CHIP. Because no holotype of *C. mirandae* has been found in CHIP, despite intensive searches, we designate the uncited isotype found in MEXU as the lectotype.

Specimens examined. Mexico. **Chiapas: Mun. Jiquipilas**, 1,170 m, 19 Jun 2018, *F. Nicolalde-Morejón et al. 2749–2759* (CIB); 1,015 m, 8 Mar 1995, *J.J. Castillo-Hernández 548* (CHIP); 1,170 m, 19 Jun 2018, *L. Martínez-Domínguez et al. 1312–1316, 1318, 1320, 1321* (CIB), *1317* (CIB, MEXU), *1319* (CIB, MEXU); 1,270 m, 25 May 1995, *M.A. Pérez-Farrera 465* (CHIP). **Mun. Villa Corzo**, 1,320 m, 12 Jul 2004, *A. Reyes-García et al. 7134* (MEXU); 1,500 m, 9 Feb 1972, *D.E. Breedlove 23999* (MEXU, MO); 1,170 m, 16 Mar 1989, *U. Bachem & R. Rojas C. 579* (CHIP, MEXU, SLPM). **Mun. Villaflores**, 960 m, 6 Apr 1995, *A.P. Vovides & M.A. Pérez-Farrera 1261* (XAL); 1,157 m, 7 Jul 2004, *D. Álvarez 9809* (MEXU); 1,277 m, 25 Jun 2018, *F. Nicolalde-Morejón et al. 2854–2863* (CIB); 1,195 m, 20 Jun 2019, *F. Nicolalde-Morejón & L. Martínez-Domínguez 3208–3213* (CIB); 1,350 m, 21 Jun 2019, *F. Nicolalde-Morejón & L. Martínez-Domínguez 3215–3224* (CIB); 1,015 m, 5 Aug 1994, *J.J. Castillo-Hernández 230* (CIB, MEXU, USCG); 1,250 m, 5 Apr 1995, *J.J. Castillo-Hernández* 595 (CHIP); 1,140 m, 6 Aug 2002, *L. Alvarado C. et al. 368* (MEXU); 1,195 m, 20 Jun 2019, *L. Martínez-Domínguez & F. Nicolalde-Morejón 1808–1813* (CIB); 1,350 m, 21 Jun 2019, *L. Martínez-Domínguez & F. Nicolalde-Morejón 1814–1825* (CIB); 1,277 m, 25 Jun 2018, *L. Martínez-Domínguez et al. 1428, 1429, 1431–1438* (CIB), *1430* (CIB, MEXU); 940 m, 22 Dec 1993, *M.A. Pérez-Farrera s/n* (XAL), *26* (CHIP, XAL); 910 m, 28 Apr 1994, *M.A. Pérez-Farrera 37* (CHIP, XAL); 1,460 m, 16 Sep 1994, *M.A. Pérez-Farrera 126* (CHIP); 950 m, 12 Apr 1996, *M.A. Pérez-Farrera 1480* (CHIP).

21. *Ceratozamia mixeorum* Chemnick, T.J.Greg. & Salas-Mor., Phytologia 83(1): 47 1998 ("1997")

Fig. 7H

Type. Mexico. Oaxaca: Cercanía a Juquila Mixe, 1,737 m, 21 May 1997. *J. Chemnick & T. Gregory 49* (lectotype, designated by Nicolalde-Morejón and Avendaño 2011, pg. 1033: XAL! [XAL0065870]).

Description. ***Stem*** 30–100 cm long, 18–25 cm in diameter, epigeous, decumbent. ***Cataphylls*** 1.8–4.6 × 1.9–4.3 cm wide at the base, persistent, triangular, reddish brown, densely brown tomentose at emergence, partially tomentose at maturity, apex acuminate. ***Leaves*** 3–17 (30), 85–246 cm long, descending, light green and glaucous at emergence with light brown trichomes, glabrous at maturity. ***Petiole*** 46–132 cm long, terete, linear, green in mature leaves; with 30–50 thin prickles, 0.20–0.38 cm long. ***Rachis*** 58–118 cm long, terete, linear, green to greenish in mature leaves, with scarce prickles. ***Leaflets*** 19–35 pairs, opposite to subopposite insertion in one plane, lanceolate, abaxially curved, rarely planar, not basally falcate, papyraceous, flat, green with adaxial and abaxial sides glabrous, distal end with entire margins, acuminate and symmetrical at the apex, attenuate at base, with conspicuous and green-light veins; median leaflets 24–39 × 1.9–2.9 cm, 1.7–3.0 cm between leaflets; articulations 0.6–1.0 cm wide, green. ***Pollen strobili*** 22–30 cm long, 4.5–7 cm in diameter, generally solitary (1–2), cylindrical, erect, greenish at emergence with reddish trichomes, greenish yellow with reddish brown trichomes at maturity; peduncle 10–15 cm long, 1.2–2.0 cm in diameter, glabrous or with trichomes scarce reddish brown to brown; microsporophylls 1.4–2.4 × 0.7–1.3 cm, obconic with a non-recurved distal face and lobate fertile portion, infertile portion 0.39–0.48 cm long and linear with straight horns 0.30–0.50 cm long, 0.50–0.93 cm and a right angle between the horns. ***Ovulate strobili*** 23–32 cm long, 12–16 cm in diameter, solitary, cylindrical, erect, yellowish green with abundant blackish trichomes at emergence, green with brown to blackish trichomes at maturity, apiculate apex; peduncle 12–23.5 cm long, 1.3–2.1 cm in diameter, pendulous, glabrous or with scarce reddish brown trichomes; megasporophylls 60–112, 6–9 orthostichies with 10–14 sporophylls per orthostichy, 2.4–2.8 × 4.0–5.1 cm, with a truncate distal face, horns straight and 0.90–1.1 cm long, 0.92–1.2 cm between horns and a right angle between the horns. ***Seeds*** 2.2–3.0 cm long, 1.6–2.0 cm in diameter, ovate, sarcotesta whitish yellow to yellow when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia mixeorum* is endemic to the southern portion of Sierra Norte of Oaxaca State (Mexico), along the highlands in the Mixe area (Fig. 21A), where it occurs in karstic rocks of cloud and oak forests between 1,170 to 2,150 m.

Etymology. The specific epithet was named in honor of the Mixe people.

Common names. Mexico. Oaxaca: Carrete (Chemnick et al. 1998).

Uses. The pollen strobilus is used as a toy (Chemnick et al. 1998).

Conservation status. (IUCN 2021). *Ceratozamia mixeorum* is listed under criteria A2cd+4cd; B1ab(ii,iii,v) as "Endangered".

Discussion. *Ceratozamia mixeorum* is similar to *C. whitelockiana*, but has prickles on the rachis of the leaf and a densely prickly petiole (up to 50 prickles), ovulate strobili with long and pendulous pedunclse up to 23.5 cm long. The fertile portion of ovulate strobilus has 6–9 orthostichies and 10–14 sporophylls per orthostichy; in contrast to 4–5 orthostichies and 5–8 sporophylls per orthostichy in *C. whitelockiana*.

Specimens examined. Mexico. **Oaxaca: Mun. Guevea de Humboldt**, 1,300 m, 30 Mar 1991, *A. Campos 3614* (MEXU). **Mun. San Juan Juquila Mixes**, 1,605 m, 7 Apr 1995, *E. Torres B. 563* (SERO, XAL); 1,595 m, 25 Sep 2020, *F. Nicolalde-Morejón et al. 3368* (CIB); 1,681 m, 25 Sep 2020, *F. Nicolalde-Morejón et al. 3369–3371* (CIB); 1,595 m, 25 Sep 2020, *L. Martínez-Domínguez et al. 2001, 2002* (CIB); 1,681 m, 25 Sep 2020, *L. Martínez-Domínguez et al. 2003* (CIB, MEXU), *2004–2006* (CIB). **Mun. San Pedro Ocotepec**, 8 Apr 1995, 1,600 m, *E. Torres B. et al. 607* (XAL). **Mun. Santa**

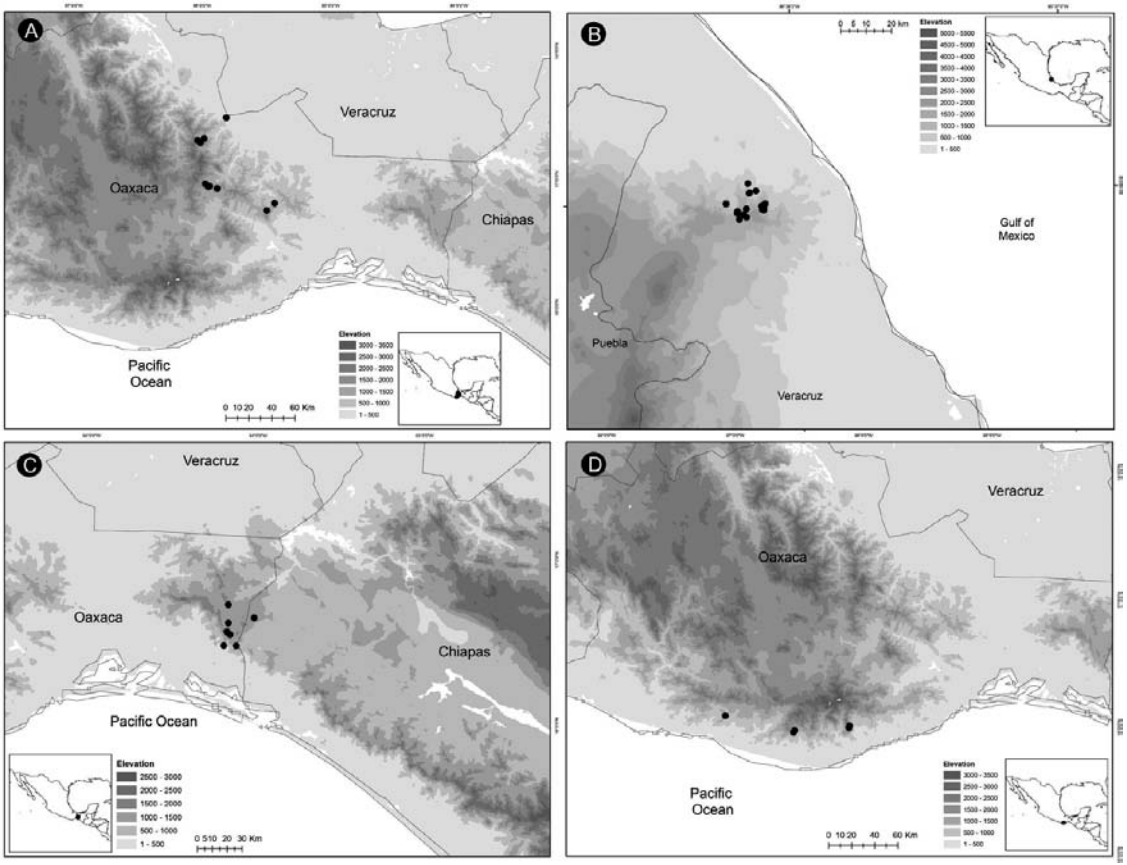

Figure 21. Distribution of *Ceratozamia* species **A** *C. mixeorum*. **B** *C. morettii*. **C** *C. norstogii*. **D** *C. oliversacksii*.

María Guienagati, 1,200 m, 25 Jan 1996, *M. Cerón C. et al. 249* (MEXU, SERO, XAL). **Mun. Santa María Tepantlali**, 1,728 m, 25 Sep 2020, *F. Nicolalde-Morejón et al.* 3362–3367 (CIB); 2,147 m, 15 May 2011, *G. Juárez-García 3871* (MEXU); 1,728 m, 25 Sep 2020, *L. Martínez-Domínguez et al. 1994–1995* (CIB, MEXU), *1996–2000* (CIB). **Mun. Totontepec Villa de Morelos**, 1,300 m, 7 Dec 1989, *E. Vargas-Ruíz 476* (MEXU); 1,900 m, 21 Apr 1986, *J. Rivera-Reyes & Gary J. Martin 246* (MEXU); 1,700 m, 15 Jul 1987, *E. Velasco-López & G.J. Martin 60* (MEXU); 1,900 m, 6 Jan 1988, *J. Rivera-Reyes & G.J. Martin 920* (MEXU); 2,000 m, 16 Sep 1987, *W. Ruíz S. 46* (MEXU).

22. *Ceratozamia morettii* Vázq.Torres & Vovides, Novon 8 (1): 87. 1998

Figs 7I–J, 10A, C

Type. Mexico. Veracruz: Mun. Landero y Coss, El Raicero, 4 km NE from Landero y Coss, 1,500 m, 7 Jan 1992, *M. Vázquez-Torres & H. Barney 4097* (holotype: CIB! [acc. # 22297UV]; isotypes: CIB! [acc. # 24578UV], MEXU [n.v.], XAL! [XAL0001061, XAL0001064])

Description. ***Stem*** 20–23 (50) cm long, 20–35 cm in diameter, epigeous, erect and decumbent. ***Cataphylls*** 4–6.5 × 3–3.5 cm wide at the base, persistent, triangular, reddish brown, densely brown tomentose at emergence, partially tomentose at maturity, apex acuminate. ***Leaves*** 3–30, 82–200 cm long, descending, light green at emergence, with brown trichomes, glabrous at maturity. ***Petiole*** 30–90 cm long, terete, linear, green in mature leaves; with 8–60 thin prickles, 0.01–0.20 cm long. ***Rachis*** 50–116 cm long, terete, linear, green in mature leaves, with prickles. ***Leaflets*** 10–23 pairs, opposite to subopposite, oblong, longitudinally curved abaxially to planar, not basally falcate, coriaceous, green with adaxial side glabrous and abaxial side glaucous, distal end with entire margins, acuminate and asymmetrical at the apex, attenuate at base, and with conspicuous and green-light veins; median leaflets 17.5–41 × 2.6–4.3 cm wide, 2.1–6 cm between leaflets; articulations 0.5–1.6 cm wide, green and yellow. ***Pollen strobili*** 12–22 (35.5) cm long, 3.8–5.2 cm in diameter, solitary, cylindrical, erect, brownish yellow at emergence, greenish yellow with blackish pubescent at maturity; peduncle 3–7 (12.5) cm long, 1.1–1.6 cm in diameter, tomentose, reddish brown to brown; microsporophylls 1.0–2.0 × 0.9–1.15 cm, obconic with a non-recurved distal face and a lobate fertile portion, infertile portion 0.37–0.75 cm long and orbicular with straight horns 0.15–0.53 cm long, 0.47–0.92 cm and an acute angle between the horns. ***Ovulate strobili*** 14–20 cm long, 8–9.9 cm in diameter, solitary, globose, erect, yellowish green with brown trichomes at emergence, green with blackish trichomes at maturity and with apiculate apex; peduncle 4–7 cm long, 1.2–1.8 cm in diameter, erect, tomentose, brown; megasporophylls 35–81 (108), 7–10 orthostichies with 5–9 sporophylls per orthostichy, 1.7–2.6 × 3.0–4.5 cm, with a truncate distal face, horns straight and 0.32–0.69 cm long, 1.05–1.6 cm between horns and a right angle between the horns. ***Seeds*** 1.5–2.3 cm, 1–1.6 cm in diameter, ovate and globose, sarcotesta whitish yellow to yellow when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia morettii* is endemic to the Sierra de Chiconquiaco in Veracruz State, Mexico (Fig. 21B), here it occurs in cloud forest on karstic rocks and cliffs between 1,150–1,850 m.

Etymology. The specific epithet honors the Italian botantist Aldo Moretti from the Orto Botanico and Istituto di Biologia Vegetale at the University of Naples Federico II, in recognition of his scientific contributions to cycad biology (Vázquez-Torres et al. 1998).

Common names. Mexico. Veracruz: Palmita (J. Rees & A.P. Vovides 1663), palmilla (J. Rees & A.P. Vovides 1676).

Uses. Leaves are used in flower arrangements to decorate the homes during religious rites (Fig. 28A).

Conservation status. (IUCN 2021). *Ceratozamia morettii* has been listed as "Endangered" under criteria B1ab(i,iv,v)+2ab(i,iv,v).

Discussion. In leaf morphology, *Ceratozamia morettii* is morphologically similar to *C. delucana*; however, there are differences in the total size of the plant and in its reproductive structures. The microsporophyll horns of *C. morettii* form an acute angle, whereas those of *C. delucana* form a right angle. The ovulate strobilus in *C. morettii* is green with blackish trichomes at maturity and an apiculate apex. In contrast, in *C. delucana*, the ovulate strobilus is green and glabrous at maturity with an acute apex.

Specimens examined. Mexico. **Veracruz: Mun. Chiconquiaco**, 1,700 m, 5 Sep 1981, *A.P. Vovides 687* (XAL); 1,850 m, 18 Dec 1981, *A.P. Vovides 704* (XAL); Sep 1829, *C.J.W. Schiede s/n* (XAL); 1,598 m, 17 Sep 2020, *F. Nicolalde-Morejón et al. 3318–3322* (CIB); 26 Nov 1974, *J. Rees 6336* (IEB); 1,700 m, 5 Jun 2018, *L. Lagunes G. 83, 84, 85, 86* (CIB); 1,600 m, 26 Apr 2018, *L. Martínez-Domínguez et al. 1280–1290* (CIB); 1,700 m, 3 Dec 2018, *L. Martínez-Domínguez et al. 1567, 1568* (CIB); 1,500 m, 15 Mar 2019, *L. Martínez-Domínguez et al. 1660, 1661* (CIB); 1,550 m, 26 Mar 2019, *L. Martínez-Domínguez et al. 1680, 1681* (CIB); 1,598 m, 17 Sep 2020, *L. Martínez-Domínguez et al. 1931–1934, 1936* (CIB), *1935* (CIB, MEXU). **Mun. Landero y Coss**, 1,750 m, 1 Jan 1976, *J. Rees & A.P. Vovides 1662–1664* (XAL); 1,750 m, 23 Sep 1976, *J. Rees & A.P. Vovides 1676* (XAL); 1,830 m, 3 Mar 2011, *L.H. Bojórquez G. et al. 23101–2316* (CIB); 1,472 m, 29 Jan 2015, *L. Martínez-Domínguez et al. 185–214* (CIB); 1,765 M, 22 Apr 2008, *M. Vázquez-Torres et al. 8349* (CIB); 3 Mar 2001, *S. Avendaño 5378* (MEXU); 1,520 m, 10 Jan 2001, *T.W. Walters 2001-01-E* (XAL). **Mun. Tenochtitlán**, 1,500 m, 10 Apr 2002, *A. Rincón G. et al. 2996, 2997* (XAL); 1,500 m, 12 Apr 2002, *A. Rincón G. et al. 2998* (XAL). **Mun. Yecuatla**, 1,400 m, 26 May 1981, *C. Gutiérrez B. & A. Montoya L. 134* (MO, XAL); 1,211 m, 28 Sep 2014, *F. Nicolalde-Morejón & L. Martínez-Domínguez 2087–2106* (CIB); 1, 200 m, 26 Sep 1976, *J. Rees 1677* (XAL); 1,211 m, 9 Aug 2014, *L. Martínez-Domínguez et al. 161* (CIB).

23. *Ceratozamia norstogii* D.W.Stev., Brittonia 34: 181. 1982

Fig. 27A

Type. Mexico. Chiapas: Rancho Fenia, Mar-Apr 1925, *C. Purpus 6* ♀ (holotype: NY! [00001116]; isotypes: F! [acc. # 1530231], MO!, US! [00620294]).

Description. ***Stem*** 30–60 cm long, 20–40 cm in diameter, epigeous, erect and decumbent. ***Cataphylls*** 4.5–6 × 2.5–6.4 cm wide at the base, persistent, long triangular, reddish brown, densely brown tomentose at emergence, tomentose at maturity, apex acuminate. ***Leaves*** 5–48, 50–126 cm long, ascending, copperish brown at emergence with whitish gray trichomes, glabrous at maturity. ***Petiole*** 10–49 cm long, terete, twisted, copperish with abundant greyish trichomes in young leaves, copperish and glabrous in mature leaves; with 30–60 robust prickles, 0.11–0.31 cm long. ***Rachis*** 40–88 cm long, terete, twisted, copperish with abundant greyish trichomes in young leaves, copperish and glabrous in mature leaves, with prickles. ***Leaflets*** 33–91 pairs, opposite to subopposite, insertion irregular to in one plane, linear, in general longitudinally planar, not basally falcate to basally falcate, coriaceous, strongly involute to caniculate, green with yellowish green base and with adaxial side glabrous and abaxial side glaucous, distal end with entire margins, acute and symmetric at the apex, attenuate at base, with conspicuous and light-green veins; median leaflets

14–48 × 0.21–0.46 cm, 0.2–1.3 cm between leaflets; articulations 0.08–0.66 cm wide, yellowish. ***Pollen strobili*** 13.2–25 cm long, 3.1–8 cm in diameter, solitary, cylindrical, erect, brown with reddish brown trichomes at emergence, yellowish green to cream at maturity; peduncle 2.0–5.5 cm long, 1.3–2.3 cm in diameter, tomentose, brown; microsporophylls 0.92–1.16 × 0.86–0.1.1 cm, discoid with a non-recurved distal face and a lobate fertile portion, infertile portion 0.36–0.45 cm long and orbicular with straight horns 0.07–0.13 cm long, 0.35–0.56 cm and an acute angle between the horns. ***Ovulate strobili*** 21–35 cm long, 8.5–10.5 cm in diameter, solitary, cylindrical, erect, green with abundant blackish trichomes at emergence, grayish green with abundant blackish trichomes at maturity, acuminate apex; peduncle 4.8–9 cm long, 1.8–2.2 cm in diameter, erect, tomentose, brown; megasporophylls 42–63, 6–7 orthostichies with 7–9 sporophylls per orthostichy, 2.6–3.0 × 3.6–4.0 cm, with a truncated distal face, horns straight and 0.37–0.58 cm long, 0.37–0.42 cm between horns with a right angle between the horns. ***Seeds*** 2.0–3.5 cm long, 1.1–1.9 cm in diameter ovate, sarcotesta whitish pink when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia norstogii* is distributed in mountainous regions in Chiapas and Oaxaca States, Mexico (Fig. 21C), where it occurs on karstic rocks in pine-oak forest and the transition between pine forest and oak forest between 800–1,650 m.

Etymology. This species was named in honor of Knut Norstog, for his extensive and significant contributions to cycad biology (Stevenson 1982).

Common names. None recorded.

Uses. None recorded.

Conservation status. (IUCN 2021). The currect category of threat to *Ceratozamia norstogii* is "Endangered" under criteria A2abd; B1ab(iii,iv,v); C1.

Discussion. *Ceratozamia norstogii* is easily diagnosable by its petiole and raquis twisted. The linear leaflets are coriaceous, strongly involute and appear caniculate and have an acute apex.

Specimens examined. Mexico. **Chiapas: Mun. Cintalapa**, 1,100 m, 19 Mar 1993, *A.P. Vovides et al. 1230, 1231, 1233, 1235* (XAL); 1,600 m, 3 Nov 1971, *D.E. Breedlove 21813* (MEXU, MO); 1,600 m, 21 Apr 1972, *D.E. Breedlove 24709* (MO); 1,600 m, 12 Oct 1979, *D.E. Breedlove 44431* (MEXU); 800 m, 4 May 1988, *E. Palacios E. 375* (CHIP, IBUG); 1,240 m, 6 Jun 1993, *E. Palacios E. 2155* (CHIP); 1,280 m, 22 May 2001, *J.M. Lázaro-Zermeño 251* (CHIP); 1,038 m, 20 Jun 2018, *F. Nicolalde-Morejón et al. 2762–2770* (CIB); 1,325 m, 20 Jun 2018, *F. Nicolalde-Morejón et al. 2771–2780* (CIB); 1,038 m, 20 Jun 2018, *L. Martínez-Domínguez et al. 1326–1334* (CIB), *1335* (CIB, MEXU); 1,325 m, 20 Jun 2018, *L. Martínez-Domínguez et al. 1337–1349* (CIB); 1,100 m, 5 Oct 1995, *M.A. Pérez-Farrera 775* (CH, CIB, XAL); *5 Dec 1996, M.A. Pérez-Farrera 1483* (HEM). **Oaxaca: Mun. San Miguel Chimalapa**, 1,120 m, 1 Apr 1996, *S. Salas-Morales et al. 1173* (SERO, XAL). **Mun. Santo Domingo Zanatepec**, 800 m, 22 Jun 2018, *F. Nicolalde-Morejón et al. 2819–2828* (CIB); 800 m, 22 Jun 2018, *L. Martínez-Domínguez et al. 1380, 1381, 1383, 1386, 1389–1391, 1394, 1396, 1399* (CIB).

24. *Ceratozamia oliversacksii* D.W.Stev., Mart.-Domínguez & Nic.-Mor., Kew Bull. 77: 212. 2022

Fig. 27B

Type. Mexico. Oaxaca: Mun. Candelaria Loxicha, Cerro Perico, 1,616 m, 10 Jun 2021, *L. Martínez-Domínguez et al. 2261* ♀ (holotype: CIB! [acc. # 23411UV]; isotypes: K!, MEXU!, NY!).

Description. ***Stem*** 30–80 cm long, 10–40 cm in diameter, epigeous, decumbent. ***Cataphylls*** 6.5–7.5 × 1.6–2.0 cm wide at the base, persistent, triangular, reddish brown, brown tomentose at emergence, glabrous at maturity, apex acuminate. ***Leaves*** 7–36 (50), 124–258 cm long, descending, green at emergence with brown trichomes, glabrous at maturity. ***Petiole*** 45–110 cm long, terete, linear, green at emergence, dark green in mature leaves; with 40–65 thin prickles, 0.50–0.70 cm long. ***Rachis*** 85–182 cm long, terete, linear, dark green in mature leaves with prickles. ***Leaflets*** 24–47 pairs, opposite to subopposite, insertion in one plane, linear, longitudinally curved abaxially to planar, not basally falcate, papyraceous, flat, green with adaxial and abaxial sides glabrous, distal end with entire margins, acuminate and symmetrical (rarely asymmetrical) at the apex, attenuate at base, with conspicuous and light-green veins; median leaflets 25–40 × 2.3–3.6 cm, 2.0–3.3 cm between leaflets; articulations 0.5–1.1 cm wide, green. ***Pollen strobili*** 20–35 cm long, 3.0–5.7 cm in diameter, solitary, cylindrical, erect, greenish with reddish trichomes at emergence, greenish yellow with reddish brown trichomes at maturity; peduncle 10–15 cm long, 1.2–1.8 cm in diameter, pubescent, reddish brown; microsporophylls 2.5–2.75 × 0.99–1.28 cm, obconic with a non-recurved distal face and a lobate fertile portion, infertile portion 0.60–0.80 cm long and a rounded with straight horns 0.08–0.18 cm long, 0.50–0.70 cm and an acute angle between the horns. ***Ovulate strobili*** 33–36 cm long, 11.5–14 cm in diameter, solitary, cylindrical, erect, greenish yellow with few reddish trichomes at emergence, green with brown to reddish brown trichomes or glabrous at maturity, acute apex; peduncle 8.0–10.0 cm long, 1.4–1.6 cm in diameter, erect, pubescent, light brown; megasporophylls 80–210, 9–10 orthostichies with 11–13 sporophylls per orthostichy, 2.3–2.9 × 3.9–5.0 cm, with a truncate distal face, horns straight and 0.31–0.53 cm long, 1.33–1.40 cm between horns with a right angle between the horns. ***Seeds*** 2.45–2.65 cm long, 1.4–1.6 cm in diameter, ovate, sarcotesta whitish yellow to yellow when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia oliversacksii* occurs along the Eastern Sierra Madre del Sur in the Oaxacan Highlands, Mexico (Fig. 21D), it is found on karstic rocks with abundant organic matter in cloud forest and the transition zone between it and pine forest between 1,040 to 1,850 m.

Etymology. The specific epithet honors Oliver Sacks, who loved cycads and was a distinguished American neurologist and historian of science. Sacks published Island of the Color Blind and Cycad Island (Sacks 1997) and Oaxaca Journal (Sacks 2002) (Martínez-Domínguez et al. 2022b).

Common names. None recorded.

Uses. None recorded.

Preliminary conservation status. *Ceratozamia oliversacksii* should be listed as "Vulnerable (V)". We visited four populations in Oaxaca in which each population size was between 50 to 300 adult plants. We observed different age classes from seedling to reproductive individuals.

Discussion. *Ceratozamia oliversacksii* is similar to *C. robusta* and *C. leptoceras* but is characterized by green leaves at emergence and the combination of linear and papyraceous leaflets. The ovulate strobili have 11 to 13 sporophylls per orthostichies and an acute apex, acute horns of megasporophylls up to 0.53 cm long, and microsporophylls with a rounded distal face and straight horns.

Specimens examined. Mexico. **Oaxaca: Mun. Candelaria Loxicha**, 1,380 m, 3 May 2005, *A. Luna-José & B. Rendón-Aguilar 1472, 1473* (XAL); 1,630 m, 23 Jun 2019, *F. Nicolalde-Morejón & L. Martínez-Domínguez 3231–3243* (CIB); 1,630 m, 23 Jun 2019, *L. Martínez-Domínguez & F. Nicolalde-Morejón 1832–1839* (CIB), *1840* (CIB, MEXU); 1,418 m, 20 April 2021, *L. Martínez-Domínguez et al. 2160, 2161* (CIB); 1,616 m, 10 Jun 2021, *M. Rios-Méndez et al. 105* (CIB). **Mun. San Agustín Loxicha**, 1,760 m, 12 Oct 2003, *A. Luna-José & B. Rendón-Aguilar 518* (XAL); 1,400 m, 23 Jun 2019, *F. Nicolalde-Morejón & L. Martínez-Domínguez 3229, 3230* (CIB); 1,400 m, 23 Jun 2019, *L. Martínez-Domínguez & F. Nicolalde-Morejón 1829–1831* (CIB). **Mun. Santa Catarina Juquila**, 1,850 m, 13 Apr 1965, *J. Rzedowski 19557* (ENCB); 1,450–1,700 m, 11 Feb 1965, *R. McVaugh 22346* (ENCB; MICH). **Mun. San Miguel del Puerto**, 1,060 m, 25 Jun 2019, *F. Nicolalde-Morejón & L. Martínez-Domínguez 3244–3249* (CIB);1,430 m, 17 Apr 2000, *J. Rivera H. et al. 2378* (FCME, SERO); 1,060 m, 25 Jun 2019, *L. Martínez-Domínguez & F. Nicolalde-Morejón 1847–1849, 1851, 1852* (CIB), *1850* (CIB, MEXU); 1,040 m, 1 Apr 2003, *L. Schibli et al. 152* (SERO).

25. *Ceratozamia osbornei* D.W.Stev., Mart.-Domínguez & Nic.-Mor., sp. nov.

urn:lsid:ipni.org:names:77305494-1
Figs 5, 6

Diagnosis. Similar to *Ceratozamia robusta* and *C. subroseophylla*, but distinguished by characters of reproductive structures. It has megasporophylls with abundant purple to wine-colored trichomes, ovulate strobili with an acuminate apex and up to 12 orthostichies with 31 sporophylls per orthostichy and pollen strobili with a long infertile portion from 0.65 to 0.80 cm long.

Type. BELIZE. Toledo: Southwestern Maya Mountains, Columbia River Forest Reserve, Union Camp. 6 Apr 1992, *B.K. Holst 4105* (holotype: NY! [01340569]; isotype: MO! [acc. # 04661737, 04661738]).

Description. ***Stem*** 30–200 cm long, 20–40 cm in diameter, epigeous, generally decumbent. ***Cataphylls*** 5.5–8 × 2–3 cm wide at the base, persistent, triangular, reddish brown, brown tomentose at emergence, glabrous at maturity, apex acuminate. ***Leaves*** 12–50, 90–300 cm long, ascending, brown at emergence, with whitish gray trichomes, glabrous at maturity. ***Petiole*** 75–100 cm long, terete, linear, greenish brown or dark brown at emergence, green in mature leaves; with 20–40 robust prickles, 0.20–0.45 cm long. ***Rachis*** 90–200 cm long, terete, linear, greenish brown or dark brown at emergence, green in mature leaves, with prickles. ***Leaflets*** 26–51 pairs, opposite to subopposite, insertion in one plane, lanceolate, longitudinally curved abaxially to planar, not basally falcate, papyraceous, flat, dark green, adaxial side glabrous, abaxial side glabrous, distal end with entire margins, acuminate and symmetric at the apex, attenuate at base, with conspicuous and light-green veins; median leaflets 30–45 × 2.5–4.0 cm, 1.7–5 cm between leaflets; articulations 0.9–1.6 cm wide, brown in young leaves and green in mature leaves. ***Pollen strobili*** 60–80 cm long, 7–9 cm in diameter, solitary, cylindrical, erect, green with wine trichomes at emergence, greenish yellow at maturity with wine to purple trichomes; peduncle 7–11 cm long, 2.5–3.5 cm in diameter, tomentose, brown; microsporophylls 2.0–3.3 × 1.1–1.85 cm, obconic with a non-recurved distal face and deeply lobate fertile portion, infertile portion 0.65–0.80 cm long and rounded with straight horns 0.20–0.29 cm long, 0.55–0.75 cm and a right angle between the horns. ***Ovulate strobili*** 30–50 cm long, 9–14 cm in diameter, solitary, cylindrical, erect, green with abundant wine to dark purple trichomes at emergence, green with abundant dark purple trichomes at maturity, acuminate apex; peduncle 12–20 cm long, 2.5–4.5 cm in diameter, erect, tomentose, reddish brown; megasporophylls 108–280, 9–12 orthostichies with 12–31 sporophylls per orthostichy, 1.8–2.3 × 2.3–4.1 cm, with a prominent distal face, horns straight and 0.36–0.50 cm long, 0.90–1.35 cm between horns with a right angle between the horns. ***Seeds*** 3.0–4.5 cm long, 2.0–3.0 cm in diameter, ovate, sarcotesta whitish pink when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia osbornei* is endemic to Belize (Fig. 22A), where it occurs in evergreen tropical forest on karstic rocks with abundant organic matter between 200 and 750 m elevation.

Etymology. The specific epithet honors Roy Osborne in recognition of his remarkable contributions to the knowledge of the cycad diversity in the world. In particular, he has actively participated in the project "The World List of Cycads" since its inception (https://www.cycadlist.org).

Common names. None recorded.

Uses. None recorded.

Conservation status. There is insufficient data for evaluation, but it is known that some populations have more than 100 plants.

Paratypes. BELIZE. **Cayo**: 19 Aug 2008, *M. Calonje et al. BZ08-042* (FTG); 20 Aug 2008, *M. Calonje et al. BZ08-056* (FTG); 25 Feb 1992, *C. Hubbuch et al.* (FTG); 6 Apr 1971, *S. Kiem s.n.* [027932] (FTG); Spring, 1973, *S. Kiem s.n.* [027929] (FTG); 550 m, 4 Aug 1980, *Sutton et al. 15* (MEXU, MO); 455 m, 12 Dec 1996, *T. Hawkins 1186* (NY, MO). **Toledo:** 750 m, 15 May 1979, *C. Whitefoord 1764* (BM); 800–1,200 m, 23–27 Apr 1976, *G.R. Proctor 35985* (BM, MO).

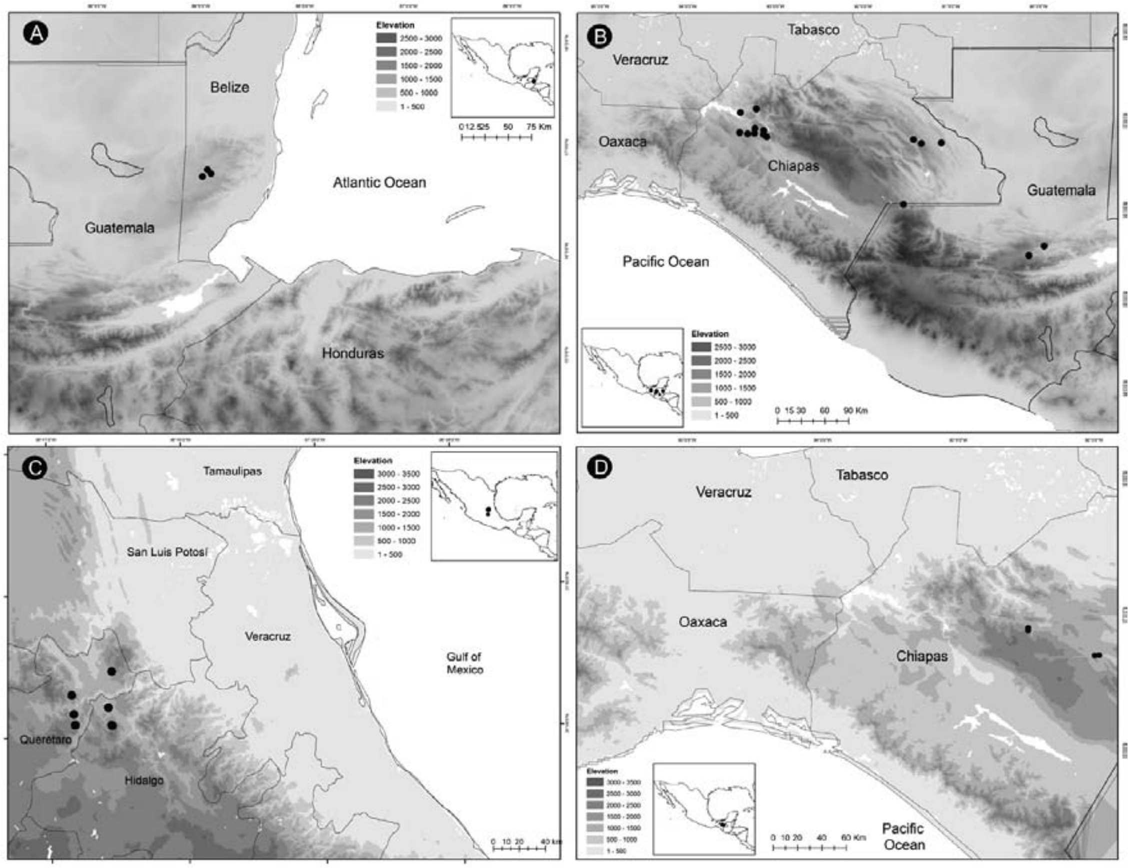

Figure 22. Distribution of *Ceratozamia* species. **A** *C. osbornei* **B** *C. robusta* **C** *C. sabatoi* **D** *C. sancheziae.*

26. *Ceratozamia robusta* Miq., Tijdschr. Wis-Natuurk. Wetensch. Eerste Kl. Kon. Ned. Inst. Wetensch. 1: 42. 1847

Fig. 27C

Ceratozamia mexicana Brongn. var. *robusta* (Miq.) Dyer, Biol. Cent.-Amer., Bot. 3: 193. 1884. Type. Based on: *Ceratozamia robusta* Miq.

Type. Mexico. Chiapas: NW of Tuxtla Gutiérrez on road through San Fernando 23.5 km from route 190, 1,000 m, 9 Jul 1983, *D.W. Stevenson 549A* (neotype, designated by Stevenson and Sabato 1986, pg. 581: NY! [00001119]).

Description. ***Stem*** 30–100 cm long, 20–40 cm in diameter, epigeous, erect and decumbent. ***Cataphylls*** 5.0–9.0 × 3.5–7.5 cm wide at the base, persistent, long triangular, reddish brown, densely brown tomentose at emergence, partially tomentose at maturity, apex acuminate. ***Leaves*** 10–50, 138–266 cm long, ascending, dark brown at emergence, green and glabrous at maturity. ***Petiole*** 56–85 cm long, terete, linear, brown in young leaves, green in mature leaves; with 27–55 robust prickles, 0.48–0.70 cm long. ***Rachi***s 60–190 cm long, terete, linear, brown in young leaves, green in mature leaves, with prickles. ***Leaflets*** 20–45 pairs, opposite to subopposite, insertion in one plane, lanceolate, in general longitudinally planar, not basally falcate, papyraceous, flat, green with adaxial side glabrous and abaxial side glaucous, distal end with entire margins, acuminate and asymmetrical at the apex, attenuate at base, with conspicuous and light-green veins; median leaflets 28–42 × 2.5–4.0 cm, 3.0–4.5 cm between leaflets; articulations 0.2–0.8 cm wide, green. ***Pollen strobili*** 50–70 cm long, 7.0–8.5 cm in diameter, solitary, cylindrical, erect, light green to yellowish green with blackish trichomes at emergence, yellowish cream with reddish brown trichomes at maturity; peduncle 10–15 cm long, 2.5–3.2 cm in diameter, tomentose, reddish brown to brown; microsporophylls 2.33–3.0 × 1.14–1.90 cm, obconic with a non-recurved distal face and deeply lobate fertile portion, infertile portion 0.45–0.65 cm long and rounded with curved horns 0.26–0.40 cm long, 0.55–0.75 cm and a right angle between the horns. ***Ovulate strobili*** 26–40 cm long, 11–14.5 cm in diameter, solitary, cylindrical, erect, dark green at emergence, dark green with scarce blackish trichomes at maturity, acuminate apex; peduncle 5.0–11.0 cm long, 2.5–4.0 cm in diameter, erect, tomentose, reddish brown; megasporophylls 153–280, 8–14 orthostichies with 17–20 sporophylls per orthostichy, 1.7–2.5 × 2.3–3.5 cm, with a prominent distal face, horns straight and 0.38–0.50 cm long, 1.35–1.60 cm between horns with a right angle between the horns. ***Seeds*** 2.5–3.5 cm long, 1.9–2.5 cm in diameter, ovate, sarcotesta whitish yellow to yellow when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia robusta* is widely distributed in Chiapas State, Mexico and northern Guatemala (Fig. 22B). It occurs in evergreen tropical forest between 400 to 1,300 m on karstic rocks with humus soil.

Etymology. The epithet is derived from the robust appearance of the species.

Common names. Mexico. Chiapas: Amendu (M.A. Pérez-Farrera 30), piña del tlacoache (A.P. Vovides & J.I. Calzada 481).

Uses. The seeds are used as food, and the leaves to decorate altars.

Conservation status. (IUCN 2021). *Ceratozamia robusta* is listed as "Endangered" under criteria A2acd. This species has a wide distribution, but some populations previously considered to be part of this entity have been assigned to described species durint the last ten years. Therefore, a reassessment of its conservation status is required.

Discussion. *Ceratozamia robusta* is similar to *C. subroseophylla* and distinguished by its dark green ovulate strobilus with sparse blackish trichomes on megasporophylls at maturity, prominent distal face, straight horns with a right angle between them, differentiating it easily from *C. subroseophylla*. The pollen strobilus of *C. robusta* is the largest in the genus (up to 70 cm long).

Specimens examined. Guatemala. **Alta Verapaz:** 200 m, 13 Jul 2002, *S. Hernández 757* (MO); 1,800-2,000 m, 27 Feb 1939, *C.L. Wilson 262* (F); 300-500 m, 17 Apr 1942, *J.A. Steyermark 45734* (US); 879 m, 15 Mar 2008, *M. Véliz et al. 19998* (BIGU). **Huehuetenango:** 2,000 m, 10 Sep 1942, *J.A. Steyermark* 52046 (NY, US); 1,150 m, 26 Jul 1943, *J.A. Steyermark 49506* (F; MO; NY); 400 m, 27 Jul 1942, *J.A. Steyermark 49682* (NY); 900-1,300 m, 3 Sep 1942, *J.A. Steyermark*

51818 (F; NY; US); 900-1,000 m, 6 Mar 2009, *M.J.M. Christenhusz et al. 5600* (MO); 1,161 m, 21 Dec 2010, *L. Vélasquez et al. 1566* (BIGU). Mexico**. Chiapas: Mun. Berriozábal**, 1,129 m, 6 Mar 2014, *F. Nicolalde-Morejón et al. 1970–1975* (CIB); 1,129 m, 6 Mar 2014, *L. Martínez-Domínguez et al. 41–47, 49, 50* (CIB), *48* (CIB, MEXU). **Mun. Copainalá**, 1,100 m, *M. Gutiérrez & T. Acero 240* (CHIP). **Mun. Ocosingo**, 900 m, 16 Mar 1981, *A.P. Vovides & J.I. Calzada 481* (XAL); 14 Apr 1967, *D.E. Breedlove 15687* (ENCB); 700 m, 18 Apr 1985, *E. Martínez S. 12067* (MEXU); 860 m, 24 Feb 1984, *J. García F. 720* (CH, CIB, XAL). **Mun. Ocozocoautla de Espinosa**, 818 m, 11 Nov 1997, *R. Álvarez G. 6* (CHIP). **Mun. Osumacinta**, 692, 18 Jul 2008, *R. Gallegos Ramos 211* (CHIP). **Mun. San Fernando**, 680 m, 29 Apr 1995, *A.P. Vovides & M.A. Pérez-Farrera 1266*^{a} (XAL); 22 Sep 1993, *Brigada T. Walters s/n* (XAL); 9 Jul 1983, *D.W. Stevenson et al. 549 B–F* (NY); 1,000 m, 9 Jul 1983, *D.W. Stevenson et al. 550 A–C* (NY), 24 Apr 1999, *M.A. Isidro V. 388* (CHIP); 29 Apr 1995, *M.A. Pérez-Farrera 293* (MEXU); 12 Jun 2009, *N. Martínez-Meléndez 2692* (MEXU); 1,200 m, 5 Dec 2000, *R.A. Palestina & I. Acosta 2707* (XAL). **Mun. Tuxtla Gutiérrez**, 1,235 m, 19 Mar 1994, *M.A. Pérez-Farrera 30* (CH, MEXU, XAL); 1,200 m, 1 Nov 1995, *M.A. Pérez-Farrera 820* (CHIP).

27. *Ceratozamia sabatoi* Vovides, Vázq.Torres, Schutzman & Iglesias, Novon 3 (4): 502. 1993

Figs 1A, 27D

Type. Mexico. Querétaro: Mun. San Joaquín, La Mojonera, 2 km on road San Joaquín-El Aguacate, 1,850 m, 15 Apr 1991, *A.P. Vovides & P. Fawcett 1205* ♀ (holotype: XAL! [XAL0005310]).

Description. ***Stem*** 8–30 cm long, 20–35 cm in diameter, epigeous, erect and decumbent. ***Cataphylls*** 3–4.5 × 2–3.5 cm wide at the base, persistent, triangular, reddish brown, densely brown tomentose at emergence, partially tomentose at maturity, apex acuminate. ***Leaves*** 3–40, 60–129 cm long, descending, dark brown at emergence with whitish gray trichomes, glabrous at maturity. ***Petiole*** 20–60 cm long, terete, linear, greenish brown in mature leaves; with 5–40 thin prickles, 0.02–0.19 cm long. ***Rachis*** 40–92 cm long, terete, linear, brown in mature leaves, with prickles. ***Leaflets*** 26–54 pairs, opposite to subopposite, insertion in one plane, linear, longitudinally curved abaxially to planar, basally falcate, papyraceous, flat, green with adaxial and abaxial sides glabrous, distal end with entire margins, acuminate and symmetrical at the apex, attenuate at base, with conspicuous and green-light veins; median leaflets 13–32 × 0.6–1.5 cm, 0.5–1.5 cm between leaflets; articulations 0.3–0.7 cm wide, brown. ***Pollen strobili*** 11–18 cm long, 3.5–4.8 cm in diameter, solitary, cylindrical, erect, greenish yellow at emergence, greenish yellow with blackish trichomes at maturity; peduncle 7–13 cm long, 1.1–1.9 cm in diameter, tomentose, reddish brown to brown; microsporophylls 1.0–1.9 × 0.85–1.90 cm, discoid with a recurved downward distal face and lobate fertile portion, infertile portion 0.33–0.43 cm long and rounded with straight horns 0.06–0.20 cm long, 0.30–0.71 cm and a right angle between the horns. ***Ovulate strobili*** 14–19.5 cm long, 5.5–8.5 cm in diameter, solitary, cylindrical, erect, yellowish green with brown trichomes at emergence, blue green with blackish trichomes at maturity, apiculate apex; peduncle 3.5–7 cm long, 1.2–2.5 cm in diameter, erect, tomentose, brown; megasporophylls 72–110, 8–10 orthostichies with 9–11 sporophylls per orthostichy, 3.5–5 × 3.8–4.5 cm, with a truncate distal face, horns straight and 0.28–0.40 cm long, 1.2–1.6 cm between horns with a right angle between the horns. ***Seeds*** 1.2–2.0 cm long, 1.2–1.5 cm in diameter, ovate, sarcotesta whitish red when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia sabatoi* is endemic to the Sierra Gorda mountain range in Mexico, particularly in Querétaro and Hidalgo States (Fig. 22C), where it occurs in the understory herbaceous layer of the transition zone between oak forest and cloud forest at 1,600–1,900 m.

Etymology. The specific epithet honors Sergio Sabato, a distinguished professor at the University of Naples Federico II for his outstanding contributions to knowledge of the biology of Zamiaceae (Vovides et al. 1993).

Common names. None recorded.

Uses. None recorded.

Conservation status. (IUCN 2021). *Ceratozamia sabatoi* is listed as "Endangered" under criteria A2c; B1ab(i,ii,iv)+2ab(i,ii,iv); C1.

Discussion. *Ceratozamia sabatoi* differs from all *Ceratozamia* species by its microsporophylls with a recurved downward distal face. In addition, this species differs from *C. kuesteriana* by its flat leaflets and descending leaves.

Specimens examined. Mexico. **Hidalgo: Mun. Jacala de Ledezma**, 1,725 m, 16 Aug 2007, *A. Frias-Castro et al. 578* (IBUG); 1,500 m, 29 Oct 1946, *H.E. Moore, Jr. 1788* (CHAPA). **Mun. Zimapán**, 2,000 m, 2 Jun 1989, *M. Vázquez-Torres & J.P. Sclavo 4035* (CIB); 1,235 m, 22 May 2003, *R. Contreras-Medina 55, 56* (XAL); 1,900 m, 13 Sep 1981, *R. Fernandez-Nava 6561* (MEXU, MO, XAL). **Querétaro**: **Mun. Cadereyta de Montes**, 1,850 m, 15 Apr 1991, *A.P. Vovides & K. Norstog 1193* (XAL); *A.P. Vovides et al. 1196–1199* (XAL), *1203* (MEXU, XAL); 1,850 m, 15 Apr 1991,); *A.P. Vovides 1201, 1205* (XAL); 1,924 m, 28 Mar 2015, *F. Nicolalde-Morejón et al. 2169, 2170* (CIB); 1,924 m, 28 Mar 2015, *L. Martínez-Domínguez et al. 313–343* (CIB); 29 Mar 1995, *R. Fernández-Nava s/n* (MEXU); 7 Dec 1990, *R. Zirahuén-Ortega V. 328* (MEXU). **Mun. Landa de Matamoros**, 1,439 m, 12 Jan 2001, *T.W. Walters 2001-05-A, B* (XAL). **Mun. Pinal de Amoles**, 1,760 m, 17 Sep 2001, *E. Carranza G. & I. Silva 6254* (MO); 1,678 m, 29 Mar 2015, *F. Nicolalde-Morejón et al. 2171, 2172* (CIB); 1,678 m, 29 Mar 2015, *L. Martínez-Domínguez et al. 344–372* (CIB); 1,700 m, 11 Dec 1988, *Rzedowski s/n* (XAL); 1,650 m, 4 Apr 1987, *R. Fernández N. 3819* (ENCB).

28. *Ceratozamia sancheziae* Pérez-Farr., Gut.Ortega & Vovides, Phytotaxa 500 (3): 209. 2021

Figs 9A, 12A, 23 D–F

Type. Mexico. Chiapas: Mun. Tenejapa, 1,500 m, 16 May 2017, *M.A. Pérez Farrera 3558* ♀ (holotype: HEM [n.v.]; isotypes: MEXU [n.v.], XAL [n.v.]).

Description. ***Stem*** 8–30 cm long, 10–20 cm in diameter, semi-hypogeous, erect. ***Cataphylls*** 2.5–5.8 × 1.3–5 cm wide at the base, persistent, triangular, reddish brown, densely brown tomentose at emergence, glabrous at maturity, apex acuminate. ***Leaves*** 4–12 (20), 100–232.5 cm long, descending, green or brown at emergence with whitish gray trichomes, glabrous at maturity. ***Petiole*** 40–134.5 cm long, terete, linear, greenish brown or green in mature leaves; unarmed or with prickles 2–28 thin, 0.05–0.34 cm long. ***Rachis*** 55–120 cm long, terete, linear, brown or green in mature leaves, unarmed or with scarce prickles. ***Leaflets*** 20–35 pairs, opposite to subopposite, insertion in one plane, linear, in general longitudinally planar, not basally falcate (sometimes basally falcate), papyraceous, flat, green with adaxial side glabrous and abaxial side glaucous, distal end with entire margins, acuminate and symmetrical at the apex, attenuate at base, with conspicuous and green-light veins; median leaflets 22–36 × 1.7–3.0 cm, 1.4–3.5 cm between leaflets; articulations 0.55–1.1 cm wide, green. ***Pollen strobili*** 15–20 cm long, 1.8–3.2 cm in diameter, solitary, cylindrical, erect, light green at emergence, green with blackish trichomes at maturity; peduncle 6–10 cm long, 0.7–1.5 cm in diameter, tomentose, reddish brown to brown; microsporophylls 0.88–1.2 × 0.65–0.80 cm, discoid with a non-recurved distal face and a lobate fertile portion, infertile portion 0.32–0.40 cm long and rounded with straight horns 0.10–0.20 cm long, 0.63–0.70 cm and an acute angle between the horns. ***Ovulate strobili*** 12–25 cm long, 7.0–8.5 cm in diameter, solitary, cylindrical, erect, yellowish green at emergence, green with scarce blackish trichomes at maturity, acuminate apex; peduncle 5.0–11 cm long, 0.9–1.1 cm in diameter, erect, tomentose, brown; megasporophylls 35–100, 7–10 orthostichies with 5–10 sporophylls per orthostichy, 0.9–1.2 × 3.5–4 cm, with a prominent distal face, horns curved to straight and 0.50–0.73 cm long, 1.2–1.4 cm between horns with a right angle between the horns. ***Seeds*** 1.9–2.3 cm long, 1.4–1.5 cm in diameter, globose, sarcotesta whitish yellow when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia sancheziae* is endemic to Chiapas State, Mexico (Fig. 22D), where it occurs in oak forest and pine-oak forest between 1,000–1,536 m.

Etymology. This species was named in honor of María Ydelia Sánchez-Tinoco, for contributions to our knowledge of the anatomy of Mexican cycad seeds (Gutiérrez-Ortega et al. 2021).

Common names. None recorded.

Uses. The community in Tenejapa use the leaves of this species for ornaments in traditional community festivities.

Preliminary conservation status. *Ceratozamia sancheziae* could be included as “Endangered” (EN) in the IUCN Red List of Threatened Species based on the number of populations, which have between 70 to 250 adult plants.

Figure 23. Vegetative comparison between *Ceratozamia zoquorum* (**A–C**) and *C. sancheziae* (**D–F**). **A** juvenile plant **B** mature leaf **C** detail of leaflets and rachis **D** juvenile plant **E, F** leaves at emergence of adult plants.

Discussion. *Ceratozamia sancheziae* is geographically close to *C. robusta*, but differs from it by its linear leaflets, leaves at emergence that are light green or reddish brown with a glaucous appearance, and ovulate strobili with curved to straight horns up to 0.73 cm long.

Here, we have recircumscribed and clarified the taxonomic identity of *Ceratozamia sancheziae*. In the description of this species, the authors mentioned Petalcingo as a municipality, but this is a locality that corresponds to Tila municipality in Chiapas (c.f. Gutiérrez-Ortega et al. 2021). The distribution range for this species was considered to extend from Tenajapa municipality in Chiapas to the mountain area close to the border with the State of Tabasco (Gutiérrez-Ortega et al. 2021). However, we found specimens from Yajalón of Chiapas (*M.A. Pérez Farrera 1635* from XAL) that do not correspond to the species description (Suppl. material 2). We carried out fieldwork and collected botanical material at the population level in the surroundings of Yajalón, Tila, Altamirano and Tenejapa. Based on the revision of herbarium specimens and the botanical material collected by us, we recircumscribed the populations for *Ceratozamia* in this region into two species: *C. sancheziae* and *C. zoquorum*. One diagnostic character for *C. sancheziae* is oblanceolate leaflets when juvenile, but we found in the field juveniles with linear leaflets only (Gutiérrez-Ortega et al. 2021: 2009; Fig. 23D). Furthermore, we did not find populations near Yajalón or Tila that correspond to *C. sancheziae*. The paratypes cited from Yajalón (*Méndez Ton 5498*, *5722* both MEXU) have some vegetative characters that do not correspond with the species description; however, these could be part of the overall species variation of *C. sancheziae*. Considering that sympatry is possible in this genus and that there is broad variation in *C. zoquorum*, research at population level with reproductive structures and molecular variation could clarify what species is represented by these plants collected near Yajalón. In addition, we have extended the range for *C. sancheziae* to the south of Chiapas (i.e., populations from Altamirano municipality).

Specimens examined. Mexico. **Chiapas: Mun. Altamirano**, 1,215 m, 16 Jul 2021, *F. Nicolalde-Morejón et al. 3691–3697* (CIB); 1,215 m, 16 Jul 2021, *L. Martínez-Domínguez 2318–2325* (CIB); 1,210 m, 28 Nov 1996, *M.A. Pérez-Farrera 1468* (CHIP); 1,200 m, 18 May 1993, *M. González-Espinosa et al.*, *1973* (CH). **Mun. Tenejapa**, 1,000 m, 5 Jun 1972, *D.E. Breedlove 25506* (MEXU); 1,536 m, 15 Jul 2021, *F. Nicolalde-Morejón et al. 3683–3690* (CIB); 1,536 m, 15 Jul 2021, *L. Martínez-Domínguez et al. 2310–2317* (CIB). **Mun. Yajalón**, 1,434 m, 26 Aug 2015, *F. Hernández-Najarro 4382* (CHIP).

29. *Ceratozamia santillanii* Pérez-Farr. & Vovides, Syst. Biodivers. 7 (4): 435. 2009

Figs 1B, 27E

Type. Mexico. Chiapas: Mun. Berriozábal, road Berriozábal-El Cairo, 15 Oct 2004, *M.A. Pérez Farrera 3030* ♀ (holotype: HEM! [HEM020981]; isotypes: XAL! [XAL0005415], MEXU [n.v.]).

Description. ***Stem*** 10–50 cm long, 11–13 cm in diameter, semi-hypogeous, erect and decumbent. ***Cataphylls*** 2–4.5 × 1.7–4 cm wide at the base, persistent, triangular, reddish brown, densely brown tomentose at emergence, with apex partiality glabrous at maturity, apex acuminate. ***Leaves*** 2–3, 50.5–79.3 cm long, descending, light green and glaucous at emergence, with whitish gray trichomes, green and glabrous at maturity. ***Petiole*** 23.5–45 cm long, terete, linear, yellowish green green in mature leaves; with 4–15 thin prickles, 0.05–0.15 cm long or unarmed. ***Rachis*** 23–40 cm long, terete, linear, yellowish green green at emergence, yellowish green in mature leaves, unarmed to armed with prickles. ***Leaflets*** 6–12 pairs, opposite to subopposite, insertion in one plane, oblong, longitudinally curved abaxially to planar, not basally falcate to basally falcate, coriaceous, flat, green with adaxial and abaxial sides glaucous, distal end with entire margins, acuminate and asymmetrical at the apex, attenuate at base, with conspicuous and green-light veins; median leaflets 17.4–30.6 × 4.2–6.5 cm, 3.5–7.1cm between leaflets; articulations 0.7–1.1 cm wide, yellow. ***Pollen strobili*** 15–20 cm long, 1.5–3.0 cm in diameter, solitary, cylindrical, erect, green with blackish trichomes at emergence, yellow-cream with blackish at maturity; peduncle 2–4 cm long, 1.0–1.3 cm in diameter, scarce pubescent, reddish brown to brown; microsporophylls 1.2–2 × 0.5–1 cm, discoid with a non-recurved distal face and a lobate fertile portion, infertile portion 0.34–0.37 cm long and linear with straight horns 0.20–0.25 cm long, 0.50–0.65 cm and an acute angle between the horns. ***Ovulate strobili*** 12–20 cm long, 7–9 cm in diameter, solitary, cylindrical, erect, green with blackish trichomes at emergence, green with scarce blackish trichomes at maturity, acute apex; peduncle 2–3 cm long, 0.9–1.3 cm in diameter, erect, tomentose, light brown; megasporophylls 21–56, 7–8 orthostichies with 3–7 sporophylls per orthostichy, 1.3–2.2 × 1.5–2.2 cm, with a prominent distal face, horns straight and 0.60–0.80 cm long, 1.65–1.90 cm between horns with an acute angle between the horns. ***Seeds*** 2.2–2.4 cm long, 1.8–2.0 cm in diameter, ovate, sarcotesta whitish pink when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia santillanii* is endemic to the northern highlands of Chiapas State, Mexico and only known from the type locality in the municipality of Berriozábal (Fig. 24A), where it was collected in evergreen tropical rain forest on karstic rocks at 800–900 m.

Etymology. The specific epithet was assigned in honor of Professor Trinidad Alemán Santillán in recognition of his academic accomplishments in the training of young biologists in botany and ecology (Pérez-Farrera et al. 2009).

Common names. None recorded.

Uses. None recorded.

Preliminary conservation status. According to IUCN criteria *Ceratozamia santillanii* should be listed as “Critically Endangered” (CR).

Discussion. *Ceratozamia santillanii* belongs to a cryptic taxonomic group with *C. zoquorum* and *C. becerrae*, which is characterized by oblong and coriaceous leaflets with an acuminate and asymmetric apex. *C. santillanii* is easily distinguished from the other taxa in this complex with internal transcribed spacer region of nuclear ribosomal (nrITS) (Martínez-Domínguez et al. 2017c), and peduncle of ovulate strobilus 3 cm long or shorter.

Specimens examined. Mexico. **Chiapas:** Known only from the type locality. 15 Oct 2004, *M.A. Pérez-Farrera 2944* (HEM).

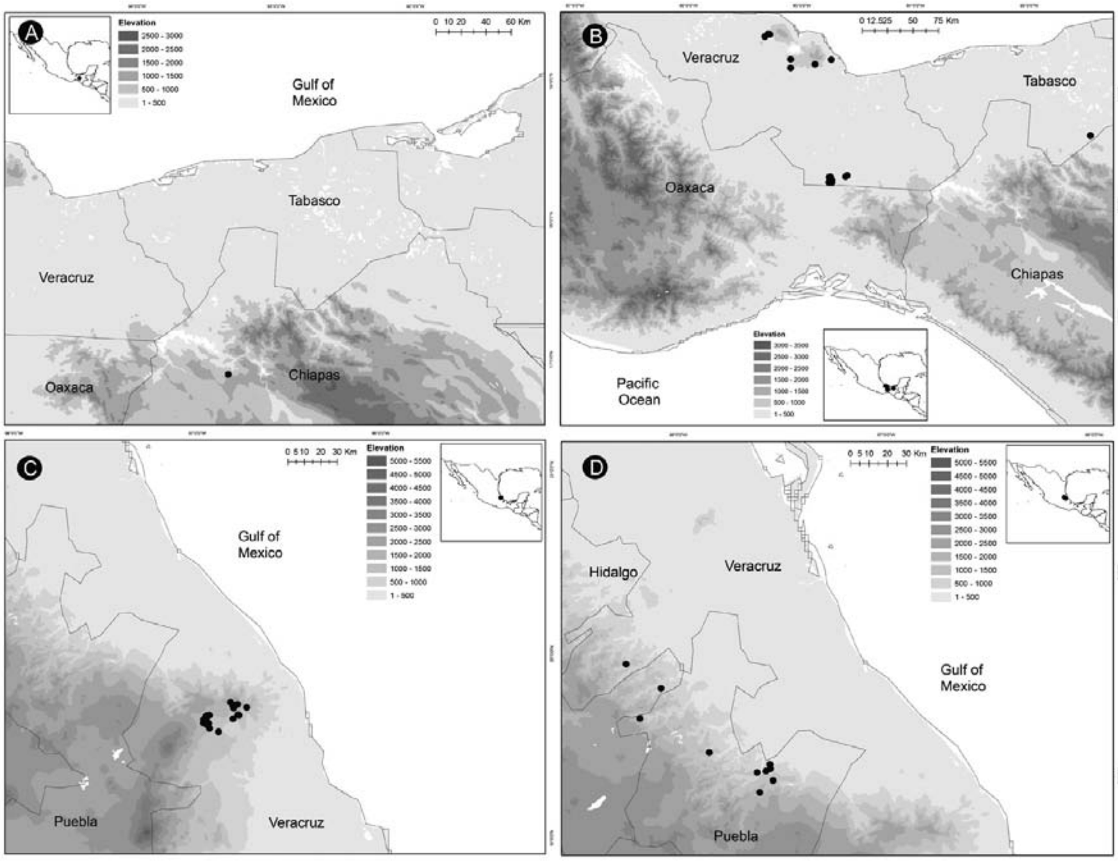

Figure 24. Distribution of *Ceratozamia* species. **A** *C. santillanii* **B** *C. subroseophylla* **C** *C. tenuis* **D** *C. totonacorum.*

30. *Ceratozamia subroseophylla* Mart.-Domínguez & Nic.-Mor., Phytotaxa 268(1): 35. 2016

Figs 12D, 25, 26

Ceratozamia dominguezii Pérez-Farr. & Gut.Ortega, Taxonomy 1: 353. 2021. Type: Mexico. Veracruz: Mun. Uxpanapa, 130 m, 29 May 2021, *M.A. Pérez-Farrera 4013* (holotype: HEM [n.v]; isotypes: MEXU [n.v], XAL [n.v]).

Type. Mexico. Veracruz: Mun. Santiago Tuxtla, hill in front Sinapán, 425 m, 15 Jul 2014, *L. Martínez-Domínguez 158* ♀ (holotype: CIB! [acc. # 16893UV]; isotypes: MEXU! [MEXU01446538–MEXU01446542], NY!).

Description. ***Stem*** 30–250 (500) cm long, 18–45 cm in diameter, epigeous, erect and decumbent. ***Cataphylls*** 5–7 × 2–2.5 cm wide at the base, persistent, triangular, reddish brown, densely brown tomentose at emergence, glabrous at maturity, apex acuminate. ***Leaves*** 10–83, 72–370 cm long, ascending, yellowish brown at emergence, with whitish gray trichomes, glabrous at maturity. ***Petiole*** 50–150 cm long, terete, linear, greenish brown or dark brown at emergence, green in mature leaves; with 25–55 robust prickles, 0.40–0.85 cm long. ***Rachis*** 80–255 cm long, terete, linear, greenish brown or dark brown at emergence, green in mature leaves, with prickles. ***Leaflets*** 23–48 pairs, opposite to subopposite, insertion in one plane, lanceolate, abaxially curved longitudinally along distal half, not basally falcate, papyraceous, flat, dark green with adaxial side glabrous and abaxial side glaucous, acuminate and symmetrical at the apex, attenuate at base, with conspicuous and light-green veins; median leaflets 18–45 × 2.5–4 cm, 1.7–6.5 cm between leaflets; articulations 0.7–1.9 cm wide, brown in young leaves and green in mature leaves. ***Pollen strobili*** 15–30 cm long, 3.1–5.4 cm in diameter, solitary, cylindrical, erect, green with reddish trichomes at emergence, greenish yellow at maturity with dark brown trichomes; peduncle 9.5–19 cm long, 1.5–2.7 cm in diameter, tomentose, reddish brown; microsporophylls 1.47–2.8 × 1.01–1.4 cm, obconic with a non-recurved distal face and lobate fertile portion, infertile portion 0.49–0.65 cm long and rounded with straight horns 0.20–0.30 cm long, 0.48–0.53 cm and an acute angle between the horns. ***Ovulate strobili*** 15.5–40 cm long, 7–11.6 cm in diameter, solitary, cylindrical, erect, yellow with reddish to purple trichomes at emergence, green and megasporophylls with base pale pink and dark brown to reddish brown trichomes at maturity, mucronate apex; peduncle 9.8–17.5 cm long, 1.8–2.3 cm in diameter, erect, tomentose, light brown; megasporophylls 110–210, 9–12 orthostichies with 11–20 sporophylls orthostichy, 1.8–2.5 × 2.5–4.47 cm, with a prominent distal face, horns straight 0.34–1.0 cm long and 0.70–1.40 cm between horns with a right angle between the horns. ***Seeds*** 2.9–3.8 cm long, 0.80–1.75 cm in diameter ovate, sarcotesta whitish pink when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia subroseophylla* occurs from southeastern Veracruz State to Tabasco State, Mexico including the montane zone of the region Santiago Tuxtla and Uxpanapa (Fig. 24B). It occurs on soils of volcanic origin and karstic rocks in evergreen tropical rain forest between 111 and 1,050 m.

Etymology. The specific epithet efers to the rosaceous (i.e., pale pink) color at the base of megasporophylls.

Common names. Mexico. Veracruz: Hymniom pekmuk (Popoluca ethnic group) (Leonti 542).

Uses. The stem is boiled to make medicinal tea to treat kidney stones (Leonti 542).

Preliminary conservation status. *Ceratozamia subroseophylla* has several populations throughout its distribution range, but anthropogenic land-use changes affect the populations of Veracruz and Tabasco. According to the IUCN Red List criteria, we recommend "Endangered" (EN) for this species.

Discussion. *Ceratozamia subroseophylla* is distinguished from *C. leptoceras* and *C. oliversacksii* by its green-brownish petiole and rachis with abundant and robust prickles, and its lanceolate and papyraceous leaflets (Fig. 25). In addition, *C. subroseophylla* has affinity with *C. robusta*, but it is easily identified by the ovulate strobilus which has mucronate apex, rosaceous base of megasporophylls rosaceous with dark brown to reddish brown trichomes at maturity (Fig. 26).

Populations from Uxpanapa (Veracruz) were recently described as *Ceratozamia dominguezii*, however, these fall within the range of variation of *C. subroseophylla* as circumscribed here. Our circumscription is based on comparative morphology, both vegetative and reproductive structures, and phenology (Figs 25, 26).

Figure 25. Vegetative characters of *Ceratozamia subroseophylla* **A** adult plant (from Uxpanapa, Veracruz) **B** young leaf (from Santiago Tuxtla, Veracruz) **C** new leaves of adult plant (from Santiago Tuxtla, Veracruz) **D** prickles on rachis (from Uxpanapa, Veracruz).

Specimens examined. Mexico. **Tabasco: Mun. Macuspana**, 185 m, 13 Jul 2021, *F. Nicolalde-Morejón et al. 3674–3680* (CIB); 185 m, 13 Jul 2021, *L. Martínez-Domínguez et al. 2295–2300* (CIB), 190 m, 13 Jul 2021, *L. Martínez-Domínguez et al. 2301* (CIB, MEXU). **Veracruz: Mun. Catemaco**, 1953, *H. Bravo 26* (MEXU); 18 May

Figure 26. Reproductive characters of *Ceratozamia subroseophylla* **A** immature pollen strobilus (from Santiago Tuxtla, Veracruz) **B** pollen strobilus at maturity (from Santiago Tuxtla, Veracruz) **C** immature ovulate strobilus (from Uxpanapa, Veracruz) **D** immature ovulate strobilus (from Santiago Tuxtla, Veracruz) **E** ovulate strobilus at maturity (Uxpanapa, Veracruz) **F** seeds.

1995, *M.A. García B. et al. 813* (XAL). **Mun. Hidalgotitlán**, 150 m, 16 Jan 1975, *Brigada Vázquez 1760* (MO, XAL); 140 m, 16 Dec 1974, *J. Rees 1655* (XAL); 16 Apr 1975, *M. Vázquez-Torres 1760* (MEXU). **Mun. Hueyapan de Ocampo**, 400 m, 14

May 2000, *Leonti 542* (MEXU). **Mun. Jesús Carranza**, 100 m, 19 Feb 2009, *D. Jimeno-Sevilla 1045* (MEXU, MO, XAL); 131 m, 27 Sep 2020, *L. Martínez-Domínguez et al. 2040* (CIB); *M. Vázquez-Torres 2430* (CHAPA, ENCB, MEXU, NY, SLPM, XAL). **Mun. Mecayapan**, 950 m, 26 Jan 1992, *M. Vázquez-Torres et al. 4122* (CIB). **Mun. Santiago Tuxtla**, 500 m, 5 Jul 1983, *D.W. Stevenson et al. 539 A–L* (NY), *540 F–H* (NY); 420 m, 28 Mar 2014, *F. Nicolalde-Morejón & L. Martínez-Domínguez 1985–1987* (CIB); 250–500 m, 30 Sep 1983, *J.F. Ortega O. et al. 358* (XAL); 420 m, 28 Mar 2014, *L. Martínez-Domínguez & F. Nicolalde-Morejón 78, 79* (NY), *80, 81* (XAL), *82–87* (CIB); 420 m, 7 Jun 2014, *L. Martínez-Domínguez & F. Nicolalde-Morejón 129* (CIB); 425 m, 15 Jul 2014, *L. Martínez-Domínguez 136–157, 159* (CIB); 500 m, 12 May 1965, *M. Sousa 2420* (MEXU); 23 Aug 1962, *R.F. Andrle 64* (US); 497 m, 17 Jan 2001, *T.W. Walters TW-2001-17* (MEXU, XAL). **Mun. Soteapan**, 21 Jun 1963, *G.N. Ross 57* (US); 1,050 m, 17 Mar 1968, *M. Sousa 3645* (MEXU); 500 m, 18 May 1986, *M. Vázquez-Torres et al. 3579* (CIB). **Mun. Tatahuicapan de Juárez**, 849 m, *C.I. Carvajal-Hernández & M. Juárez F. 796* (CIB). **Mun. Uxpanapa**, 131 m, 27 Sep 2020, *L. Martínez-Domínguez et al. 2040* (CIB, MEXU); 111 m, 27 Sep 2020, *L. Martínez-Domínguez et al. 2041* (CIB), *2042* (CIB, MEXU), *2043–2048* (CIB); 111 m, 11 Jun 2021, *L. Martínez-Domínguez et al. 2273* (CIB).

31. *Ceratozamia tenuis* (Dyer) D.W.Stev. & Vovides, Bot. Sci. 94 (2): 425. 2016
Fig. 27F

Ceratozamia tenuis Type. Cultivated in England at the Royal Botanic Gardens Kew "Hort. Kew Palm House", 1881, *Anon. s.n.* (lectotype, designated by Vovides et al. 2016, pg. 425: K! [K001092673, K001092674]). Mexico. Veracruz: Mun. Jilotepec, 19 Jan 1976, *A.P. Vovides 18* ♂ (epitype, designated by Martínez-Domínguez et al. 2018a, 117: XAL! [acc. # 16980]; isoepitype: NY!).

Ceratozamia mexicana Brongn. var. *vulgaris* J.Schust., Pflanzenr. (Engler) Heft 99, 4 fam 1: 131. 1932. Mexico. Veracruz: Mun. Xalapa, Chiltoyac, 1,270 m, 18 Oct 2016, *L. Martínez-Domínguez et al. 984* (neotype, designated by Martínez-Domínguez et al. 2018a, pg. 117: CIB! [acc. # 17988UV]).

Ceratozamia mexicana f. *tenuis* (Dyer) J.Schust., Pflanzenr. (Engler) Heft 99, 4 fam 1: 132. 1932, as "*Ceratozamia mexicana* var. *longifolia* f. *tenuis*". Type: Based on *Ceratozamia mexicana* Brongn. var. *tenuis* Dyer

Type. Based on *Ceratozamia mexicana* Brongn. var. *tenuis* Dyer, Biol. Cent.-Amer., Bot. 3: 193. 1884.

Description. ***Stem*** 20–100 cm long, 30–45 cm in diameter, epigeous, erect and decumbent. ***Cataphylls*** 2–6 × 2–5.5 cm wide at the base, persistent, triangular, reddish brown, densely brown tomentose at emergence, partially tomentose at maturity, apex acuminate. ***Leaves*** 6–56, 85–225 cm long, ascending, dark green at emergence with brown trichomes, glabrous at maturity. ***Petiole*** 30–93 cm long, terete, linear, green in mature leaves; with 10–55 thin prickles, 0.20–0.49 cm long. ***Rachis*** 56–154 cm long, terete, linear, green in mature leaves, with prickles. ***Leaflets*** 30–56 pairs, opposite to subopposite, insertion in one plane, linear, longitudinally curved abaxially to planar, basally falcate, papyraceous, involute, green with adaxial and abaxial sides glabrous, distal end with entire margins, acuminate and symmetrical at apex, attenuate at base, with conspicuous and light-green veins; median leaflets 23–50.5 × 1–2.1 cm, 0.3–2.5 cm between leaflets; articulations 0.4–1.4 cm wide, green. ***Pollen strobili*** 25–50 cm long, 4–8 cm in diameter, solitary, cylindrical, erect, greenish yellow at emergence, greenish yellow with blackish trichomes at maturity; peduncle 3.7–22 cm long, 1.2–2.5 cm in diameter, tomentose, reddish brown to light-brown; microsporophylls 1.7–2.7 × 1.2–1.9 cm, obconic with a non-recurved distal face and a lobate fertile portion, infertile portion 0.50–0.65 cm long and orbicular with recurved horns 0.25–0.46 cm long, 0.35–0.65 cm between the horns and a right angle between the horns. ***Ovulate strobili*** 22–35 cm long, (7.6) 10–14 cm in diameter, solitary, cylindrical, erect, dark green with blackish trichomes at emergence, dark green with blackish trichomes at maturity, acuminate apex; peduncle 8–23 cm long, 1.5–2.4 cm in diameter, erect or pendulous, tomentose, brown to reddish brown; megasporophylls (48) 80–196, 7–16 orthostichies with 6–14 sporophylls per orthostichy, 2.1–3.1 × 3.0–5.0 cm, with a prominent distal face, horns curved and 0.32–0.80 cm long, 0.80–1.60 cm between horns with a right angle between the horns. ***Seeds*** 2.5–3 cm long, 1.3–1.8 cm in diameter, ovate, sarcotesta whitish yellow to yellow when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia tenuis* is endemic to the central montane region in Veracruz State, Mexico (Fig. 24C), where it occurs in cloud forest at 1,200–1,920 m elevation on volcanic soils with basaltic rocks.

Etymology. The specific epithet is derived from its thin leaflets.

Common names. Mexico. Veracruz: Costilla de león (L. Martínez-Domínguez et al. 573); palma del monte (L. Martínez-Domínguez et al. 166).

Uses. Ovulate strobili are used as an insecticide; these are cut in half and mixed with milk or sugar to kill flies (L. Martínez-Domínguez et al. 980).

Conservation status. *Ceratozamia tenuis* has not been assessed for The IUCN Red List of Threatened Species. This species has populations with several adult plants (between 100 to 300); however, the total area of distribution is narrow and it is one of the areas that is highly affected by changes in land use in recent years. According to IUCN criteria this species should be considered as "Endangered" (EN) under A1acd; B1ab(iii).

Discussion. *Ceratozamia tenuis* is characterized by a petiole with thin prickles, and linear leaflets that are papyraceous and involute with a symmetric apex. The ovulate strobilus is dark green with blackish trichomes at maturity, a prominent distal face, and a right angle between the horns.

Specimens examined. Mexico. **Veracruz: Mun. Banderilla**, 1,450 m, 21 Apr 2017, *L. Martínez-Domínguez et al. 1000* (CIB). **Mun. Chiconquiaco**, 1,800 m, 26 Nov 1974, *D. Jimeno-Sevilla 754* (XAL); 1,916 m, 27 Sep 2016, *F. Nicolalde-Morejón et al. 2456–2464* (CIB); 1,800 m, 26 Nov 1974, *J. Rees 1625* (XAL), *1626*

Figure 27. Plants of *Ceratozamia* in habitat. **A** *C. norstogii.* **B** *C. oliversacksii* **C** *C. robusta* **D** *C. sabatoi* **E** *C. santillanii* **F** *C. tenuis* **G** *C. totonacorum* **H** *C. vovidesii* **I** *C. zaragozae*.

(MEXU, XAL); 1,916 m, 27 Sep 2016, *L. Martínez-Domínguez et al. 971, 973–981* (CIB), *972* (CIB, MEXU); 13 Apr 1967, *R. Fernandez-Nava 385A* (MEXU). **Mun. Coacoatzintla**, 8 Mar 1985, *F. Vazquez B. 2275* (XAL); 1,400 m, 7 Jan 1977, *G. Castillo-Campos 118* (XAL); 1,550 m, 3 Jun 2005, *L.H. Bojórquez-Galván & A.M. Zapata-Aquino 1484, 1485* (CIB); 1,435 m, 9 Jan 2015, *L. Martínez-Domínguez et* *al. 165–184* (CIB); 1, 435 m, 18 Mar 2015, *L. Martínez-Domínguez et al. 273–282* (CIB); 1,540 m, 9 Feb 2016, *L. Martínez-Domínguez et al. 759* (CIB). **Mun. Jilotepec**, 1,300 m, 29 Feb 1980, *A.P. Vovides 470* (XAL), *471* (IBUG, MEXU, XAL); 1,300 m, 14 Apr 1982, *735* (XAL); 1,385 m, 15 Nov 1978, *E. Estrada et al. 757* (MEXU); 1,316 m, 22 Aug 2014, *F. Nicolalde-Morejón* & *L. Martínez-Domínguez 2067–2086* (CIB); 1,350 m, 7 Dec 1970, *F. Ventura A. 2936* (ENCB); 1,300 m, 22 Jan 1971, *F. Ventura A. 3014* (ENCB); 1,300 m, 18 Nov 1974, *J. Rees 1620* (XAL); 1,363 m, 29 May 2015, *L. Martínez-Domínguez et al. 573–583* (CIB); 1,250 m, 23 Aug 1973, *M.G. Zola 657* (XAL), *667* (MEXU, XAL); 1,250 m, 23 Aug 1975, *R. Ortega J. 525* (XAL); Mar 2001, *S. Avendaño 5395* (MEXU). **Mun. Tepetlán**, 1,420 m, 12 Jan 2013, *F. Nicolalde-Morejón et al. 1691–1710* (CIB); 1,418 m, 21 Jun 2014, *F. Nicolalde-Morejón & L. Martínez-Domínguez 2001–2004* (CIB); 1,418 m, 22 Aug 2014, *F. Nicolalde-Morejón & L. Martínez-Domínguez 2047–2066* (CIB); 1,662 m, 10 Apr 2015, *F. Nicolalde-Morejón & L. Martínez-Domínguez 2217–2226* (CIB); 1,421 m, 26 Jul 2014, *L. Martínez-Domínguez et al. 160* (CIB); 22 Mar 2015, *L. Martínez-Domínguez & F. Nicolalde-Morejón 283–293* (CIB); 1,662 m, 10 Apr 2015, *L. Martínez-Domínguez & F. Nicolalde-Morejón 545–555* (CIB); 2 Jul 2010, *M. Vázquez-Torres et al. 9215* (CIB). **Mun. Tlacolulan**, 1,540 m, 16 Jun 2017, *F. Nicolalde-Morejón et al. 2516* (CIB); 1,540 m, 16 Jun 2017, *L. Martínez-Domínguez et al. 1041, 1042* (CIB). **Mun. Xalapa**, 1,270 m, 18 Oct 2016, *F. Nicolalde-Morejón et al. 2466–2468* (CIB); 1,270 m, 18 Oct 2016, *L. Martínez-Domínguez et al. 985, 987* (CIB), 986 (CIB, MEXU).

32. *Ceratozamia totonacorum* Mart.-Domínguez & Nic.-Mor., Brittonia 69 (4): 518. 2017

Figs 27G, 28B

Type. Mexico. Puebla: Mun. Jonotla, 600 m, 9 Jun 2015, *L. Martínez-Domínguez* & *F. Nicolalde-Morejón 618* ♀ (holotype: CIB! [acc. # 16735UV]).

Description. ***Stem*** 10–45 cm long, 10–25 cm in diameter, epigeous, erect and decumbent. ***Cataphylls*** 2–5 × 1.2–2.5 cm wide at the base, persistent, triangular, reddish brown, densely brown tomentose at emergence, glabrous at maturity, apex acuminate. ***Leaves*** 10–63, 100–265 cm long, descending, brown at emergence, with brown trichomes, glabrous at maturity. ***Petiole*** 30–80 cm long, terete, straight, green in mature leaves; with 10–40 thin prickles, 0.05–0.25 cm long. ***Rachis*** 85–185 cm long, terete, straight, green in adult leaves, with prickles. ***Leaflets*** 11–33 pairs, opposite to subopposite, insertion in one plane, oblong, longitudinally planar, not basally falcate to occasionally falcate, papyraceous, flat, green with adaxial side glabrous and abaxial side glaucous, distal end with entire margins, acuminate and symmetrical at the apex, attenuate at base, with conspicuous and light-green veins; median leaflets 17–40 × 2.7–4.2 cm, 2–5.6 cm between leaflets; articulations 0.5–1.3 cm wide, green. ***Pollen strobili*** 28–31 cm long, 5.0–6.0 cm in diameter, generally solitary (up to 2), cylindrical, erect, greenish yellow at emergence, yellow with brown trichomes at maturity; peduncle

9–12 cm long, 1.5–2 cm in diameter, tomentose, light brown; microsporophylls 1.5–2.7 × 0.9–2.0 cm, obconic with a non-recurved distal face and a lobate fertile portion, infertile portion 0.39–0.57 cm long and rounded with straight horns 0.19–0.25 cm long, 0.52–0.69 cm and a right angle between the horns. ***Ovulate strobili*** 20.5–28.7 cm long, 8.4–9.3 cm in diameter, solitary, cylindrical, erect, light green and glaucous, with orange to light brown trichomes at emergence, green with yellowish brown trichomes at maturity, acuminate apex; peduncle 10–11.2 cm long, 1.5–2.4 cm in diameter, erect or pendulous, tomentose, light brown; megasporophylls 64–120, 8–10 orthostichies with 8–13 sporophylls per orthostichy, 1.6–2.3 × 2.6–3.6cm, with a prominent distal face, horns straight and 0.55–0.80 cm long, 1.45–1.80 cm between horns with a right angle between the horns. ***Seeds*** 2.5–3.5 (4) cm long, 0.88–1.6 cm in diameter, ovate, sarcotesta whitish red when immature, cream to light brown at maturity.

Distribution and habitat. *Ceratozamia totonacorum* occurs in the Sierra Norte de Puebla and the mountain region in Hidalgo and Veracruz States, Mexico (Fig. 24D). It occurs in cloud forest and the transition zone between evergreen tropical forest and cloud forest on rocky outcrops in exposed walls up to 80 m tall at 600–1,800 m.

Etymology. The specific epithet is in reference to the Totonaco ethnic group of Santiago Ecatlán in Sierra Norte of Puebla, Mexico.

Common names. Mexico. Puebla: Kun (Totonaco ethnic group) (Martínez-Domínguez et al. 2017b).

Uses. In Sierra Norte of Puebla, the residents use the leaves of this species in local rituals to make "arcos" and altars (Fig. 28B) (Martínez-Domínguez et al. 2017b).

Preliminary conservation status. Based on total populations and the potential distribution and reduction of vegetation in the area for *Ceratozamia totonacorum*, we recommend that it should be listed as "Vulnerable" in The IUCN Red List of Threatened Species.

Discussion. *Ceratozamia totonacorum* is distinguished from *C. delucana* by its brown leaves at emergence, but this color disappears in the mature leaves which become green; besides, the leaves are descending. The ovulate strobilus is yellowish green with brown trichomes.

Figure 28. Leaves of *Ceratozamia* are used for religious ceremonies **A** *C. morettii* used during local celebrations in La Estrella (Chiconquiaco municipality, Veracruz, Mexico) **B** *C. totonacorum* used during "San Judas Tadeo" festivities in La Unión Atioyan (Nauzontla municipality, Puebla, Mexico).

Specimens examined. Mexico. **Hidalgo: Mun. Huehuetla**, 1,150 m, 2 Jun 1976, *A.P. Vovides 23* (XAL). **Puebla: Mun. Atlequizayan**, 1 Apr 2014, 867 m, *L. Caamaño Onofre & A.B. Cerón Carpio 4995* (XAL). **Mun. Cuetzalan del Progreso**, 1,470 m, 1 Nov 2017, *L. Martínez-Domínguez et al. 1180–1189* (CIB). **Mun. Jonotla**, 760 m, 13 Feb 2014, *F. Nicolalde-Morejón et al. 1948* (CIB); 600 m, 13 Feb 2014, *F. Nicolalde-Morejón et al. 1949–1955* (CIB), *1956* (MEXU), *1957* (NY), *1958–1965* (CIB); 1,006 m, 14 Feb 2014, *F. Nicolalde-Morejón et al. 1966, 1967* (CIB); 600 m, 9 Jun 2015, *L. Martínez-Domínguez & F. Nicolalde-Morejón 619* (CIB). **Mun. Pahuatlán**, 1,800 m, 12 Jan 1987, *G. Toriz et al. 226* (MEXU). **Mun. Tlapacoya**, 1,010 m, 14 Feb 1985, *E. Meza P. 14* (XAL). **Mun. Zacapoaxtla**, 1, 365 m, 30 May 2014, *L. Camaño-Onofre 5329* (XAL). **Veracruz: Mun. Tlachichilco**, 1,300 m, 12 Sep 2001, *A. Rincón G. et al. 2584* (XAL), *2585* (MEXU, XAL).

33. *Ceratozamia vovidesii* Pérez-Farr. & Iglesias, Bot. J. Linn. Soc. 153: 394. 2007

Fig. 27H

Type. Mexico. Chiapas: Mun. La Concordia, Between Finca Santa Cruz and El Puente, Rancho Las Cabañas, 1,156 m, 19 Jul 2001, *M.A. Pérez-Farrera 2620a* ♀ (holotype: HEM!; isotypes: CHIP [n.v.], MEXU [n.v.], MO [n.v.], XAL [n.v.]).

Description. ***Stem*** 50–80 cm long, 20–30 cm in diameter, epigeous, erect and decumbent. ***Cataphylls*** 5–7.5 × 2.7–5 cm wide at the base, persistent, triangular, reddish brown, densely brown tomentose at emergence, partially tomentose at maturity, apex acuminate. ***Leaves*** 3–18, 97–238 cm long, ascending, reddish brown at emergence with whitish gray trichomes, glabrous at maturity. ***Petiole*** terete, linear, 40–164 cm long, green in adult leaves, with 15–55 thin prickles, 0.14–0.38 cm long. ***Rachis*** terete, linear, 54–153 cm long, green in mature leaves, with prickles. ***Leaflets*** 30–85, opposite to subopposite, insertion in one plane, lanceolate, mostly longitudinally planar, generally basally falcate, papyraceous, flat, green with adaxial side glabrous and abaxial side glaucous, distal end with entire margins, acuminate and symmetrical apex, attenuate at base, with conspicuous and light-green veins; median leaflets 20–45 × 0.7–1.4 cm, 0.3–2.4 cm between leaflets; articulations 0.2–0.8 cm wide, green. ***Pollen strobili*** 15–45 cm long, 3.5–5 cm in diameter, solitary, cylindrical, erect, yellowish green with reddish brown trichomes at emergence, yellowish cream with reddish brown trichomes at maturity; peduncle 6–9.5 cm long, 1.6–1.9 cm in diameter, tomentose, reddish brown to brown; microsporophylls 1.2–1.5 × 0.8–1.04 cm, obconic with a non-recurved distal face and lobate fertile portion, infertile portion 0.37–0.50 cm long and rounded with straight horns 0.30–0.42 cm long, 0.52–0.84 cm with a right angle between the horns. ***Ovulate strobili*** 26–40 cm long, 7.1–9.6 cm in diameter, solitary, cylindrical, erect, greyish green with abundant reddish brown trichomes at emergence, green with abundant blackish trichomes at maturity, acuminate apex; peduncle 7–15 cm long, 1.7–2.2 cm in diameter, erect or pendulous, pubescent, brown; megasporophylls 60–70, 6–8 orthostichies with 7–12 sporophylls per row, 3.8–4.5 × 4–5 cm, with a prominent distal face, horns straight and 0.60–0.80 cm long, 0.99–1.40 cm between horns with an obtuse angle between the horns. ***Seeds*** 2.2–2.7 cm long, 1.3–1.7 cm in diameter, ovate, sarcotesta whitish yellow to yellow when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia vovidesii* is distributed along the Sierra Madre of Chiapas State in Mexico to Guatemala. It occurs on karstic rocks in cloud forest between 800 and 1,850 m elevation (Fig. 29A).

Etymology. The specific epithet honors Andrew P. Vovides in recognition of his systematic and ecological studies and efforts in the conservation of Mexican cycads, including the creation of the Mexican National Cycad Collection at the Francisco Javier Clavijero Botanic Garden in Xalapa (Veracruz, Mexico) (Pérez-Farrera et al. 2007).

Common names. None recorded.

Uses. Decorative (M.A. Pérez-Farrera 2620a).

Conservation status. (IUCN 2021). *Ceratozamia vovidesii* is listed as "Vulnerable" (V) under criteria D2. Here, we extended the distribution range for this species to Guatemala. Based on number of populations and data obtained during fieldwork we recommend not changing the current status.

Discussion. *Ceratozamia vovidesii* shares several vegetative characteristics with *C. mirandae*. However, there are differences in their pollen strobili; *C. vovidesii* has obconic microsporophylls with rounded infertile portions, whereas those of *C. mirandae* are elliptic with a linear infertile portion.

There is some confusion concerning the holotype for *C. vovidesii*. In 2012 the gathering *Pérez-Farrera 2620*[a] also was used as the holotype in the protologue of *Zamia grijalvensis* Perez-Farr., Vovides & Mart.-Camilo (Pérez-Farrera et al. 2012). The specimen, *Pérez-Farrera 2620*[a] is clearly a *Ceratozamia* and not a *Zamia* and corresponds to the protologue of *C. vovidesii*. Another specimen, *Pérez-Farrera 3026*, is labelled as the holotype for *Z. grijalvensis* and it clearly matches the protologue of that taxon. We are treating this miscitation of *Pérez-Farrera 2620*[a] in the protologue of *Z. grijalvensis* as a typographical error, which is now corrected.

Specimens examined. Guatemala. **Huehuetenango:** 900–1,300 m, 3 Sep 1942, *J.A. Steyermark 51818* (NY; US); 1,630 m, 9 Jul 2006, *M. Véliz & V. Davila 17042, 17043, 17044* (BIGU); 30 May 1906, *O.F. Cook 51* (US); 1,629 m, 10 Jul 2006, *V. Davila & M. Véliz 1050* (BIGU); 1,622 m, 10. Jul 2006, *V. Davila & M. Véliz 1052, 1053* (BIGU). Mexico**. Chiapas: Mun. Ángel Albino Corzo**, 730 m, 23 Jan 1968, *Alush Shilom Ton 3554* (ENCB); 14 Dec 1980, 1,380 m, *D.E. Breedlove 48678* (NY); 800–1,000 m, 8 Nov 1945, *E.H. Xolocotzi & A.J. Sharp 402* (CHAPA, ENCB, MEXU); 1,000 m, 2 Jun 1987, *E.M. Martínez S. et al. 21586* (MEXU); 1,819 m, 13 Aug 2009, *H. Gómez-Domínguez 2316* (MEXU); 1,650 m, 18 May 1982, *J.I. Calzada et al. 9131* (XAL); 5 Mar 1989, *U. Bachem C. & R. Rojas 405* (CHIP). **Mun. Jaltenango de la Paz**, Jun 1995, *E. Matuda s/n* (MEXU); 1,500 m, 23 Jun 1990, *M. Heath & A. Long 1287* (CHIP); 25 Feb 1995, *Miranda 7042* (MEXU). **Mun. La Concordia,** 1,000 m, 5 Jun 1988, *E. Palacios E. 1050* (CHIP); 1,840 m, 26 Jun 2018, *F. Nicolalde-Morejón et al. 2864–2875* (CIB); 1,840 m, 26 Jun 2018, *L. Martínez-Domínguez et al. 1439–1451* (CIB); 1,156 m, 19 Sep 2001, *M.A. Pérez-Farrera 2621* (XAL); 1,600 m, *M.A. Pérez-Farrera s/n* (CHIP); 1,120 m, 17 Jun 2014, *M.G. Díaz M. 961* (CHIP);

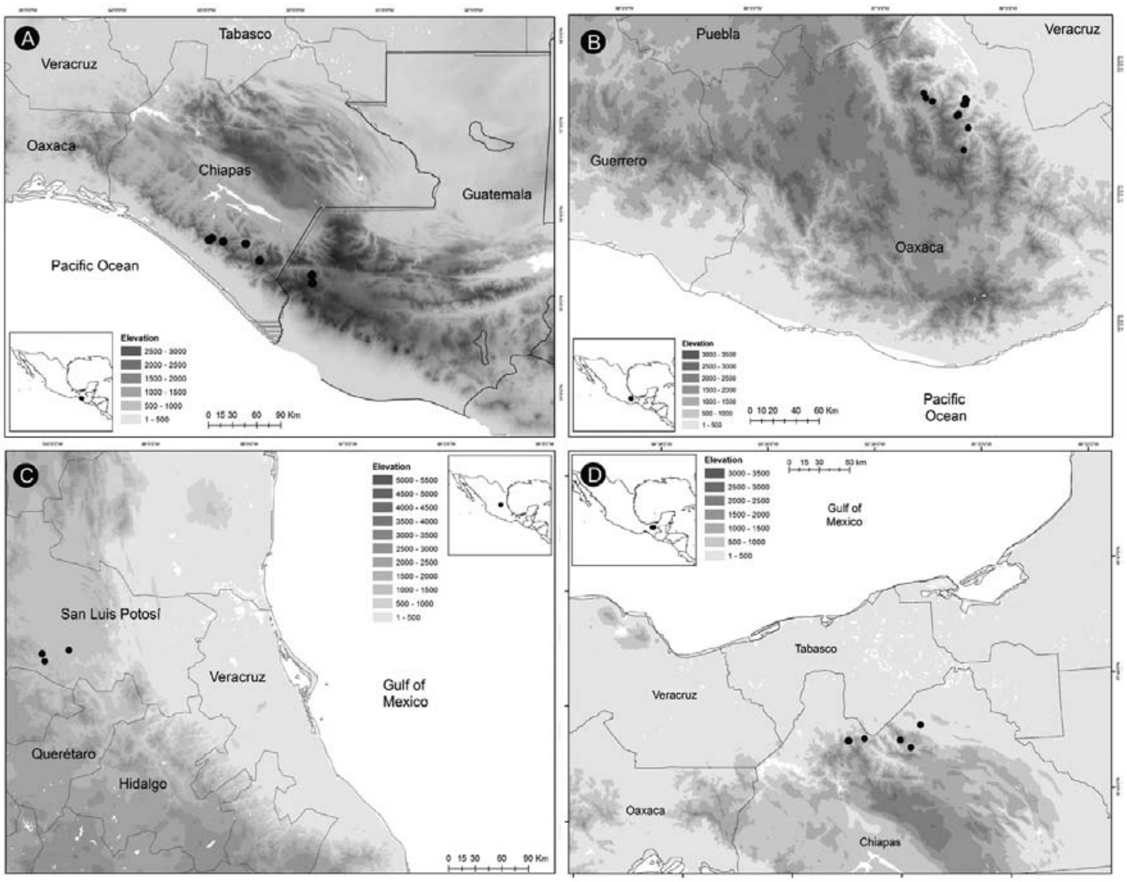

Figure 29. Distribution of *Ceratozamia* species. **A** *C. vovidesii* **B** *C. whitelockiana* **C** *C. zaragozae* **D** *C. zoquorum.*

24 Mar 2001, *R. Martínez-Camilo 54* (CHIP); 1,100 m, 11 Jun 1988, *T.G. Cabrera-Cachón 74* (CHIP); 1,700 m, 1 Jun 1989, *U. Bachem C. & R. Rojas 795* (CHIP). **Mun. Mapastepec**, 1,750 m, 13 May 1982, *J.I. Calzada et al. 8874* (IBUG, MEXU, MO, XAL). **Mun. Siltepec**, 28 Feb 2000, *O. Farrera S. 1958* (CHIP).

34. *Ceratozamia whitelockiana* Chemnick & T.J.Greg., Phytologia 79(1): 51. 1996 ("1995")

Fig. 30

Type. Mexico. Oaxaca: Vicinity of Metates, south of Valle Nacional, 628 m, 10 May 1995, *J. Chemnick & T. Gregory 5* (holotype: HNT [n.v.]; isotypes: FTG!, XALU [n.v.]).

Description. ***Stem*** 20–80 cm long, 18–30 cm in diameter, epigeous, decumbent. ***Cataphylls*** 2–5 × 2–5 cm wide at the base, persistent, triangular, reddish brown, densely brown tomentose at emergence, partially tomentose at maturity, apex acuminate. ***Leaves*** 3–10, 129–250 cm long, descending, light green and glaucous at emergence with whitish gray trichomes, glabrous at maturity. ***Petiole*** 60–140 cm long, terete, linear, green in mature leaves; with 3–22 thin prickles, 0.06–0.39 cm long.

Rachis 70–160 cm long, terete, linear, greenish in mature leaves, unarmed. ***Leaflets*** 20–48 pairs, opposite to subopposite, insertion in one plane, lanceolate, generally longitudinally planar, not basally falcate, papyraceous, flat, green with adaxial and abaxial sides glabrous, distal end with entire margins, acuminate and symmetrical at the apex, attenuate at base, with conspicuous and green-light veins; median leaflets 25–38.5 × 2.0–3.7 cm, 1.6–3.1 cm between leaflets; articulations 0.5–1.4 cm wide, green. ***Pollen strobili*** 20–30 cm long, 3–5 cm in diameter, generally solitary (1–2), cylindrical, erect, greenish at emergence with reddish trichomes, greenish yellow with reddish brown trichomes at maturity; peduncle 15–25 cm long, 1.2–1.9 cm in diameter, glabrous or with trichomes scarce reddish brown to brown; microsporophylls 1.5–3.0 × 0.8–1.5 cm, elliptic with a non-recurved distal face and a lobate fertile portion, infertile portion 0.40–0.50 cm long and linear with straight horns 0.38–0.50 cm long, 0.50–1.0 cm and a right angle between the horns. ***Ovulate strobili*** 14–20 cm long, 7–10 cm in diameter, solitary, cylindrical, erect, yellowish green with abundant blackish trichomes at emergence, green with brown to blackish trichomes at maturity, apiculate apex; peduncle 1–4 cm long, 1.0–1.8 cm in diameter, erect, glabrous or with trichomes scarce, reddish brown; megasporophylls 24–40, 4–5 orthostichies with 5–8 sporophylls per orthostichy, 1.7–2.2 × 4.5–5.1 cm, with a truncate distal face, horns straight and 0.98–1.4 cm long, 0.95–1.3 cm between horns and an acute angle between the horns. ***Seeds*** 2.1–2.7 cm long, 1.4–1.8 cm in diameter, ovate, sarcotesta whitish yellow to yellow when immature, light brown at maturity.

Distribution and habitat. *Ceratozamia whitelockiana* is endemic to the Sierra Norte of Oaxaca (Mexico), between 500 to 1,800 m in La Chinantla area (Fig. 29B). It occurs in the elevational gradient of evergreen tropical forest with *Quercus* sp. and cloud forest with *Oreomunnea mexicana* (Standl.) J.-F.Leroy on karstic rocks.

Etymology. This species was named in honor of Loran Whitelock for his contributions to cycad biology (Chemnick and Gregory 1996).

Common names. None recorded.

Uses. None recorded.

Conservation status. (IUCN 2021). *Ceratozamia whitelockiana* is listed as “Endangered (EN)” under criteria A2c; B1ab(i,ii,iii,v)+2ab(i,ii,iii,v); C1.

Discussion. *Ceratozamia whitelockiana* is similar to *C. mixeorum* in leaf morphology; the only difference is the sparse prickles in *C. whitelockiana* (between 3 to 22, in number), whereas *C. mixeorum* has more than 28 prickles. Additionally, the length of the petiole is very long in relation to the total size of the leaf in *C. whitelockiana*. In reproductive structures, the ovulate strobili in *C. whitelockiana* have a long peduncle 12–23 cm and the fertile portion has 4–5 orthostichies with 5–8 sporophylls per orthostichy and the pollen strobili have a long peduncle that is the same size as the fertile part or longer (Fig. 30 A, C). In contrast, *C. mixeorum* has ovulate strobili with a short peduncle from 1–4 cm long and pollen strobili with peduncles shorter than the fertile area.

Specimens examined. Mexico. **Oaxaca**: 660 m, 29 Jun 1977, *T.B. Croat 39751* (MO). **Mun. Ixtlán de Juárez,** 1,640 m, 29 Jan 1998, *Y. Arellanes C. et al. 283*

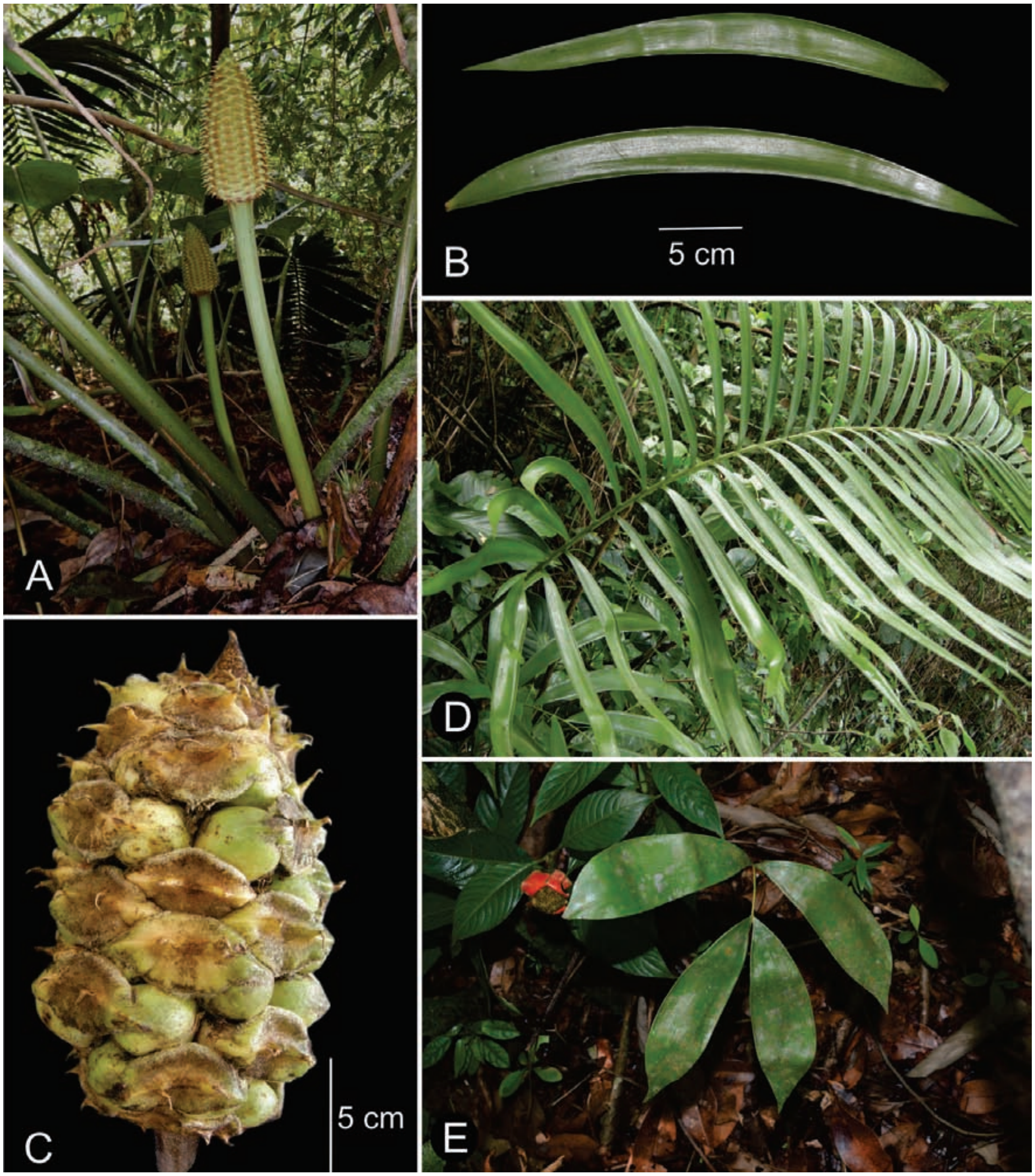

Figure 30. *Ceratozamia whitelockiana* **A** immature pollen strobili **B** leaflet variation **C** mature ovulate strobilus **D** leaves **E** seedling.

(SERO); 1,640 m, 21 Aug 1998, *Y. Arellanes C. et al. 413* (MEXU, MO, SERO). **Mun. San Felipe Usila**, 24 Oct 1994, *P. Osorio H. 312* (MEXU). **Mun. San Juan Bautista Valle Nacional**, 650 m, 24 Sep 2020, *F. Nicolalde-Morejón et al. 3346* (CIB); 650 m, 24 Sep 2020, *L. Martínez-Domínguez et al. 1968* (CIB, MEXU); 500 m, 22 Jan 2001, *S. Avendaño R. 5375* (MEXU); 518 m, 22 Jan 2001, *T.W. Walters 2001-39-D,E* (XAL). **Mun. San Juquila Vijanos**, 1,900 m, 15 Nov 1996, *X. Munn et al. 233* (XAL). **Mun. San Pedro Sochiapam**, 1,682 m, 2 Nov 2016, *M.B. Velasco-Pichardo et al. 225* (MEXU). **Mun. San Juan Tepeuxila**, 1,538 m, 9 May 2008, *J.E. Rivera-*

Hernández 4289 (MEXU, XAL); 1,538 m, 30 Jul 2008, *J.E. Rivera-Hernández 4380* (MEXU). **Mun. Santiago Comaltepec**, 1,750 m, 8. Jan 1995, *A. Rincón G. et al. 516* (MEXU, MO, XAL); 560 m, 24 Sep 2020, *F. Nicolalde-Morejón et al. 3348–3355* (CIB); 560 m, 24 Sep 2020, *L. Martínez-Domínguez et al. 1973–1975, 1980* (CIB, MEXU), *1976, 1978, 1979, 1981* (CIB), *1977* (CIB, MEXU, NY); 1,600 m, 10 Jun 1988, *R. López-Luna & G.J. Martin 285* (MEXU), 1,760 m, 26 Jan 1988, *R. Torres C. & E. Martínez S. 11345* (MEXU).

35. *Ceratozamia zaragozae* Medellín-Leal, Brittonia 15: 175. 1963

Fig. 27I

Type. Mexico. San Luis Potosí: Mun. Río Verde, 22 Jul 1962, *F. Medellín-Leal 1452* ♀ (holotype: SLPM! [acc. # 003530]; isotypes: ENCB! [ENCB003716], GH! [00003279], MEXU! [MEXU00162859, MEXU0053418], MICH! [1192896], US! [00011997]).

Description. ***Stem*** 10–20 cm long, 10–15 cm in diameter, semi-hypogeous, erect. ***Cataphylls*** 1.8–2.5 × 1–2 cm wide at the base, persistent, triangular, reddish brown, densely brown tomentose at emergence, partially tomentose at maturity, apex acuminate. ***Leaves*** 3–27, 95–202 cm long, ascending, reddish brown at emergence with whitish gray trichomes, glabrous at maturity. ***Petiole*** 11–36 cm long, terete, twisted, green in mature leaves, unarmed. ***Rachis*** 40–77 cm long, terete, twisted, green in mature leaves, unarmed. ***Leaflets*** 25–46, opposite to subopposite, insertion in one plane, linear, generally longitudinally planar, basally falcate, membranaceous, strongly involute, green with adaxial and abaxial sides glabrous, distal end with entire margins, acute and symmetrical at the apex, attenuate at base, with conspicuous and light-green veins; median leaflets 17–31.5 × 0.4–0.7 cm, 0.8–2.3 cm between leaflets; articulations 0.2–0.3 cm wide, yellow. ***Pollen strobili*** 15–19 cm long, 2–3.5 cm in diameter, solitary, cylindrical, erect, greenish with reddish brown trichomes at emergence, reddish brown at maturity; peduncle 5–8 cm long, 1.5–1.8 cm in diameter, tomentose, reddish brown to brown; microsporophylls 0.8–1.2 × 0.3–0.6 cm, obconic with a non-recurved distal face and a lobate fertile portion, infertile portion 0.25–0.35 cm long and rounded with straight horns 0.20–0.30 cm long, 0.22–0.30 cm and an obtuse angle between the horns. ***Ovulate strobili*** 7–12 cm long, 5.5–7.3 cm in diameter, solitary, cylindrical, erect, green with scarce reddish brown trichomes at emergence, dark green at maturity, acute apex; peduncle 6–9 cm long, 0.9–1.2 cm in diameter, erect, tomentose, brown; megasporophylls 24–49, 5–7 orthostichies with 5–6 sporophylls per orthostichy, 2.0–2.6 × 2.2–3.7 cm, with a truncate distal face, horns straight and 0.33–0.45 cm long, 1.95–2.35 cm between horns with an obtuse angle between the horns. ***Seeds*** 2–2.8 cm long, 1.8–2 cm in diameter, ovate, sarcotesta light brown at maturity.

Distribution and habitat. *Ceratozamia zaragozae* is endemic to Mexico in a small mountain range in San Luis Potosí (Fig. 29C), where it occurs in pine-oak forest on karstic rocks at 1,500–1,950 m.

Etymology. The specific epithet refers to General Ignacio Zaragoza, who was a hero in the Battle of Puebla against the French Army in May of 1862.

Common names. None recorded.

Uses. None recorded.

Conservation status. (IUCN 2021). *Ceratozamia zaragozae* is listed as "Critically Endangered (CR)" under criteria A2acd; B1ab(iii,iv,v)+2ab(iii,iv,v); C1. Castillo-Lara et al. (2018) evaluated the population structure and spatial distribution for this species and found that the populations have several individuals with a density variable from four to 209 plants in an area of 2500 m2, but low population growth. These authors suggest that the status for this species could be modified to "Endangered (EN)".

Discussion. *Ceratozamia zaragozae* and *C. norstogii* are the only species in the genus with twisted petiole and rachis, but the first has an unarmed petiole, whereas *C. norstogii* has abundant and robust prickles on the petiole. Additionally, leaflets are membranaceous in *C. zaragozae*, whereas in *C. norstogii* they are coriaceous.

Specimens examined. Mexico. **San Luis Potosí: Mun. Río Verde**, 1,700 m, 29 Mar 1984, *A.G. Mendoza & L. Vargas 1389* (MEXU); 1,750 m, 20 Sep 1979, *A.P. Vovides 435* (XAL); 22 Jul 1962, *E. Molseed 34* (MEXU; MICH); 1,860 m, 24 Jan 1994, *F. García S. s/n* (SLPM); 1,800 m, 22 Jul 1962, *F. Medellin-Leal 1451* (SLPM; US); 28 Oct 1965, *F. Medellin-Leal s/n* (SLPM); 1,900 m, Sep 1994, *F. Medellin-Leal s/n* (SLPM); 1,956 m, 18 Mar 2016, *F. Nicolalde-Morejón et al. 2307–2319* (CIB); 1,750 m, 13 Apr 1968, *J. Rzedowski 25658* (ENCB; MICH); 1,956 m, 18 Mar 2016, *L. Martínez-Domínguez et al. 792, 794–796, 798–808* (CIB), *793, 797, 799* (CIB, MEXU); 2031 m, 31 Jul 2017, *P. Chávez C. et al. 98* (SLPM); 1,532 m, 13 Jan 2001, *T. Walters et al. TW-2001-07* (MEXU, XAL). **Mun. Zaragoza**, 1,869 m, 9 Nov 2012, *P. Castillo-Lara et al. 593* (SLPM); 1,847 m, 14 Jul 2016, *P. Castillo-Lara et al. 1073* (SLPM).

36. *Ceratozamia zoquorum* Pérez-Farr., Vovides & Iglesias. Bot. J. Linn. Soc. 137 (1): 77. 2001

Figs 1D, 23 A–C

Type. Mexico. Chiapas: Northern mountain range, 18 Nov 1998, *M.A. Pérez-Farrera 1732* ♂ (holotype: CHIP [n.v]).

Description. ***Stem*** 12–30 cm long, 8–15 cm in diameter, epigeous, semi-hypogeous, erect. ***Cataphylls*** 2.5–6.9 × 1.3–4.5 cm wide at the base, persistent, triangular, reddish brown, densely brown tomentose at emergence, glabrous at apex when mature, apex acuminate. ***Leaves*** 2–17, 46–216.5 cm long, descending, light green or brown, glaucous at emergence with whitish gray trichomes, glabrous at maturity. ***Petiole*** 23.3–111.5 cm long, terete, linear, pink at emergence, yellowish green green at mature leaves; unarmed to armed with 3–27 thin prickles 0.08–0.15 cm long. ***Rachis*** 17.6–114.5 cm long, terete, linear, pink at emergence, yellowish green at mature leaves, generally unarmed. ***Leaflets*** 6–17 pairs, opposite to subopposite, insertion in one plane, oblong to oblanceolate, longitudinally curved abaxially to planar, generally basally falcate, coriaceous, flat, green with adaxial and/or abaxial side glaucous, distal end with entire margins, acuminate (rarely acute) and asymmetrical (rarely symmetrical in apical leaflets) at the apex, attenuate at base, with conspicuous and light-green veins; median leaflets 22–37 × 3.9–5.6 (7) cm, 4.6–11.1 cm between leaflets; articulations 0.5–1.5 cm wide, green and yellowish. ***Pollen strobili*** solitary (up to 2), 10.8–25 cm long, 2.8–4.3 cm in diameter, cylindrical, erect, green with blackish trichomes at emergence, yellow-cream with blackish trichomes at maturity; peduncle 5–13 cm long, 1.5–1.8 cm in diameter, tomentose, light brown; microsporophylls 0.9–1.4 × 0.7–0.9 cm, discoid with a non-recurved distal face and a deeply lobate fertile portion, infertile portion 0.35–0.40 cm long and linear with straight horns 0.30–0.45 cm long, 0.73–0.80 cm and an acute angle between the horns. ***Ovulate strobili*** 15–26 cm long, 7.5–9.5 cm in diameter, solitary, cylindrical, erect, green with reddish brown trichomes at emergence, green with brown to blackish trichomes at maturity, acute apex; peduncle 5–18.5 cm long, 1.6–2 cm in diameter, pendulous and erect, tomentose, light brown; megasporophylls 30–56, 6–8 orthostichies with 5–7 sporophylls per orthostichy, 1.5–2.0 × 3.5–4.0 cm, with a prominent distal face, horns curved to straight and 0.70–0.90 cm long, 0.92–1.56 cm between horns with a right angle between the horns. ***Seeds*** 2–2.8 cm long, 1.8–2 cm in diameter, ovate, sarcotesta whitish pink at emergence, light brown at maturity.

Distribution and habitat. *Ceratozamia zoquorum* is endemic to the northern mountains of Chiapas, Mexico (Fig. 29D), where it occurs on karstic outcrops in evergreen tropical forest and oak forest between 500 and 1,150 m.

Etymology. The specific epithet was established in honor of the Zoque culture (Pérez-Farrera et al. 2001).

Common names. None recorded.

Uses. None recorded.

Conservation status. (IUCN 2021). *Ceratozamia zoquorum* is listed as "Critically Endangered" under criteria A2c+4c; B1ab(i,ii,iii,v).

Discussion. *Ceratozamia zoquorum* has oblong and coriaceous leaflets and leaves with scarce thin and short prickles. It belongs to a cryptic taxonomic group, and is geographically close to populations of *C. becerrae* and *C. santillanii*, the other two species in this group. The three taxa are distinguishable with the nrITS (Martínez-Domínguez et al. 2017c). Morphologically, *C. zoquorum* differs from *C. santillanii* by its peduncle of ovulate strobili more than 3 cm long.

Specimens examined. Mexico. **Chiapas: Mun. Solosuchiapa**, 530 m, 23 Jan 2014, *F. Nicolalde-Morejón et al. 1931, 1932* (CIB); 550 m, 23 Jan 2014, *F. Nicolalde-Morejón et al. 1933–1935* (CIB); 682 m, 24 Jan 2014, *F. Nicolalde-Morejón et al. 1936–1947* (CIB); 531 m, 23 Jan 2014, *L. Martínez-Domínguez et al. 1–5* (CIB); 500 m, 23 Jan 2014, *L. Martínez-Domínguez et al. 6–14* (CIB); 682 m, 23 Jan 2014, *L. Martínez-Domínguez et al. 15–34* (CIB); 520 m, 17 Apr 1996, *M.A. Pérez-Farrera 905* (CH, CHIP, HEM); 520 m, 16 Apr 1996, *M.A. Pérez-Farrera s/n* (HEM); 19 Jan 2001, *S. Avendaño 5216* (MEXU); 531 m, 20 Jan 2001, *T.W. Walters 2001–2028-A* (XAL). **Mun. Tila**, 1,135 m, 16 Jul 2021, *F. Nicolalde-Morejón et al. 3698–3702* (CIB); 1,135 m, 16 Jul 2021, *L. Martínez-Domínguez et al. 2326–2330* (CIB).

"Names" (designations) not validly published

Ceratozamia angustifolia Linden, Illustr. Hort. 28: 32. 1881, nomen nudum, name in list, no description and diagnosis.

Ceratozamia ensiformis hort. ex J.Schust., Pflanzenr. 99: 130. 1932, pro syn.

Ceratozamia eriolepis hort. ex J.Schust., Pflanzenr. 99: 132. 1932, pro syn.

Ceratozamia fusca hort. ex J.Schust., Pflanzenr. 99: 132. 1932, pro syn.

Ceratozamia fuscata hort. ex J.Schust., Pflanzenr. 99: 132.1932, pro syn.

Ceratozamia ghiesbreghtii Brongn., Compte Rendue 81: 303. 1875, nomen nudum, name in list, no description and diagnosis.

Ceratozamia × *hybrida* J.Schust., Pflanzenr. 99: 132. 1932, pro syn.

Ceratozamia karsteniana hort. ex Dyer, Biol. Cent.-Amer., Bot. 3: 192. 1884, pro syn. Thiselton-Dyer cited this name as synonym of *C. latifolia.*

Ceratozamia longipinnata hort. ex J.Schust., Pflanzenr. 99: 130. 1932, pro syn.

Ceratozamia miquelii hort., Vilm. Blumengärtn., ed. 3. 1: 1246. 1895, pro syn.

Ceratozamia muricata Miq. ex Linden, Illustr. Hort. 32. 1881, nomen nudum.

Ceratozamia ottonis hort. ex J.Schust., Pflanzenr. 99: 130. pro syn. 1932, pro syn.

Ceratozamia purpurea Matte, Recherches Appareil Libéro-Lign. Cycad. 125. 1914, nomen nudum.

Dipsacozamia Lehm. ex Lindl., The Vegetable Kingdom, 225. 1846, nomen nudum, not validly published; no diagnosis and description (Art. 39).

Dipsacozamia mexicana Liebm. ex Dyer, Biol. Cent.-Amer., Bot. 3: 193. 1884, pro syn. Thiselton-Dyer cited this name as synonym of *C. mexicana.*

Excluded names

Ceratozamia boliviana Brongn., Ann. Sci. Nat., Bot. ser. 3, 5: 9. 1846. Lectotype: P [P02441739]. Taxonomic Status: synonym of *Zamia boliviana* (Brongn.) A.DC.

Ceratozamia katzeriana Regel, Trudy Imp. S.-Peterburgsk. Bot. Sada 4(4): 298. 1876. Lectotype: LE [LE00009045]. Taxonomic Status: synonym of *Zamia katzeriana* (Regel) E.Rettig.

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Supplementary material 1

Glossary

Authors: L. Martínez-Domínguez, F. Nicolalde-Morejón, D. W. Stevenson

Data type: Text

Explanation note: Glossary for characters associated to reproductive structures.

Link: https://doi.org/10.3897/phytokeys.208.80382.suppl1

Supplementary material 2

Herbarium specimens for *Ceratozamia sancheziae* and *C. zoquorum*

Author: L. Martínez-Domínguez

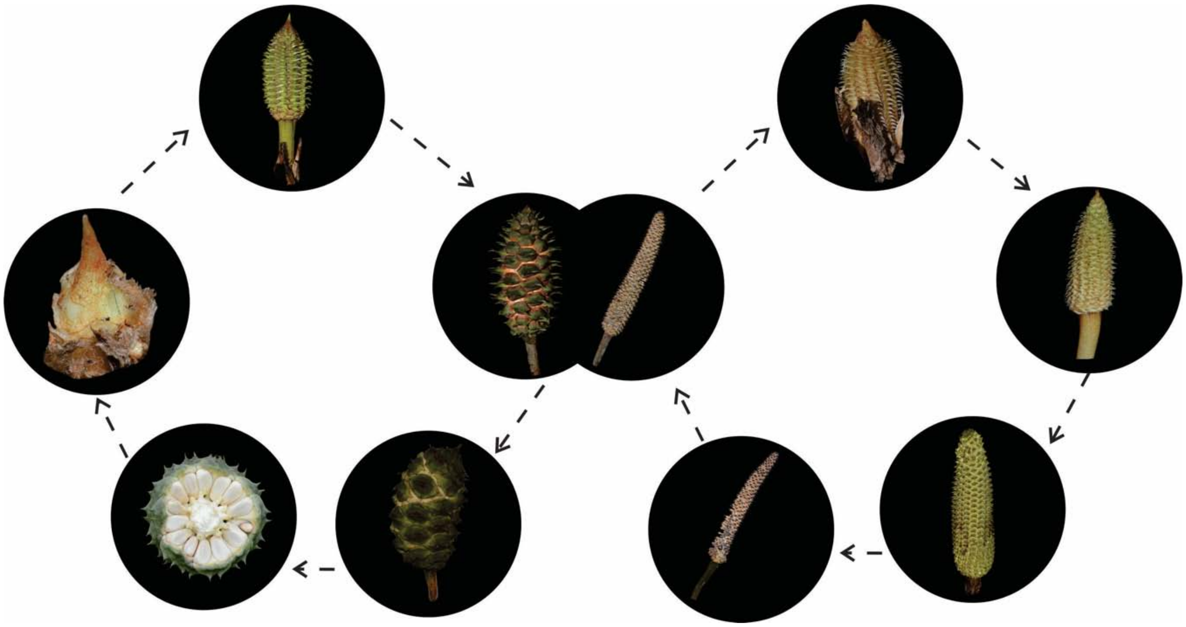
Data type: Images

Explanation note: Specimens: M.A. Pérez-Farrera 1635; D.E. Breedlove 25506.

Link: https://doi.org/10.3897/phytokeys.208.80382.suppl2

II.III. Capítulo III.

Fenología reproductiva en *Ceratozamia*: clave para los estudios evolutivos en cícadas



II.III.I. Temporal shifts in reproductive phenology of cycads: a comparative study in *Ceratozamia*. 2022. Botany, 100: 827–838. DOI:10.1139/cjb-2022-0053

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Article

Temporal shifts in reproductive phenology of cycads: a comparative study in *Ceratozamia*

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Abstract

The reproductive phenology in plants consists of successive life cycle phases leading to reproductive success. In seed plants, cycads and other dioecious groups have complex reproductive systems, where individuals require synchronizations among two sexes and populations of the same species. Here, we analyzed phenology between populations of three geographically close species in the cycad genus *Ceratozamia* Brongn. We described the lifespan of pollen and ovulate strobili and their morphological changes throughout ontogeny and evaluated synchrony among reproductive events, focusing on the timing and abundance of the receptivity and open pollen phases. Our results showed that the reproductive timing in the three species was highly synchronous, and that the overlap between the receptivity and open pollen phases could point to gene flow among populations of different species. We identified a correlation between the reproductive patterns and the temperature and precipitation regimes. Pollen and ovulate strobili were produced during the rainy season, whereas the period of synchrony between receptivity and pollen occurred during the dry season. The seed release occurred during the rainy season, which could contribute to keep the seed moist and prevent the embryo from drying out. Finally, we highlight the utility of studies in wild populations for the ecological and evolutionary understanding of phenological patterns in cycads.

Key words: dioecy, evolution of phenological patterns, gymnosperm ecology, phenophase, *Zamiaceae*

Résumé

La phénologie de la reproduction chez les plantes consiste en phases successives du cycle de vie menant au succès reproductif. Chez les plantes à graines, les cycades et d’autres groupes dioïques possèdent des systèmes reproductifs complexes, où les individus ont besoin d’une synchronisation entre les deux sexes et les populations de la même espèce. Les auteurs ont analysé ici la phénologie entre les populations de trois espèces géographiquement proches du genre *Ceratozamia* Brongn. Ils ont décrit la durée de vie du pollen et des strobiles ovulifères et leurs changements morphologiques tout au long de l’ontogenèse et évalué la synchronisation entre les événements reproductifs, en se concentrant sur la coordination et l’abondance des phases de réceptivité et polliniques ouvertes. Leurs résultats ont montré que la coordination de la reproduction des trois espèces était hautement synchrone, et que le chevauchement entre les phases de réceptivité et polliniques ouvertes pourrait indiquer un flux génétique entre les populations de différentes espèces. Ils ont identifié une corrélation entre les patrons de reproduction et les régimes de température et de précipitations. Le pollen et les strobiles ovulifères étaient produits pendant la saison des pluies, tandis que la période de synchronisation entre la réceptivité et le pollen avait lieu pendant la saison sèche. La libération des graines avait lieu pendant la saison des pluies, ce qui pourrait contribuer à maintenir la graine humide et à empêcher l’embryon de se dessécher. Enfin, les auteurs soulignent l’utilité des études sur les populations sauvages pour la compréhension écologique et évolutive des modèles phénologiques chez les cycades. [Traduit par la Rédaction]

Mots-clés : dioécie, évolution des modèles phénologiques, écologie des gymnospermes, phénophase, *Zamiaceae*

Introduction

The temporal patterns of biological events in plants, such as leaf production and timing of flowering or coning, constantly interact with the prevailing environmental dynamics of an area (Cleland et al. 2006; Arcanjo-Bruno et al. 2019). These plant life-cycle events are often linked with climatic variables (Menzel 2002). Recently, phenological studies have increased in number because climate change could cause mismatches in the interactions between plants and their ecological role in communities (Walther et al. 2002; Gordo and Sanz 2010; Numata et al. 2022). The variable responsiveness of plants to local conditions could even have considerable consequences within reproductive systems, and negatively impact species survival (Walther et al. 2002; Forrest and Miller-Rushing 2010; Gordo and Sanz 2010; Stucky et al. 2018). The degree of response to environmental seasonality within these types of events can vary in magnitude of influence, both across different developmental events — or phenophases — and between species (Martins et al. 2021).

Because seasonal droughts can be long in arid and semi-arid regions, the close relationship between environmental patterns and reproductive events is more conspicuous, particularly in relation to temperature and precipitation regimes, which could initiate the phenophases (Renzi et al. 2019; Sakai and Kitajima 2019). In tropical forests, seasonal timing and factors that may explain phenological changes have been highly diverse or have shown large variation (Sakai and Kitajima 2019). Associations of phenological patterns with climate variables at the community level have been the focus of several phenological research projects (Stradic et al. 2018). This large-scale approach has allowed for the detection of macrogeographic patterns. Although the phenology in closely related species tends to be similar, the approaches that are restricted to species within the same taxonomic group allow the visualization of the heterogeneity of phenological patterns, as well as the discovery of the drivers of phenological transitions and how these are synchronized between related species (Davies et al. 2013; Gerst et al. 2017). Phenological patterns provide crucial data to understand plant reproduction, because seasonal timing in phenology is directly linked to fertilization (Inouye et al. 2003). Shifts in reproductive timing can act as reproductive isolation mechanisms or as drivers of speciation (Morellato et al. 2010; Christie and Strauss 2018). Conversely, shifts that lead to congruent phenologies can reduce reproductive barriers and lead to hybridizations (Miller-Rushing et al. 2010; Clugston et al. 2016). In line with this, phenology can also provide evidence to infer evolutionary adaptations and phylogenetic relationships (Clugston et al. 2016). Phenological studies in dioecious species — particularly those that require external vectors for successful pollination — could have outstanding research value due to their susceptibility to environmental changes (Miller-Rushing et al. 2010; Clugston et al. 2016). These species require spatial and seasonal reproductive synchronization both between plants that produce male and female gametes and between species (Lazcano-Lara and Ackerman 2018). However, most plant phenological research has focused on monoecious species (Escobedo-Sarti and Mondragón 2016; Martins et al. 2021), leaving dioecious species with long life cycles relatively unattended.

Gymnosperms in the order Cycadales are a group of tropical to subtropical dioecious taxa that are characterized by cross-pollination. Specialized mutualistic interaction between cycads and their pollinator insects is essential for their mutual survival (Terry et al. 2014). In paleobotanical terms, cycads are an ancient group which dates back to the Upper Paleozoic (265–290 million years ago), but their relatively higher diversity in the past contrasts with their current status as one of the most threatened plant groups with a significant extinction risk (Nicolalde-Morejón et al. 2014). Thus, efforts to characterize their phenological cycles and the traits that characterize them become more pressing from a plant conservation biology viewpoint.

Recently, cycad phenology has been approached from phylogenetic and taxonomic perspectives (Clugston et al. 2016; Martínez-Domínguez et al. 2018*a*; Segalla et al. 2021). In Mexico, these types of studies are promising due to the occurrence of three genera — namely, *Zamia* L., *Dioon* Lindl., and *Ceratozamia* Brongn. The latter genus is the most diverse in this area and its species are pollinated by insects of the Erotylidae family (Nicolalde-Morejón et al. 2014; Tang et al. 2018). *Ceratozamia* species have long reproductive cycles of approximately one year, but phenological phases have been described in only one species, *Ceratozamia tenuis* (Dyer) D.W.Stev. & Vovides (Martínez-Domínguez et al. 2018*a*).

Generally, the traits evaluated in phenological studies are duration, onset, and termination of phenophases (Renzi et al. 2019). Peaks in these parameters are crucial to show the tendency that related species might have to exhibit either the same phenological profile or overlaps in their patterns (Renzi et al. 2019). The research presented here was designed to fully document and characterize the reproductive cycles, including phases in the ontogenetic development of reproductive structures in three species of *Ceratozamia* that inhabit in the central region of Veracruz State (Mexico): (*i*) *Ceratozamia morettii* Vázq.Torres & Vovides; (*ii*) *Ceratozamia brevifrons* Miq.; and (*iii*) *Ceratozamia delucana* Vázq.Torres, A.Moretti & Carv.-Hern. Additionally, we explored the relationships among phenophases and climatic variables in these species. Thus, the aims of this study were as follows: (*i*) to characterize the phenophases in each species; (*ii*) to examine the onset, termination, duration, and peaks of phenophases, as well as their relationship with climatic variables; and (*iii*) to compare the degree of synchrony among species.

Materials and methods

Species and sites of study

Ceratozamia morettii and *C. brevifrons* each have a narrow distribution. The former is a little-known cycad that occurs in the cloud forest, on karstic rocks and cliffs between 1200 and 1850 m of elevation, whereas *C. brevifrons* inhabits karstic rocks in the oak forest and the transition zone between oak forest and cloud forest between 500 and 1350 m. In contrast, *C. delucana* is a species with wide distribution from the northern central region of Veracruz down to the Puebla state. It grows in the cloud forest, between 500 and 1650 m of elevation. Morphologically, these three species bear close similarities, but they can be distinguished by certain vegetative characters, such as type and number of prickles and insertion of leaflets on the rachis. In particular, *C. delucana* is a polymorphic species similar in both vegetative and reproductive morphology to *C. morettii* (cf. Martínez-Domínguez et al. 2018*b*).

The three species occur in the cloud forest region, informally known as "mountain range of Puebla-Chiconquiaco", which is part of the Sierra Madre Oriental (CONABIO 2008). The study was carried out in two populations of each species occurring along this area, within the distribution range of the three species (Fig. 1). The Sierra Madre Oriental is a mountain range located in Eastern Mexico, spanning from the central coastal plain of the Gulf of Mexico toward the central high plateau of Mexico (Eguiluz de Antuñano et al. 2000).

Phenological observations

Observations for reproductive phenological events in all wild populations were carried out from August 2016 to August 2021. A directed sampling scheme was conducted to register each reproductive individual and the ontogenetic stage of their corresponding reproductive structures. Because the study area has steep slopes, the most accessible individuals were studied to guarantee covering the largest possible area containing plants in each of the populations. The populations of *C. morettii* were difficult to monitor because the plants occur in steep and exposed rocky walls. Thus, the number of adult individuals observed in both populations was lower than in populations of the remaining two species included in the study. These populations were visited monthly, when the stage of the reproductive structures (pollen and ovulate strobili) indicated the start of the phenophases associated with pollination and fertilization (i.e., receptivity and open pollen). Sometimes, the individual plants were visited more than once a month. We recorded the phenophases of each individual and the number of reproductive structures at the population level (Table 1). These phenophases were described for each species, following previous descriptions by Martínez-Domínguez et al. (2018*a*).

Statistical analysis

Circular statistics was applied for observations of receptivity and open pollen using the ORIANA software (Kovach 1994). Months of the year were converted to angles at 30° intervals of the circumference from January to December (Morellato et al. 2010). We calculated the mean angle (μ), which indicates the central tendency of data, the mean vector (r), and Rayleigh's test (z) to evaluate deviations (Zar 2010). We tested data for normality and homoscedasticity tests. We analyzed the correlation among phenological and temperature and precipitation parameters using the non-parametric Spearman correlation. The monthly temperature and precipitation data were obtained directly from the nearest meteorological station of the National Meteorological System of Mexico for the species of interest (Misantla, for populations of *C. brevifrons*; Atzalan, for *C. delucana*; and Naolinco, for *C. morettii*). Because we had incomplete data from the meteorological stations, analyses were carried out using the average of temperatures and precipitation for 2016–2018. For *C. delucana*, temperature data were not incorporated because the meteorological station only registered precipitation data during this period. This set of analyses was performed using the R software (R Core Team 2013).

Results

During this time period, we registered 43 polliniferous plants and 21 ovuliferous plants for *C. morettii*. A total of 24 ovuliferous plants and 53 polliniferous plants were registered for *C. brevifrons,* and 67 ovuliferous plants and 102 polliniferous plants for *C. delucana*. Ovuliferous plants produced only one strobilus per apex, while polliniferous plants produced up to two strobili per apex. The strobili of both polliniferous and ovuliferous plants emerged from the shoot apex surrounded by cataphylls. The ovuliferous plants did not produce a strobilus each year, while most of the polliniferous plants did produce a strobilus each year.

Morphological description and lifespan

Pollen and ovulate strobili pass through four stages of the development over their lifespan. In pollen strobili, the first phase was the emergence (EP) stage, which began in August for all species and lasted between three and four months for each pollen strobilus (Fig. 2A). In *C. morettii*, some pollen strobili were recorded earlier in July. At the population level, these structures emerged continuously during the next three months (September, October, and November). This phenophase was characterized by tightly packed microsporophylls, which were yellowish to greenish with orange trichomes. The closed pollen (CP) stage lasted one month and, in a few individuals, up to two months. It was characterized by the complete development of microsporophylls and the pre-elongation of the central axis (Figs. 3B and 4B). Microsporophylls exhibited the same color that would have until maturity. Pollen strobili were greenish to yellowish green with black trichomes during CP in the three species. *Ceratozamia morettii* had abundant trichomes on the distal face of microsporophylls, in contrast to the other two species. The open pollen (OP) phase occurred during the elongation of central axis and dehiscence of the microsporangia. During OP, the microsporangia were visible following the separation of the microsporophylls, and finally dehisced synangium units released the pollen (Fig. 2C). The OP lasted three to four weeks in each individual plant (Fig. 4C). At the population level, OP occurred from February to June in *C. brevifrons*, from December to June in *C. morettii*, and from January to June in *C. delucana* (Fig. 5). The highest number of OP strobili was recorded from January to March, for *C. brevifrons* and *C. delucana*, and in January for *C. morettii* (Fig. 5D-F). The number of individual plants in OP progressively decreased from April onward. The final phase of lifespan was senescence (SP), in which the apex of pollen strobilus became curved and microsporophylls turned brown as the strobilus structure dried up (Figs. 2D, 3C, and 4D). The SP phase lasted one to two weeks.

The emergence of ovulate strobili (EO) at the population level for all species started during August and finished in September (Figs. 5A-C). This phenophase began with the emergence of ovulate strobili from the shoot apex and lasted until megasporophylls were distinct enough from each other and the peduncle (sterile part of strobilus) was visible (Figs. 2E and 3D). The ovulate strobili were yellowish with orange trichomes, and throughout ontogeny they turned green to dark green with blackish trichomes. On average, the receptivity

Fig. 1. Distribution map of *Ceratozamia brevifrons*, *Ceratozamia delucana*, and *Ceratozamia morettii*. The localities where the phenological monitoring was carried out are represented with large symbols. Map source data were collected from the "Geoportal of CONABIO". The map was created in ArcMap 10.2 by L. Martínez-Domínguez. [Colour online]

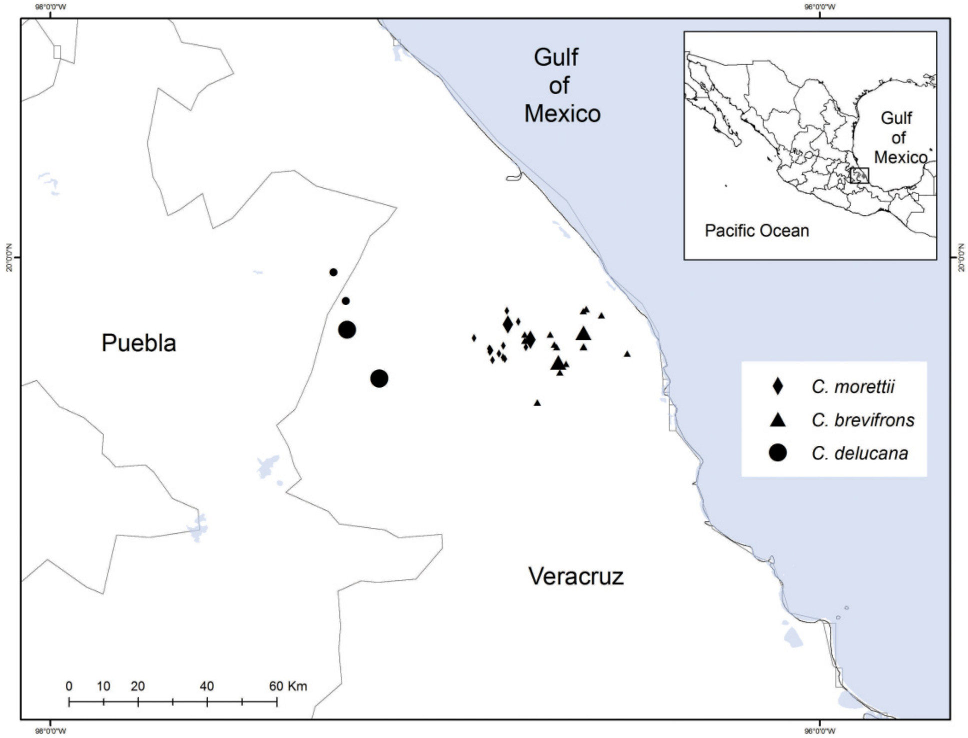


Table 1. Summary of the main descriptive variables in circular statistical analysis for the three *Ceratozamia* species.

	C. brevifrons		*C. delucana*		*C. morettii*	
Variables	Receptivity	Open pollen	Receptivity	Open pollen	Receptivity	Open pollen
Mean vector (°)	56.168	84.653	44.09	56.123	9.418	29.313
Length of mean vector (r)	0.979	0.83	0.951	0.843	0.99	0.78
Median angle, μ (°)	45	75	45	45	15	15
Rayleigh test, z	23.01	36.52	60.63	72.42	20.59	26.15

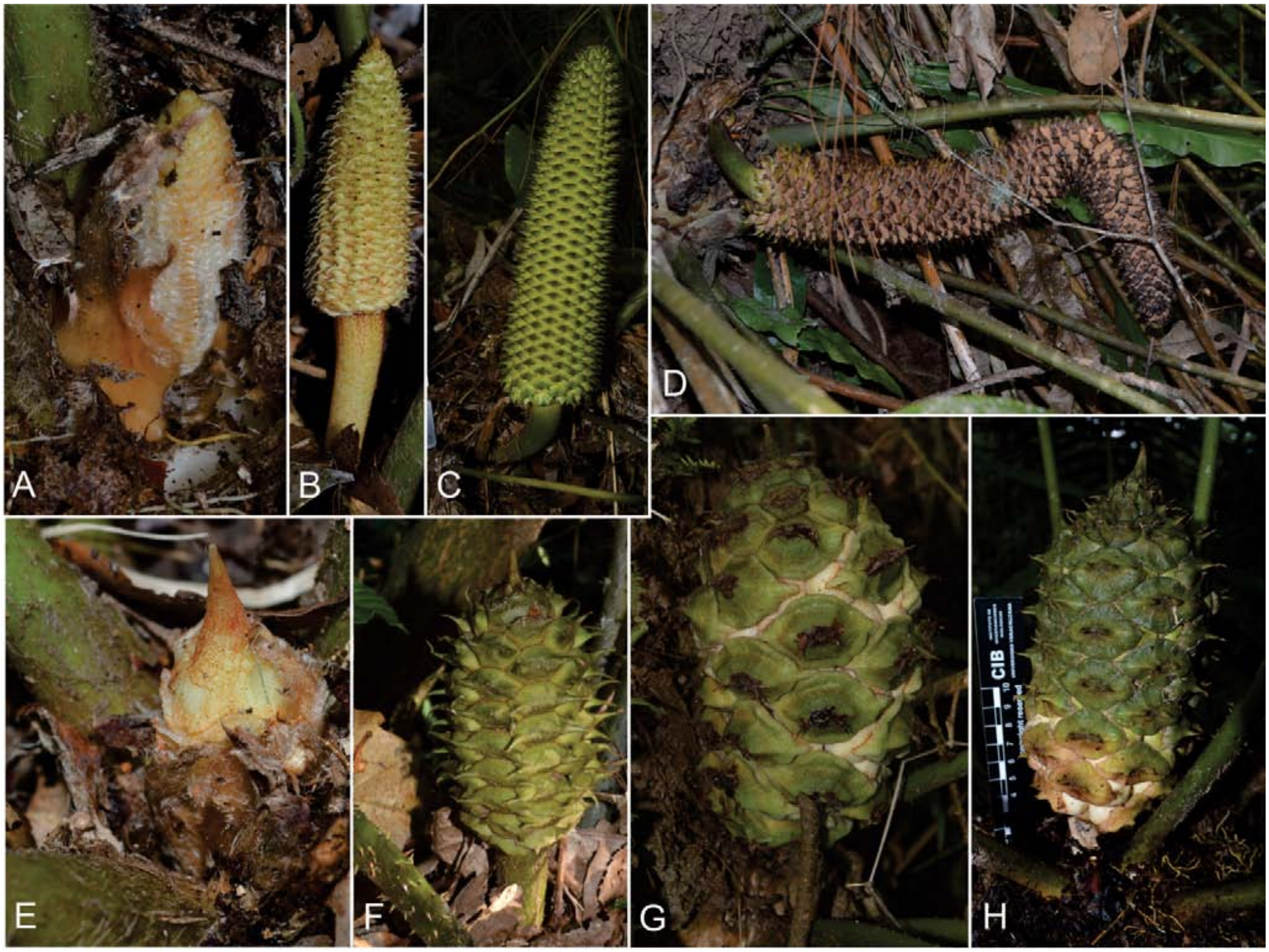
(R) phase lasted three weeks. This phase involved the opening and closing of cracks between megasporophylls, and included three events: (*i*) pre-receptive, in which the base of megasporophylls changed from green to reddish color (8–10 days long); (*ii*) open-receptivity, in which a reddish color intensified at the base of the megasporophylls, as well as a separation between them (5–10 days long); and (*iii*) post-receptivity, when the cracks were closed and megasporophylls gradually turned green to yellowish green or light-green at the base (10–15 days long). *Ceratozamia brevifrons* was characterized by yellowish to greenish-yellow megasporophylls during all developmental stages after receptivity. The late ovulate (LO) was the longest phase recorded in which the ovules increased in size and the sarcotesta color became visible. This phase lasted five to six months in each individual plant. In LO, the pollinated ovules began their development but the distal color of megasporophylls remained unchanged. Finally, the disintegration (DO) phase was characterized by the release of megasporophylls from the central axis, and the initiation of sarcotesta degradation. During this phase, megasporophylls in all species were brownish green. In all species, DO occurred from August to October; however, most plants released their seed in September.

Synchrony of phenological patterns

In general, we observed synchrony between the two populations monitored for each of the species studied. However, polliniferous plants finished the season asynchronously in *C. morettii* and *C. brevifrons* populations. It was not possible to obtain sufficient data for a more detailed comparison between populations of the same species because some populations were small (i.e., composed of approximately 50 individuals, with few of them in reproductive stages) and due to the disparity of reproductive events between years. Monitoring showed that, after a given year with several reproductive individuals in a population, the next yearly period had no ovuliferous strobili and few pollen strobili.

The central tendency of mean angles in phenophases showed that observations were concentrated in the same direction (Table 1). Phenograms showed partial synchroniza-

Fig. 2. Reproductive phenology of *Ceratozamia morettii*. Phenophases for polliniferous plants: (A) emergence (August), (B) emergence (fully emerged, 30 days from beginning emergence), (C) closed pollen (March), (D) senescence (May). Phenophases for ovuliferous plants: (E) emergence (August), (F) pre-receptivity (10 days from beginning receptivity phase — December), (G) late ovulate (May), (H) disintegration (August). [Colour online]



tion in the receptivity and open pollen phases within the same species (Fig. 5). We observed an asynchrony between R and OP during most of the months in the reproductive cycle. This is because at the population level, the R phase occurred for two or up to three months, whereas OP occurred continuously for three to four months after receptivity. Thus, asynchrony involves extra time for pollen shedding. The peaks between these phenophases overlapped within each species. At the population level for all species, the R phase was shorter than OP. In addition, these phenophases showed an overlap between all species, including the general pattern of beginning and end of these phenophases (Fig. 5). *Ceratozamia brevifrons* and *C. delucana* matched in peaks for the R phase, which occurs during February. The peak for the OP phase in *C. brevifrons* occurred in March, while in *C. delucana* occurred in February. In *C. morettii*, the peaks of the OP and R phases matched with each other during January (Fig. 5). The OP phase progressively decreased throughout the year for all species: in *C. brevifrons*, it started in January and lasted until June; in *C. morettii*, it went from December until May; and in *C. delucana* it started in January and lasted until June. In particular, *C. morettii* was the species where the OP and R phenophases began earlier, during December.

Influence of climate on phenology

In general, the R and OP phases turned out to be correlated with the climatic variables evaluated (Figs. 6–8). There was low negative correlation between the precipitation and R phase, with the exception of *C. delucana* (Table 2). The R phase occurred during the mid-dry season for all species. This phenophase was correlated with low monthly precipitation, but the temperature displayed an irregular pattern (Figs. 6A, 7A, and 8). Despite the similarity in the precipitation regime for the three species, lower rainfall was observed in *C. brevifrons* (Fig. 6). In *C. delucana*, the highest precipitation occurred between September and October (Fig. 8), whereas in *C. morettii*, a longer period of rainfall was recorded beginning in August (Fig. 7). The monthly temperature was lower during the period of receptivity. The peaks for all species occurred

Fig. 3. Reproductive phenology of *Ceratozamia brevifrons*. Phenophases for polliniferous plants: (A) emergence (fully emerged — December), (B) closed pollen (February), (C) senescence (March). Phenophases for ovuliferous plants: (D) emergence (fully emerged, six months from beginning emergence — February), (E) receptivity (March), (F) late ovulate (July), (G) disintegration (October). [Colour online]

within this general pattern, without a clear correlation with the beginning or ending of dry and cold seasons.

The OP phase was more consistently related with the temperature (Table 2). At higher temperatures, the number of pollen strobili decreased (Figs. 6 and 7). The peaks occurred at relatively low temperatures. Precipitation did not increase or decrease consistently between consecutive months. However, precipitation values were the highest during the months of the year when we did not observe pollen strobili (from July to October).

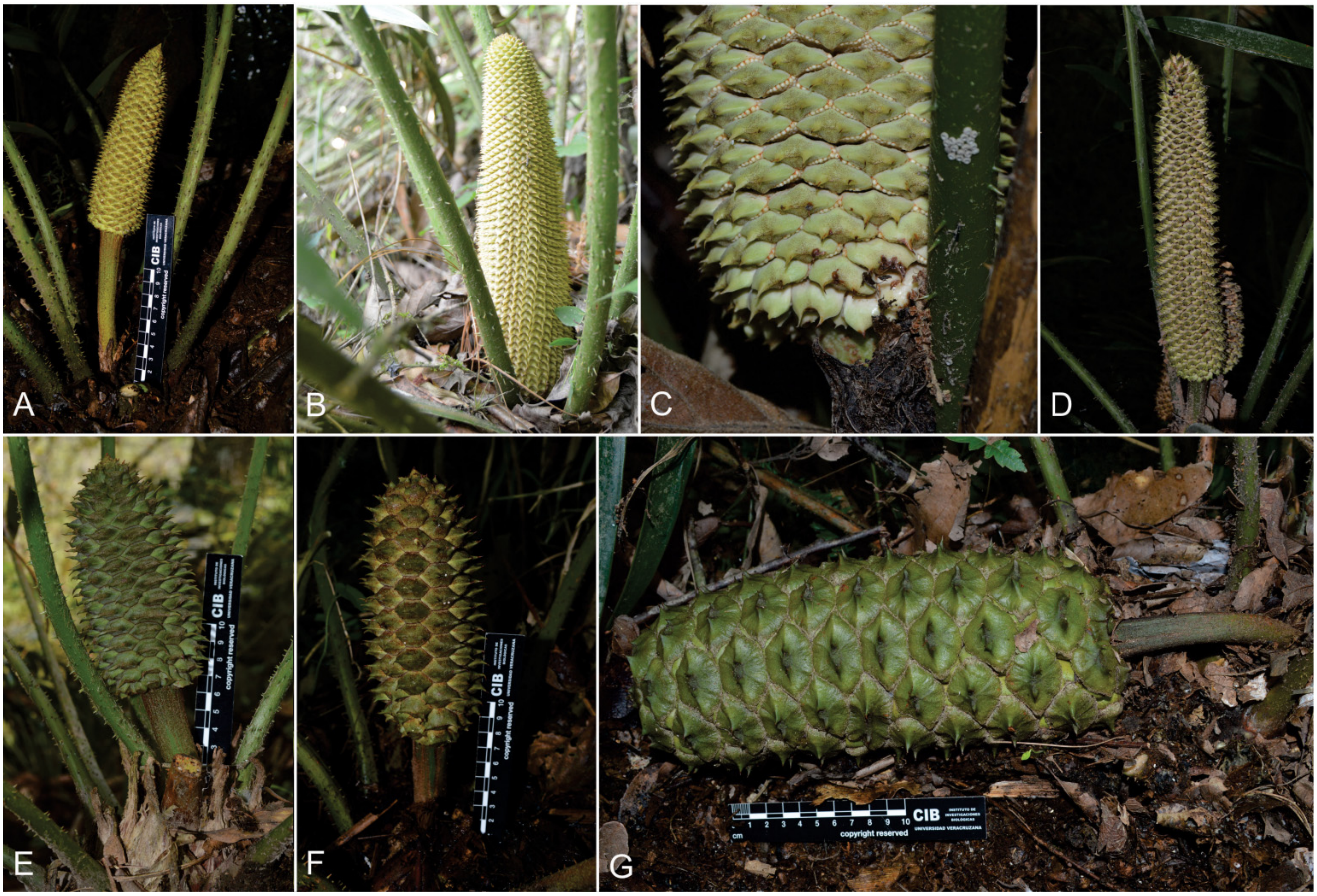
The timing of the E phase, both in pollen and ovulate strobili, occurred during the rainy and warm season. The development of pollen strobili did not show a clear correspondence with seasonality, whereas the development of ovulate strobili occurred during the dry season and the beginning of the warm season. The DO phase occurred at the beginning of the rainy season.

Discussion

Reproductive phenology is a critical part of plant life cycles, particularly with regards to reproductive success (Inouye et al. 2003; Tang et al. 2016). Because cycads are long-lived and have reproductive structures with long-lifespan, their phenology has been little explored (Stevenson 1981; Clark and Clark 1987; Tang 1990; Ornduff 1991). Recently, phenological data have been used for describing lifespans, analyzing patterns from a phylogenetic context, and synchrony among events (Lopez-Gallego and O'Neil 2010; Martínez-Domínguez et al. 2018*a*, 2021; Segalla et al. 2021). Living collections and herbarium specimens have been used as a resource for the systematic study of phenology (Calonje et al. 2011; Griffith et al. 2012; Clugston et al. 2016). However, phenological monitoring from wild populations provides complementary relevant data for exploring the biological drivers of phenological transitions.

Phenological studies in cycads provide data for taxonomy, ecology, conservation, and could improve our understanding of evolutionary processes responsible for the current diversity (Griffith et al. 2012). Characters of reproductive structures have been useful to identify species in genera such as *Zamia* and *Ceratozamia* (Nicolalde-Morejón et al. 2009; Martínez-Domínguez et al. 2018*b*, 2021; Calonje et al. 2019). In particular, the description of ontogenetic stages for ovu-

Fig. 4. Reproductive phenology of *Ceratozamia delucana*. Phenophases for polliniferous plants: (A) emergence (fully emerged — December), (B) closed pollen (March), (C) open pollen (March), (D) open pollen and senescence in the same plant. Phenophases for ovuliferous plants: (E) pre-receptivity (March), (F) post-receptivity (30 days from beginning receptivity phase — March), (G) late ovulate (August). [Colour online]



late and pollen strobili are relevant to detect the stage in which to evaluate the morphological characters that characterize a species (Martínez-Domínguez et al. 2018*a*). Here, we found that the shape and sporophyll color in pollen and ovulate strobili remained the same during most ontogenetic stages in the three species of *Ceratozamia* (Figs. 2–4). The corresponding character states can be assessed during CP and OP phases in pollen strobili and LO in ovulate strobili.

The reproductive cycle in cycads has two essential phases for reproduction: R and OP, for ovuliferous and polliniferous plants, respectively. These phases must occur simultaneously because pollination is dependent on interactions with insects, which carry pollen from pollen-bearing plants in the OP phase to ovulate strobili that are in the R phase (Stevenson et al. 1998; Terry et al. 2005). Our results showed a slight time difference between these two phases (Fig. 5). At the population level, plants in the R phase were synchronic, whereas plants in the OP phase were partially asynchronous (Fig. 5). Although the number of pollen strobili decreases during the year, some individuals continue to complete their ontogenetic development until reaching the OP phase. This disparity has been described in other cycad species (Martínez-Domínguez et al. 2018*a*; Segalla et al. 2021), which could be related to dynamics involving insect pollinators. We have observed individuals of *Pharaxonotha tenuis* (Santiago-Jiménez, 2019) in the last polliniferous strobili of a population in July, and up to four generations of insect pollinators during a year in populations of *C. tenuis*. However, it has not been reported if the insects go into diapause until the next reproductive period as other cycad species such as *Zamia furfuracea* L.f. (Norstog and Fawcett 1989; Norstog et al. 1986). It is not even known if and when the insects stop looking for other pollen strobili, or what are the factors that promote their diapause.

Our results also demonstrated that the phenological patterns of *C. brevifrons*, *C. delucana*, and *C. morettii* are similar to each other, with the latter species displaying a slight disparity (Fig. 5). The R phase started first in *C. morettii*; however, all three taxa do have some plants in the R phase at the same time. This would allow for cross-pollination between all three species; thus, making the production of hybrids possible because these species have similar reproductive peaks (R and OP phases). This temporal overlap could allow some gene flow among populations between these three species. Additionally, *C. morettii* and *C. brevifrons* occur in adjacent areas, and in some localities, they have an interpopulation distance of only 1–1.5 km (Fig. 1) increasing the prob-

Fig. 5. Circular histograms of reproductive phenological events. Phenophases of *Ceratozamia brevifrons*: (A) receptivity and (D) open pollen. Phenophases of *Ceratozamia morettii*: (B) receptivity and (E) open pollen. Phenophases of *Ceratozamia delucana*: (C) receptivity and (F) open pollen. [Colour online]

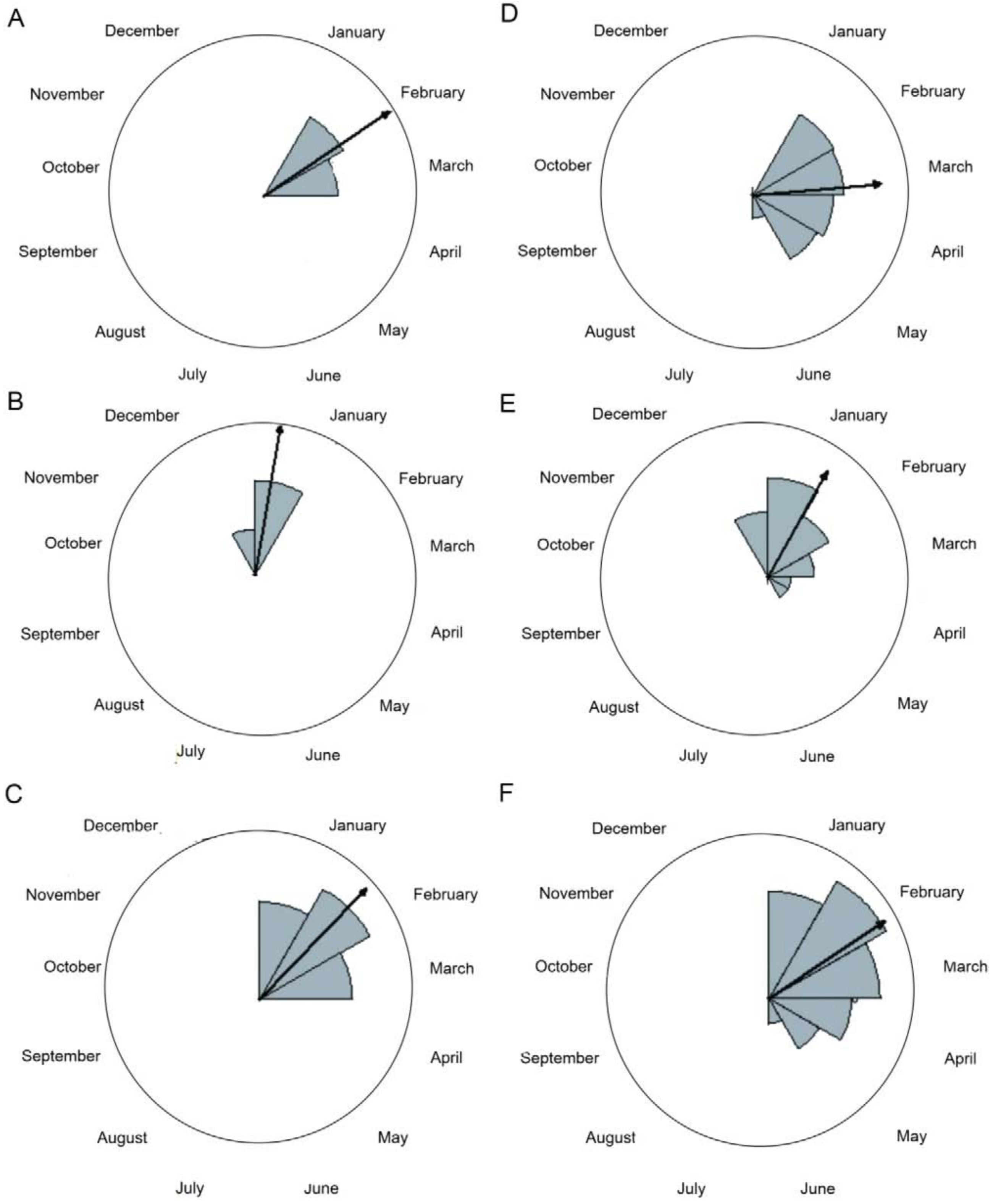


Fig. 6. Average monthly climate for receptivity and open pollen phenophases in *Ceratozamia brevifrons*: (A) precipitation and (B) temperature. [Colour online]

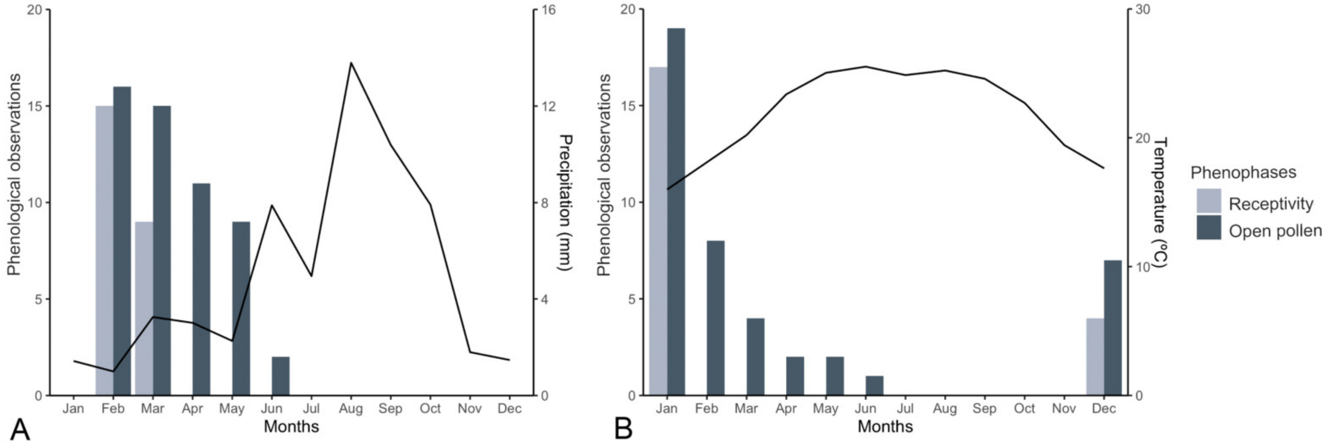


Fig. 7. Average monthly climate for receptivity and open pollen phenophases in *Ceratozamia morettii*: (A) precipitation and (B) temperature. [Colour online]

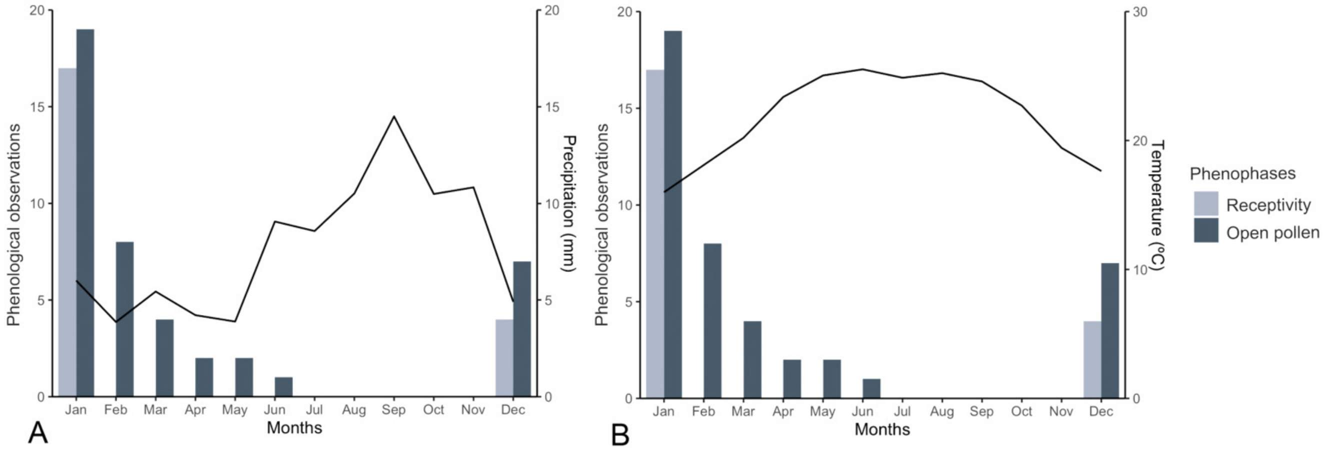


Fig. 8. Average of precipitation for receptivity and open pollen phenophases in *Ceratozamia delucana*. [Colour online]

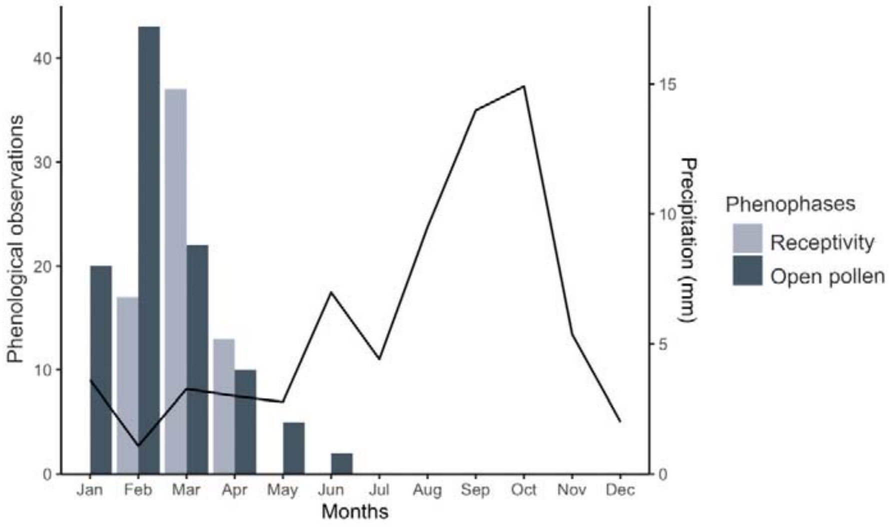


Table 2. Spearman correlation coefficients of phenophases with climatic variables (precipitation and temperature) for the three *Ceratozamia* species.

	C. brevifrons		*C. delucana*		*C. morettii*	
Phenophases	*P*	*T*	*P*	*T*	*P*	*T*
Receptivity	−0.3547	−0.3328	−0.5140	Null	−0.4497	−0.6504
Open pollen	−0.3197	−0.0779	−0.6121	Null	−0.7737	−0.6066

Note: *P* is average monthly precipitation and *T* is average monthly temperature.

ability of hybridization. More detailed studies on the pollinators of these species are required. Of particular interest is *C. tenuis,* another species that is found relatively near to our study area and in which more than one species of insect pollinator has been reported to be associated with its pollen strobili (Santiago-Jiménez et al. 2019). This suggests the possibility that pollinators may move pollen among species. Additionally, the same pollinator species has been found in up to four geographically close species of *Ceratozamia* — for example, the pollinators, *Ceratophila picipennis* (Tang, Skelley & Pérez-Farrera, 2018), and *Ceratophila chiapensis* (Tang, Skelley & Pérez-Farrera, 2018) are all found in strobili of *Ceratozamia vovidesii* Pérez-Farr. & Iglesias, *Ceratozamia alvarezii* Pérez-Farr., Vovides & Iglesias, *Ceratozamia mirandae* Vovides, Pérez-Farr. & Iglesias, and *Ceratozamia norstogii* D.W.Stev. (Tang et al. 2018). Thus, hybridization is a distinct possibility in these *Ceratozamia* species. However, because no hybrids have been reported in *Ceratozamia*, prezygotic or postzygotic or both, other isolation mechanisms could be involved.

Reproduction in *Zamia portoricensis* Urb. is influenced by the spatial distribution of the polliniferous and ovulate plants; however, reproductive success in this particular case is variable (Lazcano-Lara and Ackerman 2018). In addition, the close relationship between gymnosperm plants and their insect pollinators through mutualism, where both have dual benefits present challenges to our understanding of reproductive biology of cycads (Marler 2010; Salzman et al. 2021). Studies focusing on the nursery-deception pollination system of cycads and their phenological patterns provide a perspective into the evolution of this interaction, improving our understanding of long-term persistence of cycads. In turn, these patterns could enlighten how genetic exchange might occur over time under these conditions. We acknowledge the limitations of a five-year study window for investigating phenological patterns in cycads (Fig. 5); however, our results showed the relevance of inclusion of longer-term data for the study of reproductive dynamics in these gymnosperm taxa.

The influence of climatic variables in the phenological patterns observed in these *Ceratozamia* species should not be downplayed. The R and OP phases occurred during the dry season (Figs. 6–8). It is possible that heavy rains might inhibit pollination. The effect of rain on cross-pollination in several angiosperms varies and may be related to pollen resistance to water and (or) mechanical and energetic constraints of pollinators (Lawson and Rands 2019). In cycads, pollen grains are short lived (Chamberlain 1926), a condition which could hinder the arrival of viable pollen after storms. In addition, the DO phase occurred during the rainy season for the three species. Because seeds of *Ceratozamia* are susceptible to desiccation, this pattern allows for the seeds to be moist for a longer period of time, thus preventing the embryo from desiccation. This is very necessary in *Ceratozamia* for the embryo to properly finish its development in approximately one year after the onset of the DO phase (Norstog and Nicholls 1997). This observed pattern in *Ceratozamia* differs from that recorded in species of other cycad genera — e.g., *Zamia boliviana* (Brongn.) A.DC., in which R and OP occur between August and November and maturation of seeds was recorded during the dry season (Segalla et al. 2021). Our results revealed that, in addition to precipitation, the temperature is also more constant during the months of the seed development in ovulate strobili. Even though these results were obtained from climate data from areas adjacent to the cycad populations, there is a different precipitation regime between their distribution ranges (Figs. 6 and 7). This approach indicates the need to directly collect climate variables at the sample sites. Overall, we suggest that more precision is required to evaluate the influence of climate in this phenological pattern, including other climatic variables (Renzi et al. 2019).

Considering the high extinction risk for cycad species, a better understanding of their phenological patterns would allow for a more refined evaluation of the anthropogenic activity-driven factors — such as habitat fragmentation and (or) size reduction of natural populations — that affect reproductive population dynamics (Morellato et al. 2016; Martínez-Domínguez et al. 2021). Ultimately, these data would be valuable to propose conservation strategies. It should also be noted that under adverse conditions such as fire, cycads could still generate viable strobili (Tang 1990). Ovulate plants do not produce strobili every year as a result of their longer lifespans, while polliniferous plants do so every year, or at least comparatively more often (Clark and Clark 1987; Pérez-Farrera and Vovides 2004; Calonje et al. 2011). These differences are related to the energy drain derived from producing many seeds, each with a well-developed megagametophyte, containing storage material for the germinated seed and seedling. It is common that some cycads do not even produce new flush of leaves in the particular season that they produce a strobilus (Stevenson 1981); thus, the energy drain on seed producing plants is considerably more than that for pollen-producing plants. That is pollen plants are transitory in reproduction; this is, in turn, related to differential resource investments in reproduction by pollen-producing plants (Calonje et al. 2011). Finally, the correlations in timing patterns between ovuliferous and polliniferous plants described in this study could serve as resources for future integrative biological studies in Neotropical cycads, particularly under climate change scenarios, where the alterations in seasonal patterns could affect their population dynamics in relation to animal pollinators and, therefore, their mutual survival.

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Data availability

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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Author contributions

L.M.D. collected data in the field, analyzed the data, and wrote the manuscript; F.N.M. collected data in the field and wrote the manuscript; F.V.S. and D.W.S. contributed to the revision and writing of the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they do not have conflict of interest.

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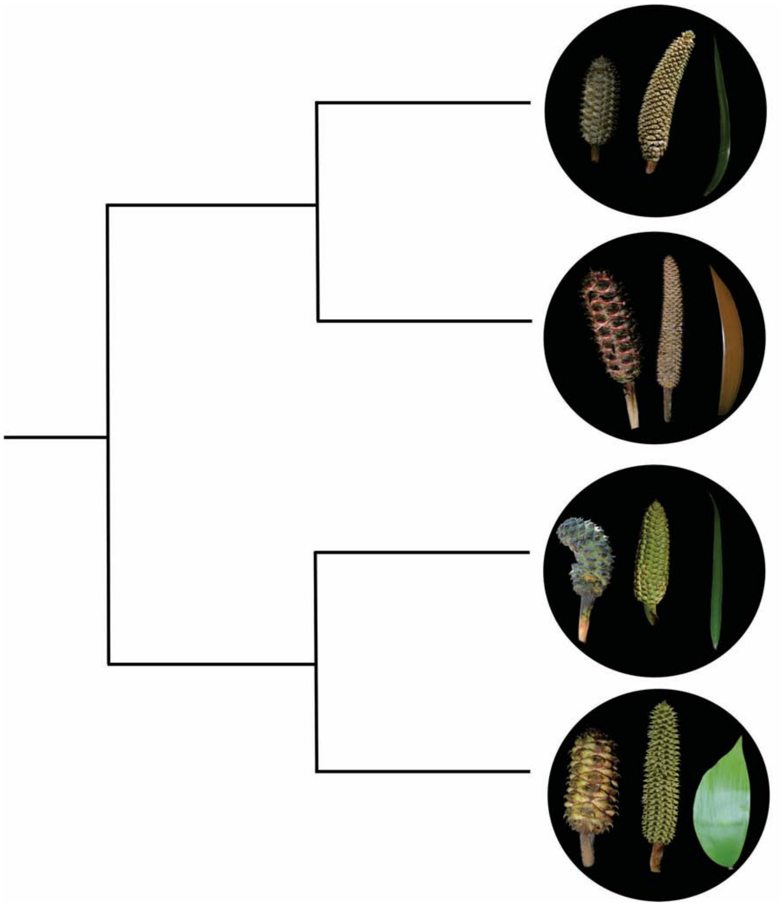
II.IV. Capítulo IV.

Relaciones filogenéticas en *Ceratozamia*: fósiles, reconstrucción de caracteres ancestrales y trayectorias evolutivas



II.IV. Morphological evolution of reproductive structures in *Ceratozamia*, a diverse genus in the Cycadales. Artículo sometido a 'Annals of Botany' (23377).

Morphological evolution of reproductive structures in *Ceratozamia*, a diverse genus in the Cycadales

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Evolution of reproductive structures in cycads

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ABSTRACT

• Background and Aims *Ceratozamia* is a dioecious Mesoamerican gymnosperm genus. All species have pollen and ovulate strobili specialized for aspects of sexual reproduction. Morphologically, the reproductive structures of cycads have received special attention in terms of pollination and innovations related to the emergence of the Spermatophytes. However, variability of pollen and ovulate strobili, evolutionary trajectories and the role of environment in the evolution of cycad species by selecting for phenotypic reproductive variation have not been well documented.

• Methods In the present study, we sampled 1400 individuals of 36 *Ceratozamia* species to explore patterns in the evolution of their reproductive phenotypes to identify and evaluate factors that potentially drove their evolution. We analyzed characters for both pollen and ovulate strobili within a phylogenetic framework. Thus, we used different methods and characters (molecular and both quantitative and qualitative morphological) to infer phylogenetic relationships in the genus. Using this phylogenetic framework, evolutionary models of trait evolution for strobilar size were evaluated. In addition, quantitative morphological variation and its relation to environmental variables across species were analyzed.

• Key Results We found contrasting phylogenetic signals between characters of pollen and ovulate strobili. These structures exhibited high morphological disparity in several characters concerning size. Results of analyses of evolutionary trajectories suggested a stabilizing selection model. In regards to phenotype-environment, the analysis produced mixed results and differences for groups in the vegetation type where the species occur; however, a positive relationship with climatic variables was found.

• **Conclusions** The integrated approach synthesized reproductive phenotypic variation with current phylogenetic hypotheses and provided explicit statements of character evolution for pollen and ovulate strobili. The characters of volume for ovulate strobili in this context were the most informative, which could provide a reference for further study of the evolutionary complexity in *Ceratozamia*. Finally, the heterogeneous environments, which are under changing weather conditions, promote variability of reproductive structures.

Key words: *Ceratozamia,* cycads, gymnosperm, models of character evolution, phenotype, phylogenetic signal, strobilar size, Zamiaceae

INTRODUCTION

Exploring the morphological variation of phenotype in seed plants offers an opportunity to identify and evaluate factors that drive the evolution of morphological characters (Pigliucci, 1996; Dkhar and Pareek, 2014; Weber *et al.,* 2020). In cycads, an ancient order of nonflowering seed plants, several studies, largely focused on vegetative morphologies, have shown that leaflet shape is homoplasious and leaflet size is strongly influenced by environmental factors (Newel, 1985; Limón *et al.,* 2016; Calonje *et al.,* 2019). This group has separate pollen-producing and seed-producing strobili whose morphological characteristics have been of great interest because of questions related to dispersal and the change from leaf-like reproductive structures into determinate and/or indeterminate strobili in the case of ovulate plants of *Cycas* L. (Stevenson *et al.,* 1998; Brenner *et al.,* 2003). However, the full range of variation in pollen and ovulate strobili has not been completely described and evaluated in detail (Klavins *et al.,* 2013; Elgorriaga and Atkinson, 2023). Reproductive morphology in cycads has been addressed in morphological terms as primary resources for classification at family and generic levels (Stevenson, 1990, 1992). The incomplete and scarce preservation of reproductive structures in scientific collections and prolonged development, which lasts from one to two years as well as a lack of knowledge of reproductive phenological patterns have resulted in a paucity of data to close this gap (Norstog and Nicholls, 1997; Martínez-Domínguez *et al.,* 2022 *a*).

Gymnosperm strobili appear to be relatively undiversified but there are some dioecious species in cycads and Gnetales with fleshy structures associated with their seeds, which is a condition correlated with animal-dispersal (Leslie *et al.,* 2017; Nigris *et al.,* 2021). In particular, the extant cycad genera show different fleshy covering the ovule and strobilus colors (Nigris *et al.,* 2021). All these characteristics together with strobilus size are key to plant reproduction and dispersal of diaspores. Evolutionary patterns of strobilus and seed size have been studied in the largest lineage of gymnosperms, the Pinaceae (Leslie, 2011 *a*; Leslie *et al.,* 2014, 2017; Leal-Sáenz *et al.,* 2020) but neglected in other groups. Size changes could occur also in different evolutionary pathways between pollen and ovulate strobili (Gleiser *et al.,* 2019). These evolutionary trajectories are the result of different selective pressures and intricate interactions between ecological factors and developmental constraints (Gleiser *et al.,* 2019; Leslie, 2011 *a*).

Cycadales, with a diversity of approximately 372 species worldwide in ten genera, is characterized by simple strobili and compound leaves (Norstog and Nicholls, 1997; Calonje *et al.,* 2013-2023). *Ceratozamia* is a genus endemic to the Mesoamerican dominion with a broad distribution in the mountainous areas of Mexico, Belize, Guatemala and Honduras (Martínez-Domínguez *et al.,* 2022 *b*). Currently, the diversity of this dioecious genus comprises approximately 36 to 39 species that are distributed in humid environments (Calonje *et al.,* 2013-2023; Martínez-Domínguez *et al.,* 2022 *b*). It has high phenotypic variation, but with similarities among closely related species (Martínez-Domínguez *et al.,* 2022 *b*). Interspecific variation in leaf size and other vegetative characters such as number of leaves and leaflets as well as anatomical characters such as the number and length of perivascular fibers have been widely evaluated and used as basic sources of information for recognizing evolutionary patterns (Martínez-Domínguez *et al.,* 2016; Medina-Villarreal and González-Astorga, 2016; Vovides *et al.,* 2020). In terms of shape, foliar morphological characters exhibit a high degree of homoplasy and are correlated with climatic conditions, particularly those associated with water stress (Stevenson *et al.,* 1986; Medina-Villarreal *et al.,* 2019). The convergence that characterizes vegetative morphology in this group has hindered phylogenetic inferences and evolution of phenotypes. Also, the morphological stasis present in phenotypes in cycads is even found in the genome (Renner, 2011; Wu and Chaw, 2015) and this in turn has discouraged the study of reproductive structures that have been considered uninformative. Moreover, morphological variation in reproductive structures has been poorly addressed (Martínez-Domínguez *et al.,* 2022 *c*). However, the morphology in this genus has been augmented by the description of additional morphological characters of both pollen and ovulate strobili (Martínez-Domínguez *et al.,* 2018, 2022 *b*). Some of the shapes of reproductive characters were analyzed through the reconstruction of ancestral characters in the most recent phylogeny of genus (Habib *et al.,* 2023). In contrast, intra-interspecific variation of strobilus size and the subsequent causes of phenotypic variation among species remain poorly understood. Surprisingly, morphological characters of ovulate strobili were correlated with temperature annual range, i.e., minimum temperature of the coldest month and the maximum temperature of the warmest month (Martínez-Domínguez *et al.,* 2022 *c*).

Despite a rich history of research in phylogenetics (e.g., González and Vovides, 2002, 2012; Vovides *et al.,* 2004; Medina-Villarreal *et al.,* 2019; Habib *et al.,* 2023), the morphological variation in *Ceratozamia* has long confounded systematists, with each new phylogeny seemingly producing new hypotheses of relationships. The first two molecular phylogenetic studies of *Ceratozamia* focused at the infrageneric level and resulted in poor resolution and low support values (González and Vovides, 2002, 2012). Based on these phylogenetic trees, which included *ITS*, *trnL-F* and *D-SCAR*, seven species complexes were proposed (González and Vovides, 2012). Consequently, a phylogeny using six molecular markers produced two major clades ('Mexicana Clade' and 'Miqueliana Clade'), again with relatively low support for species relationships within each clade (Medina-Villarreal *et al.,* 2019). More recently, a phylogeny with 80% of the species using transcriptome data from multiple single-copy nuclear genes (SCGs) was published for the genus (Habib *et al.,* 2023) that recovered three major clades ('Mexicana, 'Miqueliana', and 'Matudae') with six subclades that, in part, correspond to the species complexes of González and Vovides (2012). Overall, two later phylogenies (Medina-Villarreal *et al.,* 2019; Habib *et al.,* 2023) exhibit some discrepancies in the phylogenetic position of species within subclades.

Incorporating fossil cycad taxa into phylogenetic analyses could contribute to a more robust understanding of their evolution such as has been done for other gymnosperm groups (Leslie *et al.,* 2018). The fossil record of cycads is primarily represented by vegetative structures that are often incomplete and difficult to assign to a genus (Kvacek, 2002, 2004, 2014; Hermsen *et al.,* 2006; Martínez *et al.,* 2012). However, recent microscopy techniques have helped to clarify some fossil identities between the genera *Zamia* and *Ceratozamia* whose macromorphological vegetative differences are inconspicuous. Four fossil species have been assigned to *Ceratozamia,* all of which only have vegetative structures of the leaves preserved. Two species were described based on shape and length of leaflets*, C. wrightii* Hollick from the Eocene of Alaska and *Ceratozamites vicetinus* Meschinelli from the Oligocene of Italy. The latter is dubious as a cycad as pointed out by Hollick (1932) and other authors (e.g., Kräusel, 1928). *Ceratozamia floersheimensis* (Engelhardt) Kvaček and *C. hofmannii* Ettingsh., from the lower Oligocene and the lower Miocene of Europe, respectively, were described not only from macromorphological structures, but also based on anatomical characters (Erdei *et al.,* 2012; Kvacek, 2014). In particular, epidermal structures such as cuticles and stomata have been found to be more informative for assigning these fossils to extant cycad genera (Erdei *et al.,* 2012; Kvacek, 2014). These last two species can be unambiguously assigned to *Ceratozamia* based on articulate leaflets inserted laterally and attached via the entire width of the leaflet with the former characterizing the Subfamily Zamioideae (Stevenson, 1992), and the latter *Ceratozamia* within that group along with anatomical characters of stomata (Kvacek, 2014). Unfortunately, no fossil reproductive structures of *Ceratozamia* are known to date. In general, the fossil record is equivocal, in part, because of low quality of preservation and similarities with other unrelated groups that could result in inaccurate taxonomic assignment (Klavins *et al.,* 2003; Elgorriaga and Atkinson, 2023).

The goal of this study was to quantify variation of reproductive structures in plants that produce pollen and seeds on separate individuals in populations of all species of *Ceratozamia*. Thus, we analyzed the pattern of variation in pollen and ovulate strobili in *Ceratozamia*. This included evaluating the degree of reproductive morphological differentiation among the species. All data were analyzed within an explicit phylogenetic framework through phylogenetic reconstruction with morphological data that included strobilus shape and size. We then compared the patterns of change across time and phylogenetic signal using Blomberg's K, Pagel's λ, Moran's and Abouheif's indices. We also tested for climate influence on reproductive morphology in all species under all environments and elevations where *Ceratozamia* occurs. Additionally, we used the fossils of *Ceratozamia* to discuss vegetative morphological change and its evolutionary trajectory.

MATERIAL AND METHODS

Taxon sampling

This work follows the most recent circumscription of *Ceratozamia* in the recent monograph by Martínez-Domínguez *et al.* (2022 *b*). Of the 36 species attributed to the genus, we analyzed 1500 individuals for vegetative characters from a total of 96 populations (10–20 per population) and 1400 individuals for reproductive characters from 79 populations (5–12 per population) located throughout the distribution range of the genus (Supplementary Data Table S1). Leaf samples were pressed and dried for further measurements with all vouchers deposited in the herbaria of CIB, MEXU and TEFH. Five leaflet samples from each of these populations were used from these collections. Some morphological characters were measured and coded directly in the field for each species such as the number of leaves and the characters associated with ovulate and pollen strobili. In total, we evaluated 788 pollen strobili and 612 ovulate strobili from 76 and 70 populations, respectively. The reproductive structures were measured at the same ontogenetic stage. Thus, the pollen strobili had visible microsporangia and showed a slight separation of the microsporophylls. The megasporophylls of ovulate strobili had fully developed ovules. We developed a database with all geographic coordinates and elevations for each sampled population. This included all herbarium records for all species to produce a full distribution map of the genus (Fig. 1).

Character and character state coding

A total of 89 vegetative and reproductive morphological characters were coded as binary, multistate or a range (Supplementary Data Table S2 and S3). We evaluated 44 morphological vegetative characters with 17 and 27 of those either quantitative or qualitative, respectively (Supplementary Data Table S2). For each reproductive structure, 22 quantitative and 23 qualitative characters were evaluated (Supplementary Data Table S2 and S3). A qualitative character was coded as polymorphic when more than one character state occurred in a species (e.g., symmetry of leaflet lamina in *Ceratozamia delucana*). The quantitative characters for the phylogenetic analysis were expressed as intervals (1 standard error –SE– of the mean, to the mean +1 SE) to address the diminished significance problem (i.e., the attribution of different character states to terminals when they do not differ significantly from each other; Goloboff *et al.,* 2006). To evaluate the variation of reproductive structures and their correlation to the environment through other statistical analysis, a database with these characters for each individual was created to establish a record of total range.

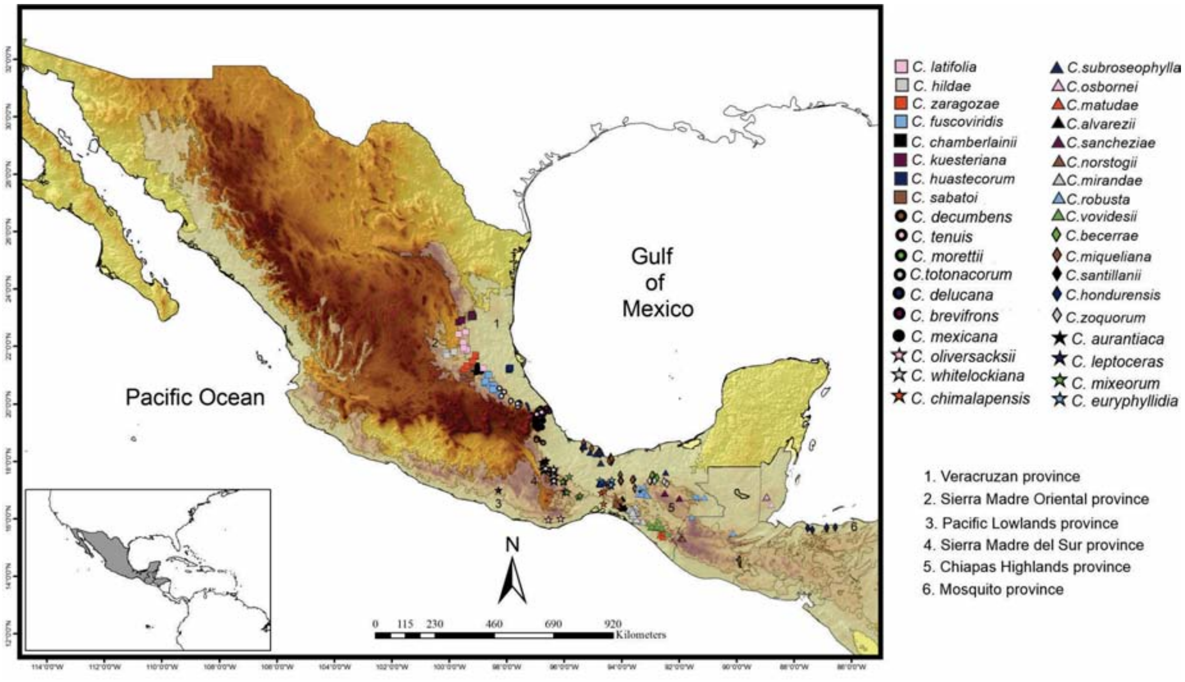

FIG. 1. Distribution map and vegetation types of *Ceratozamia* species. Populations covered in this study are indicated with black circles, whereas the rest of the distribution is indicated with gray circles. The Neotropical provinces represented by dashed lines are according to Morrone *et al.,* 2022.

Molecular markers, sequence editing and alignment

Total genomic DNA was extracted from 100 mg of silica-dried leaflets for all *Ceratozamia* species following the protocol of the DNeasy Plant Mini Kit (QIAGEN, Germantown, MD, USA). Also, we included three outgroup species: *Dioon sonorense* (De Luca, Sabato & Vázq.Torres) Chemnick, T.J.Greg. & Salas-Mor, *Bowenia serrulata* (W.Bull) Chamb. and *Zamia furfuracea* L.f. We selected 9 molecular markers from nuclear and chloroplast genome regions used in the phylogenetic studies of *Ceratozamia* and other Cycadales genera (Salas-Leiva *et al.,* 2013; Condamine *et al.,* 2015; Medina-Villarreal *et al.,* 2019; Martínez-Domínguez *et al.,* 2020; Xiao *et al.,* 2020). The loci GroES, GTP, HTS, CyAG and PEX4 are cycad-specific single-copy nuclear genes (SCNGs); the nuclear ribosomal ITS region provided most of the phylogenetic signal in *Ceratozamia*; and three plastid DNA regions, *matK* (the maturase K, coding region), and two intergenic spacer *psbK/I* and trnS-G that have high diagnostic sites. PCR amplifications were performed as reported by in previous published works (Shaw *et al.,* 2005; Nicolalde-Morejón *et al.,* 2011; Salas-Leiva *et al.,* 2014). PCR products were purified using a QIAquick PCR Purification Kit (QIAGEN). The PCR amplifications were evaluated by electrophoresis using 1% agarose gel stained with ethidium bromide. Purified products were sent to Macrogen Inc. (Seoul, South Korea) for automated Sanger sequencing.

Nucleotide sequences were manually edited with Sequencher v.4.8 (Gene Codes Corp., Ann Arbor, MI, USA). Sequences for each locus were aligned using the 'multiple alignment' option of Clustal X in BioEdit v.7.0.9 (Thompson *et al.,* 1997; Hall, 1999). The aligned lengths ranges from 615 bp (GTP) to 1254 bp (CyAG). Sequences were deposited in GenBank (Supplementary Data Table S4). Sequences of each marker were assembled with SequenceMatrix v.1.7.8 (Vaidya *et al.,* 2011).

Phylogenetic analysis

We analyzed both separate and combined matrices. Four datasets were created: (1) all morphological data, (2) all molecular data, (3) a combined matrix with morphological and molecular data of only extant species, and (4) a combined matrix with morphological and molecular data for extant and extinct species. The total length of the concatenated molecular data was 7890 nucleotide sites with no molecular data for the fossils.

All data sets were analyzed using parsimony. The qualitative and molecular characters were treated as equally likely, and quantitative characters, both continuous and meristic, as additive. We carried out a heuristic search using 1000 random sequence additions replicates and TBR branch swapping. Internal branches were considered unsupported and collapsed during lookups if ambiguously supported (when optimization is missing, TNT rule 1; Coddington and Scharff, 1994). For character-weighting, we used implied weighting across different constant values of concavity *K* (see Goloboff, 1993, 1995, Goloboff *et al.,* 2008). This "sensitivity analysis" (Goloboff *et al.,* 2008) was performed with *K* values of 0, 3, 10, 20, 25, 30, and 35. These values are compared with the previous results of combined data represented over the trees in the form of boxes of "Navajo rug" (Wheeler, 1995). The consistency index (CI) and the retention index (RI) were calculated. Two supporting indices were calculated to assess the stability of the clades, bootstrap and jackknife with 36% cutoff and 1000 replicates. All analyses were carried out using TNT v.1.5 (Goloboff *et al.,* 2008).

Simultaneous analyses using Maximum Likelihood (ML) and Bayesian Inference (BI) with the concatenated matrix of molecular data were implemented. ML was carried out in W-IQ-TREE (Trifinopoulos *et al.,* 2016) using the ultrafast bootstrap approximation method with 1000 replicates. Divergence times were calculated through BI using BEAST v.2.6.7 software (Drummond and Rambaut, 2012). We used the Bayesian Information Criterion in jMODELTEST v.2.1.10 (Posada, 2008) to select the best-fit model of nucleotide substitution for each dataset (Supplementary Data Table S5). A lognormal relaxed-clock model and a birth-death process were used as branching process priors in BEAUti (Drummond and Rambaut, 2012). The calibrations were conducted using fossil calibrations from previous studies in Cycadales: uniform priors between 0 to 10 with a starting value of 0.1 for the mean growth rate, uniform priors between 0 to 10 with a starting value of 0.5 for the relative death rate, and exponential prior with a mean of 0.33 on the standard deviation and a uniform prior between 0 to 1 on the mean of the model (Condamine *et al.,* 2015; Calonje *et al.,* 2019). Two independent runs of 50 million Markov chain Monte Carlo (MCMC) generations were performed, sampling every 1000 iterations. Log and tree files were combined using Logcombiner v. 2.6.7 (included in BEAST). The log output was evaluated using Tracer v.1.7.1 (Rambaut *et al.,* 2018). To elaborate a maximum clade credibility tree, we used Tree Annotator v.2.4.4 (Drummond and Rambaut, 2012), discarding 10 % of the trees as burn-in. FigTree v.1.4.4 was used to visualize the results.

The tree from BI was used to explore the evolution of reproductive morphological character state reconstruction though parsimony as implemented in Mesquite v.3.70 (Maddison and Maddison, 2017). In total, we used 11 characters considered informative and used to diagnose species within this genus, nine of which are reproductive characters (microsporophylls shape, infertile portion shape of microsporophylls, fertile portion shape, direction of microsporophyll horns and ovulate strobilus apex shape, number of megasporophylls per row, length fertile portion of ovulate strobilus, length fertile portion of pollen strobilus and width of microsporophylls) and two vegetative characters (leaf color at emergence and leaflet shape).

Variation of reproductive quantitative characters between species

To explore the differences among *Ceratozamia* species, we used the ANOSIM test (analysis of similarities). This procedure measures the overlap among the clusters and was performed with 999 permutations (Clarke and Ainsworth, 1993) in R package vegan v.2.3-5 (Oksanen *et al.,* 2018).

Analysis of strobili size evolution

We used four indices to test phylogenetic signal from all quantitative reproductive characters that included both pollen and ovulate strobili (20 reproductive characters, of which 11 correspond to the ovulate strobili and nine to the pollen strobili). These are: (1) fertile portion length of ovulate strobilus-LOH, (2) fertile portion diameter of ovulate strobilus-DOS, (3) length of ovulate strobilus peduncle-LOSP, (4) diameter of ovulate strobilus peduncle-DOSP, (5) length of megasporophylls-LMe, (6)width of megasporophylls-WMe, (7) horn length of megasporophylls-HLMe, (8) distance between horns of megasporophylls-DHMe, (9) number of megasporophylls-NMe, (10) number of orthostichies-Nor, (11) number of megasporophylls per row-NMer, (12) fertile portion length of pollen strobilus-LPS, (13) fertile portion diameter of pollen strobilus-DPS, (14) length of pollen strobilus peduncle-LPSP, (15) diameter of pollen strobilus peduncle-DPSP, (16) length of microsporophylls-LMi, (17) width of microsporophylls-WMi, (18) horn length of microsporophylls-HLMi, (19) distance between horns of microsporophylls-DHMi, (20) length of infertile portion of microsporophylls-LIpMi. We estimated Blomberg's K and Pagel's λ using the function phylosig from R phytools (Revel, 2012), which are based on an explicit Brownian Motion model of character evolution (BM) (Pagel, 1999; Blomberg *et al.,* 2003; Münkemüller *et al.,* 2012). Also, we estimated two indices of autocorrelation that do not rely directly on an evolutionary model, Moran's I and Abouheif's C_{mean}, using the function mora.abouheif from the R package adephylo (Jombart *et al.,* 2010; Münkemüller *et al.,* 2012). The first describes the relation of phylogeny to cross-taxonomic character variation and considers branch length (Gittleman and Kot, 1990). The second is based on topology (not branch length) and measures autocorrelation among terminals by a phylogenetic approximation matrix (Abouheif, 1999). We made a comparison between ovulate and pollen strobili sizes for all *Ceratozamia* species. Data were scaled using min-max normalization by applying the formula $Z_i=[X_i–\min(X)]/[\max(X)–\min(X)]$, which scales data to a linear range from 1 as highest and 0 as lowest.

We tested a suite of four different evolutionary models for each character that have been widely used in several groups, in particular, as related to phylogenetic conservatism and morphological stasis (Leslie *et al.,* 2017; Glade-Vargas *et al.,* 2018; Gleiser *et al.,* 2019): (1) Brownian motion (BM), a stochastic model of evolution in which characters move away from the ancestral value tracking an optimum that drifts neutrally (Felsenstein, 1988); (2) Ornstein-Uhlenbeck process (OU), a stabilizing selection model of evolution with one optimum where a character evolves towards an optimal value of the ancestral character value, i.e. integrates random walk with a deterministic tendency (Harmon *et al.,* 2010; Ingram *et al.,* 2012); (3) early burst model (BM), a model based on a random direction of evolution of characters but with a rate of diffusion that decreases over time (Ingram *et al.,* 2012); and (4) white noise (WN; non-phylogenetic) model, characterized by loss of tracks that indicate shared ancestry or random draw (Münkemëller *et al.,* 2015; Gleiser *et al.,* 2019). To increase the power of the test, we estimated standard errors according to our data for each species. The fitting of these models was evaluated using the Akaike Information Criterion (AIC), and low AIC values together with AIC weight where values nearest 1 indicate the best fitting model (Butler and King, 2004, Diniz-Filho *et al.,* 2012). The models with $\Delta\text{AIC} \leq 2$ values showed more substantial support or evidence for the model with best fit (Burnham and Anderson, 2002). These analyses were performed using the R package geiger (Harmon *et al.,* 2008).

Relationship of reproductive quantitative characters with climate

Separately, we ran two principal component analyses (PCA) for all populational of species data: (1) pollen strobili, and (2) ovulate strobili. PCA allowed for the visualization of reproductive morphological variation among species. A categorical variable to describe the type of vegetation for each population was created. Additionally, comparisons of the values of all reproductive character within each vegetation type were represented using boxplots.

To evaluate the relationship of the reproductive morphological characters with climate across the range of *Ceratozamia*, we used the average values for each population, considering 19 bioclimatic variables from the WorldClim project (Hijmans *et al.,* 2005; Supplementary Data Table S6) at a spatial resolution of 30 arc-sec and elevation. A redundancy analysis (RDA) was implemented with R package vegan (Oksanen *et al.,* 2018) to determine the combination of environmental variables that explain the morphological variation in both pollen and ovulate strobili. All climatic variables were standardized to the same scale. We conducted the analyses with all environment variables using R v.4.1.2 (R Core Team, 2022).

Results

Phylogenetic analysis

The molecular matrix of only extant taxa included 7890 characters and with characters equally weighted under parsimony resulted in 27 most parsimonious trees (L= 2412 steps, CI = 0.831, RI=0.781) (Supplementary Data Figure S1A). The strict consensus was poorly resolved but the majority rule consensus tree recovered an almost completely resolved topology with three species as a polytomy (Supplementary Data Figure S1A). The morphological matrix included 95 characters and recovered a single most parsimonious tree (L=1428.617, CI=0.417, IR=0.570, *K*=25) (Supplementary Data Figure S1B). This topology has several differences in the topology of species within each of the two clades, 'Mexicana' and 'Miqueliana', when compared to the topology based on the molecular matrix (Supplementary Data Figure S1).

The quantitative and qualitative morphology and molecular data when concatenated included 7985 characters. The parsimony analysis had low support values, but each analysis from the implied weighting recovered only one most-parsimonious tree and the species in each of the two clades recovered were highly consistent across concavity values (*K*) with few changes in relationship among some taxa representing those clades (Fig. 2). The analysis with only extant taxa found a single most-parsimonious tree with a length of 3942.663 steps, CI=0.659, and IR=0.647 (*K*=25; Supplementary Data Figure S1B). Also, the analysis with the same data concatenated for both extant and extinct taxa found a single tree with a length of 3955.351 steps, CI=0.657 and RI=0.647 (*K*=10). All reconstructions with different *K* values for this matrix showed the same clades with few changes among sister groups (Fig. 2). The topologies recovered from both analyses, with and without fossils species, were highly similar with only a few reordered relationships among sister species. *Ceratozamia,* including the two extinct taxa, was recovered as monophyletic with the two extinct species as a sister clade to the extant ones.

All phylogenetic analyses (parsimony, ML, BI) recovered the same broad topology consisting of two major clades: (1) a 'Miqueliana Clade', and (2) a 'Mexicana Clade' (Figs. 2, 3; Supplementary Data Figure S1). Most subclades were supported by values greater than 80% bootstrap for ML (Supplementary Data Figure S1). Some relationships within each clade showed some discrepancies. For example, *Ceratozamia norstogii*, *C. alvarezii* and *C. santillanii* were monophyletic in the parsimony trees, but in different clades in the ML tree (Supplementary Data Figure S1). *C. zoquorum* was recovered in different positions and clades in separate analyses (Figs. 2, 3; Supplementary Data Figure S1). The other species within each major clade were consistent in all the topologies.

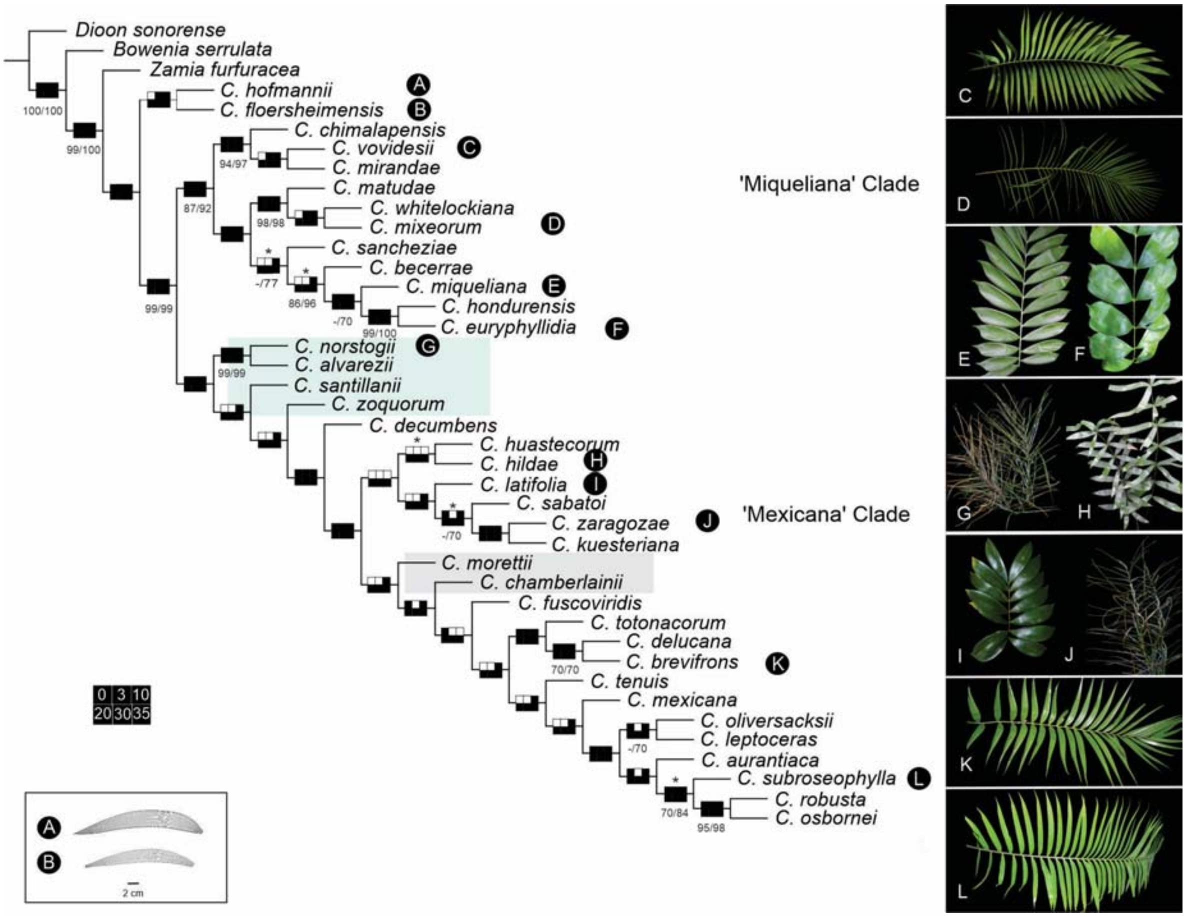

FIG. 2. Phylogeny of *Ceratozamia* with fossil taxa included based on the combined morphology (qualitative and quantitative characters) and DNA data sets. Single most parsimonious tree recovered from parsimony analysis under *K*=25 (CI=0.647, RI=0.631, tree length= 4018.739). The groups not recovered by Maximum Likelihood and Bayesian Inference without the inclusion of fossils are indicated with the colored boxes. The support values from different resampling analysis are indicated on each branch only for values >70% (bootstrap, jackknife).

The two major clades are partially congruent with morphological similarities, geographic affinity, or both. The 'Miqueliana Clade' (Fig. 3) was composed of *Ceratozamia alvarezii, C. norstogii, C. miqueliana, C. euryphyllidia, C. hondurensis, C. santillanii, C. becerrae, C. sancheziae, C. matudae, C. chimalapensis, C. mirandae, C. vovidesii, C. zoquorum* and *C. whitelockiana*, which are species primarily distributed south of the Trans-Mexican Volcanic Belt in the Chiapas highlands province, the Mosquito province and south of the Veracruzan province. There were two exceptions to this: *C. whitelockiana* and *C. mixeorum.* Morphologically, the 'Miqueliana Clade' has two types of leaves and leaflets. This clade has one species group with relatively long leaves with a few broad leaflets, and another group that has shorter leaves with several very narrow leaflets (c.f. Figs. 2, 3). In contrast, the 'Mexicana Clade' comprised species with moderately narrow leaflets and long leaves, which occur to the north of the Trans-Mexican Volcanic Belt, particularly in the Sierra Madre Oriental province and the north of the Veracruzan province with the exception of *C. robusta, C. osbornei, C. subroseophylla, C. aurantiaca, C. oliversacksii* and *C. leptoceras*. These latter species are found in southern Mexico, are monophyletic within the *C. robusta* complex (Fig. 2), and are morphologically similar (Fig. 2L).

The mean stem age of *Ceratozamia* was estimated at 32.57 Ma during the Oligocene (95% HPD 27.62-37.57) and the crown age at 16.32 Ma (95% HPD 13.88-18.92) during the mid-Miocene. The two major clades, 'Miqueliana' and 'Mexicana', have similar ages (Fig. 3). The split among subclades within the 'Mexicana Clade' was estimated to be 10.13 Ma (95% HPD 7.45-17.18 Ma) for these Sierra Madre Oriental species. Speciation within the 'Miqueliana Clade' began approximately 10.02 Ma (5.91-14.09 Ma), which is consistent when compared to the most comprehensive previous result (Habib *et al.,* 2023).

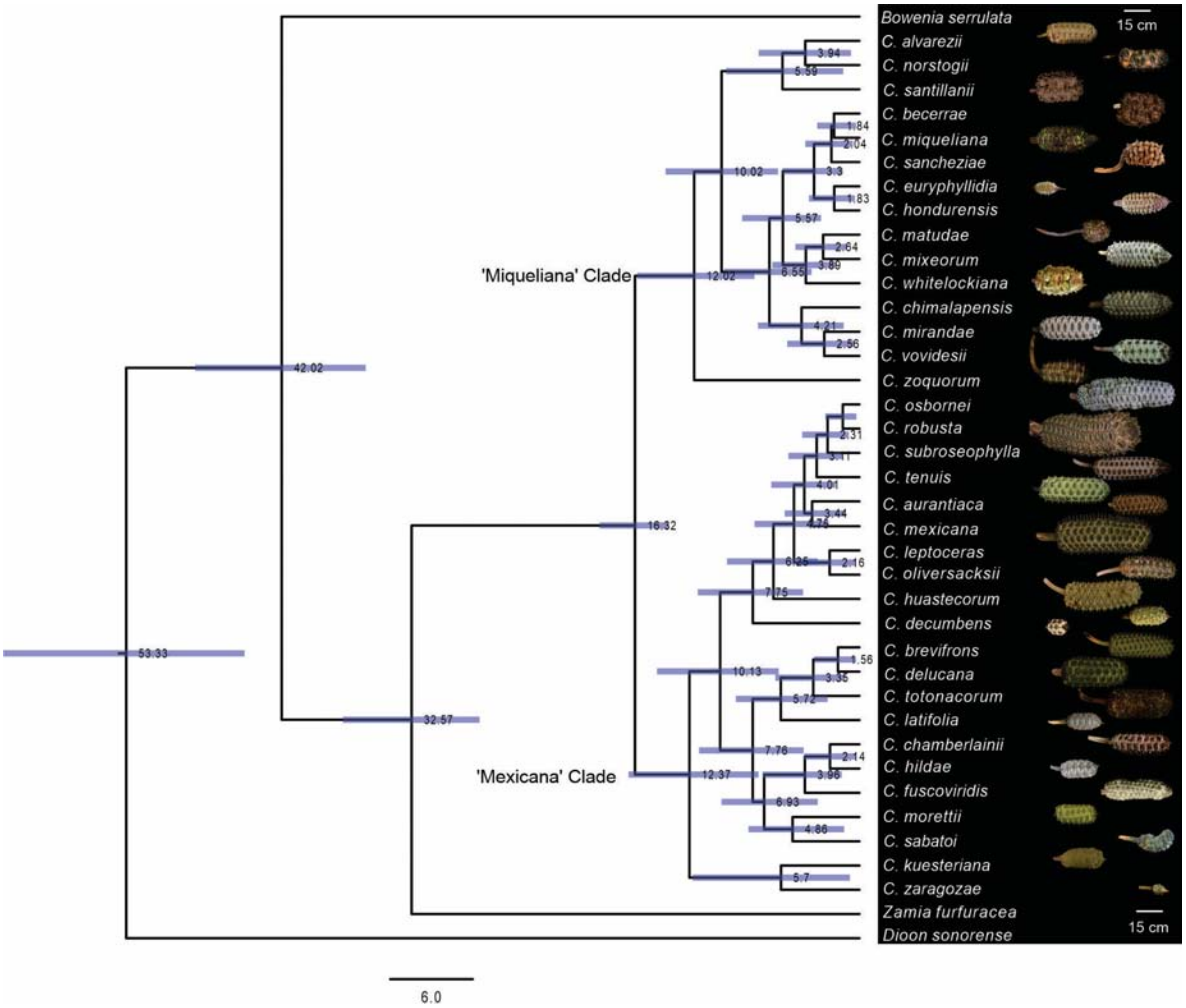

FIG. 3. Maximum clade credibility tree chronogram obtained using single-copy nuclear genes, ITS and plastid loci. Numbers on branches are mean estimated ages of each clade (Ma.). The purple bars indicate the 95% highest posterior density (HPD) indices.

Evolution shape: ancestral character state reconstruction

The ancestral states recovered were: (1) microsporophylls with a lobate fertile portion, (2) ovulate strobilus with an acuminate apex, (3) oblong leaflets, and (4) whitish gray trichomes on leaves. In terms of continuous characters, the reconstruction showed ovulate strobili characterized by a small size with a low number for NMer and LOH, which suggested relatively small ovulate strobili. Overall, the reconstruction suggested a tendency towards a higher number of megasporophylls with the total length of ovulate strobili becoming longer, but in a few species tending to have become reduced (*Ceratozamia matudae, C. becerrae, C. santillanii, C. zaragozae, C. sancheziae, C. zoquorum*). We found a similar size distribution for pollen strobili. Regarding WMi, the smallest and largest (extreme) sizes for microsporophylls were recovered only in the 'Mexicana Clade' (Supplementary Data Figure S2J).

All qualitative morphological characters exhibited homoplasy. However, microsporophyll shape (Fig. S1C), infertile portion shape of microsporophylls (Fig. S1B), and leaflet shape (Fig. S1D) revealed trends that identify subclades. The obconic shape of microsporophylls was inferred to occur almost entirely in the 'Mexicana Clade' (Fig. S1C), whereas the elliptic shape was absent in this clade and rare in the 'Miqueliana Clade' (Supplementary Data Figure S2). An orbicular shape of the infertile portion of microsporophylls was rare in both clades and seems to have evolved separately several times, the linear shape was mainly within the 'Miqueliana Clade' and the rounded shape was within the 'Mexicana Clade' (Fig. S1C). With respect to vegetative characters, leaflet shape was extremely homoplasious, appearing to arise several times (Fig. S1D). The only leaflet shape character exclusive for sister species was obovate leaflets, which is a synapomorphy for *Ceratozamia hondurensis* and *C. euryphyllidia* (Fig. 2 E, F).

Variation of quantitative reproductive characters among Ceratozamia *species*

Our analysis of ovulate strobili characters showed a high level of dissimilarity between *Ceratozamia* species (R=0.7762) with a significance of 0.001. Most species were highly dissimilar in the size of the ovulate structures (Supplementary Data Figure S2). Species with a high dissimilarity were *C. robusta, C. subroseophylla*, *C. osbornei*, *C. zoquorum*, *C. becerrae*, *C. matudae*, *C. decumbens*, *C. whitelockiana* and *C. zaragozae*. The first three species showed high similarity among themselves (Supplementary Data Figure S3). At the same time, the rest of these species exhibited high similarity among species groups; in particular, the following pairs of species were not significantly differentiated: *C. becerrae* with *C. zoquorum*, and *C. decumbens* with *C. zaragozae* and *C. whitelockiana.* In contrast, *C. matudae* was dissimilar to all the other species even when compared with its closely similar species (Supplementary Data Figure S2).

Our analysis of pollen strobili characters indicated an R-value closer to 1 (R=0.7806) with a significance of 0.001, which suggests more dissimilarity among species for characters of the pollen strobili. Several species showed high dissimilarity related to their congeners (Supplementary Data Figure S3). In particular, *Ceratozamia osbornei* and *C. robusta* showed high segregation from several species, but high similarly between themselves. Other species with high dissimilarity compared to most species within the genus were *C. totonacorum, C. latifolia, C. leptoceras, C. santillanii, C. zaragozae* and *C. kuesteriana* (Supplementary Data Figure S3). Overall, the pollen strobili characters showed strong patterns of dissimilarity between sister species.

Evolutionary patterns of reproductive structures size between ovulate and pollen strobili

Strobilar size within *Ceratozamia* showed different evolutionary patterns when comparing pollen and ovulate strobili characters to each other (Figs. 4, 5). The length range observed in these reproductive structures overlapped in most species (Fig. 4). The relative strobili size comparisons between the length of pollen and ovulate strobili across all species showed a positive trend where the pollen strobili size increases as do the ovulate strobili (r=0.71; Fig. 4). In both pollen and ovulate strobili, *C. matudae* and *C. latifolia* independently have the smallest strobili and the clade comprised *C. robusta* and *C. osbornei* have the largest strobili. Relative length variations were found in some species. This disparity was wider between ovulate and pollen strobili in those species that had long strobili. For most species, pollen strobili were longer than ovulate strobili. A few species (e.g., *C. latifolia*) had ovulate and pollen strobili of equal length (Fig. 4). Another character with an equal length size pattern was the peduncle of both pollen and ovulate strobili in several species including those with long strobili such as *C. robusta* (r=0.75; Fig. 5D).

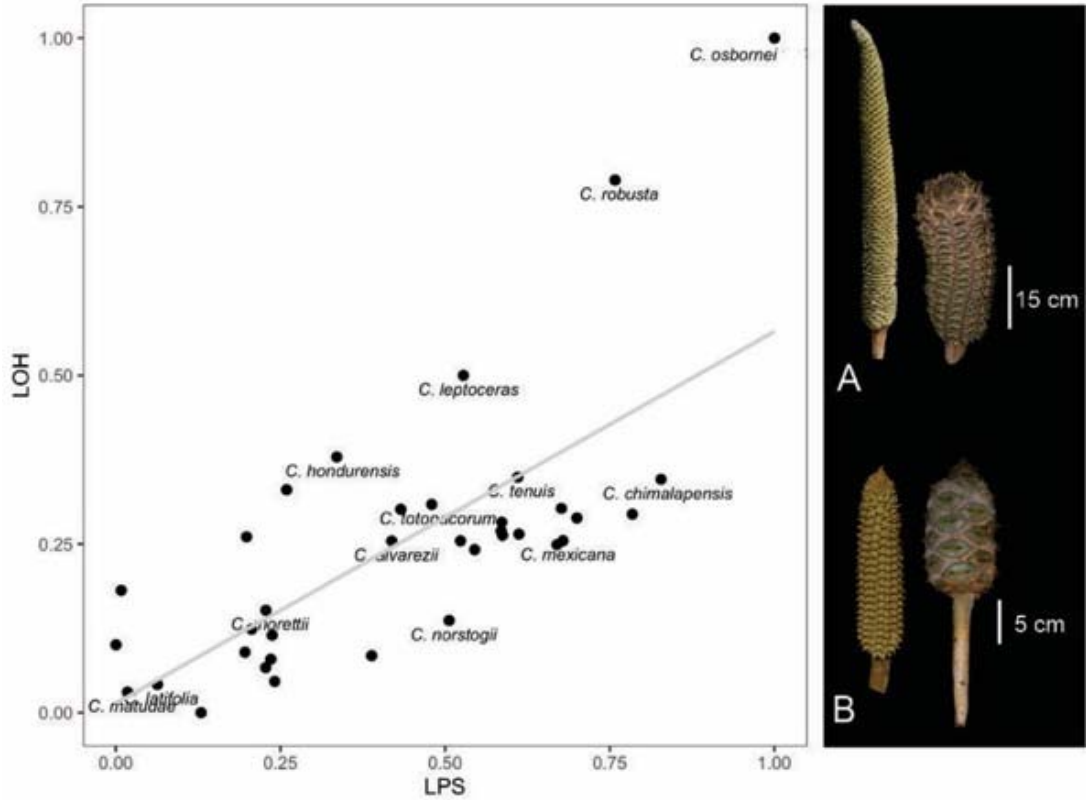

FIG. 4. Comparisons between ovulate and pollen strobili length (fertile portion of the strobilus) for *Ceratozamia* species. Strobili of *C. robusta* (A) and *C. latifolia* (B) on the right as examples of the disparity and similarity in sizes of ovuliferous and polliniferous strobili, respectively.

The rest of the species had similar patterns where most had ovulate and pollen strobili that did not exhibit parallel changes in length (Fig. 5). The diameter of pollen and ovulate strobili did show a positive trend, which was parallel in a few species such as *Ceratozamia tenuis* and *C. chimalapensis* (r=0.65; Fig. 5A). In contrast, the characters directly related to the microsporophylls and megasporophylls showed the greatest size dissimilarity (Fig. 5C, E, F). This is particularly developed in the case of the distance between the horns of sporophylls as shown by the clear separation from the line across all species in Fig. 5C (r=0.02).

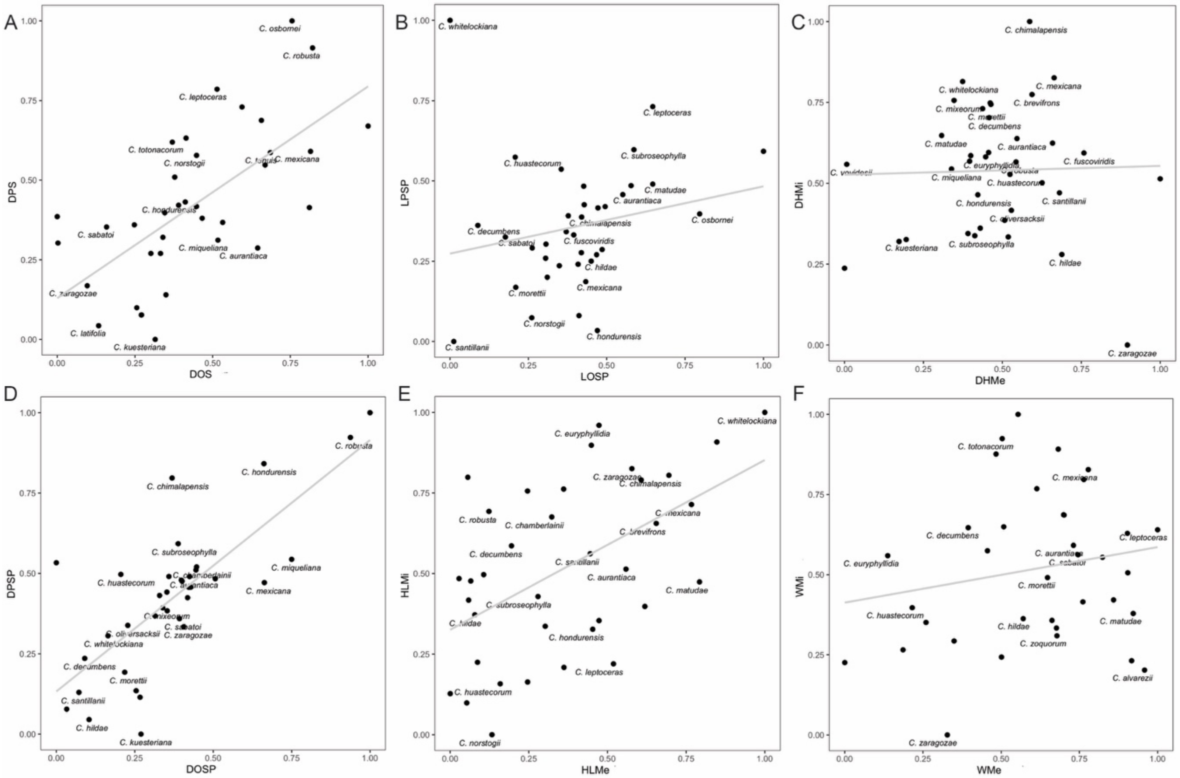

FIG. 5. Comparison between ovulate and pollen strobili size for *Ceratozamia* species. (A) Diameter of strobilar fertile portions. (B) Length of strobilar peduncles. (C) Distance between the horns of sporophylls. (D) Diameter of strobilar peduncles. (E) Horn length of sporophylls. (F) Width of sporophylls.

TABLE 1. *Outcomes of the phylogenetic signal tests for all quantitative characters for pollen and ovulate strobili of the* Ceratozamia *species.*

Strobilus type	Quantitative character	Blomberg's *K*	Pagel's λ	Abouheif's C_{mean}	Moran's *I*
		K/*P*	λ/P	C_{mean}/SO*/*P*	Moran's *I*/SO*/*P*
Ovulate strobilus	LOH	0.561/0.011	0.746/0.130	0.262/2.418/0.015	0.237/2.183/0.019
	DOS	0.387/0.161	0.192/0.288	0.124/1.138/0.139	0.108/1.256/0.117
	LOSP	0.416/0.118	<.001/1	0.026/0.233/0.384	-0.009/0.215/0.375
	DOSP	0.335/0.396	<.001/1	0.153/1.436/0.084	0.139/1.428/0.085
	LMe	0.409 /0.105	<.001/1	0.205/1.982/0.034	0.185/1.769/0.049
	WMe	0.291/0.677	<.001/1	0.019/0.182/0.41	-0.003/0.270/0.354
	HLMe	0.392/0.1	0.074/0.645	0.205/1.894/0.036	0.185/1.849/0.048
	DHMe	0.373/0.278	0.067/0.547	-0.042/-0.451/0.65	-0.085/-0.563/0.700
	NMe	0.627/0.001	0.591/0.009	0.359/3.249/0.005	0.342/3.295/0.003
	NOr	0.606/0.001	0.565/0.010	0.340/3.079/0.006	0.326/3.162/0.001

	NMer	0.627/0.003	0.644/0.019	0.379/3.386/0.002	0.360/3.583/0.001
Pollen strobilus	LPSP	0.523/0.003	0.482/0.154	0.319/2.781/0.006	0.304/3.033/0.002
	DPSP	0.509/0.009	<.001/1	0.165/1.488/0.081	0.138/1.612/0.061
	LPS	0.450/0.052	0.526/0.138	0.246/2.218/0.016	0.225/2.280/0.014
	DPS	0.398/0.14	<.001/1	0.117/1.080/0.143	0.086/0.995/0.168
	LMi	0.658/0.001	0.693/0.165	0.326/2.885/0.004	0.305/2.924/0.007
	WMi	0.813/0.001	0.784/0.0004	0.435/3.885/0.001	0.407/3.829/0.001
	HLMi	0.368/0.213	0.056/0.670	0.073/0.610/0.25	0.044/0.613/0.278
	DHMi	0.511/0.025	0.633/0.081	0.183/1.771/0.045	0.130/1.533/0.071
	LIpMi	0.515/0.008	0.596/0.141	0.199/1.932/0.031	0.173/1.749/0.055

*SO shows deviation from random expectation.

TABLE 2. *Parameter estimates and statistical support for the evolutionary models of ovulate strobili size characters.*

Quantitative	Parameters			Models (AIC/ΔAIC/AICw)			
characters	α	σ^2	z_0	BM	EB	NW	OU
LOH	0.176	20.178	21.450	254.033/3.707/0.117	256.033/5.707/0.043	254.563/4.237/0.090	250.326/0/**0.749**
DOS	0.289	2.374	9.270	169.540/10.983/0.002	171.541/12.984/0.000	159.175/0.618/0.422	158.557/0/**0.574**
LOSP		10.559	9.192	203.536/10.316/0.003	205.536/12.316/0.001	193.220/0/**0.691**	194.867/1.647/0.303
DOSP		0.304	1.892	82.290/15.602/0.000	84.290/17.602/0.000	66.978/0.29/0.463	66.688/0/**0.536**
LMe		0.514	2.597	90.577/7.018/0.019	92.577/9.018/0.007	83.559/0/**0.667**	85.129/1.57/0.304
WMe		1.013	3.516	121.042/13.957/0.000	123.042/15.957/0.000	107.085/0/**0.730**	109.085/2/0.268
HLMe	-	-	-	-1.889/6.137/0.016	-8.026/0/**0.357**	-8.026/0/**0.357**	-7.450/0.576/0.268
DHMe	-	-	-	47.952/6.367/0.014	41.585/0/**0.347**	41.585/0/**0.347**	41.935/0.35/0.291
NOr	0.129	0.941	7.905	149.819/2.56/0.208	154.484/7.225/0.020	154.484/7.225/0.020	147.259/0/**0.750**
NMer	0.175	0.175	8.516	374.000/3.321/0.157	380.755/10.076/0.005	380.755/10.076/0.005	370.679/0/**0.831**
NMe	0.139	474.647	70.723	192.580/5.213/0.066	195.318/7.951/0.016	195.318/7.951/0.016	187.367/0/**0.899**

- Akaike weights are equal.

Based on the inferred phylogenetic relationship, we estimated Pagel's λ, Blomberg's K, Abouheif's C_{mean} and Moran's *I* to determine the phylogenetic signal of pollen and ovulate strobili size variation. In the analysis of both ovulate and pollen strobili in all species, size exhibited strong phylogenetic signals for these reproductive characters and the size of related species was more similar than expected under Brownian motion (Table 1). The WMi had the strongest phylogenetic signal recovered under all tests (Table 1).

While several ovulate strobili size characters did not show significant phylogenetic signal (Table 1), three other reproductive structure characters (NOr, NMer and NMe) had high phylogenetic signal values. The remaining characters of ovulate strobili sizes such as DOS and LMe had a random distribution across the phylogeny. In relation to pollen strobili sizes, most characters showed only moderate phylogenetic signal (Table 1). The signal of LPSP and LMi was significantly smaller than those found for ovulate strobili, but nevertheless were recovered as relatively high values under all tests.

For strobilar size diversification, the model-based analysis using AIC weight supported OU as the best model as compared with alternative evolutionary models (Table 2). Most of the size characters for ovulate strobili showed uncertainty ($\Delta AIC \leq 2$) indicating that the models EB, OU and NW were not distinguishable from each other. Only LOH, NMer, NOr and NMe characters recovered AIC values that indicated substantial support for an OU model of character evolution (Table 2). In particular, the ΔAIC values of DOS, DOSP, LOSP, LMe and WMe characters do not provide supporting evidence to distinguish between NW and OU models, HLMe between the EB and NW models (Table 2). For pollen strobilar size, the model of evolution tests showed that LPS and LMi fits an OU model with a weight of 0.93 and 0.71, respectively (Table 3). The LPS character was indistinguishable among BM, NW and OU models, whereas WMi was not distinguishable between BM and OU models.

Strobilar size patterns associated with vegetation types

Ceratozamia inhabits five vegetation types: ETF (evergreen tropical forest), MCF (mountain cloud forest), OF (oak-forest), POF (pine-oak forest) and TDF (tropical subdeciduous forest). Most species are restricted to one vegetation type and primarily occur in ETF or MCF (Fig. 1). There was a difference between the morphology of pollen strobili and the vegetation types, whereas in ovulate strobili there was no a clear morphological separation in relation to vegetation types (Fig. 6). For the ovulate strobili, the first two components of PCA explained 64.19% of the accumulated variance with the higher loading values associated with characters of size in fertile portion of the ovulate strobili (LOH, DOS, NMe and NOr) and two megasporophylls variables (WMe and DHMe) (Fig. 6A; Supplementary Data Table S7). In general, ovulate strobilar size was overlapping in all the types of vegetation where most of the species occur (Fig. 6A); however, morphological variability was different within each vegetation type. Morphological variation of species in each vegetation type overlapped among different species, particularly, in ETF, which had the widest variation. Morphological size had some differences in terms of variability range. POF species had the greatest variation even though few species inhabit this vegetation type. In contrast, MCF showed the least morphological variation with some extreme values that overlapped with the other vegetation types (Fig. 6A). This occurred even in the variables that most contributed to the explanation of the variation such as in LOH (Fig. 7C).

For the morphological variation of pollen strobili, 58.41% of the accumulated variance was explained by the first two components (42.48% and 15.93%; Supplementary Data Table S8). DHMi and HLMi had the highest loading values (Fig. 6B; Supplementary

Data Table S8). The species that occurred in TDF were clearly separated in sizes from species that occurred in POF and OF as well as differing marginally from species in ETF and MCF (Fig. 6B). The pollen strobili of species in ETF showed the greatest morphological variation, whereas those in TDF showed the lowest variation. The mean values overlap for the variables evaluated in most vegetation types except in TDF (Fig. 7). Morphological variation of species in each vegetation type showed similar sizes for variables such as LMi, PPSP and LIpMi (Fig. 7).

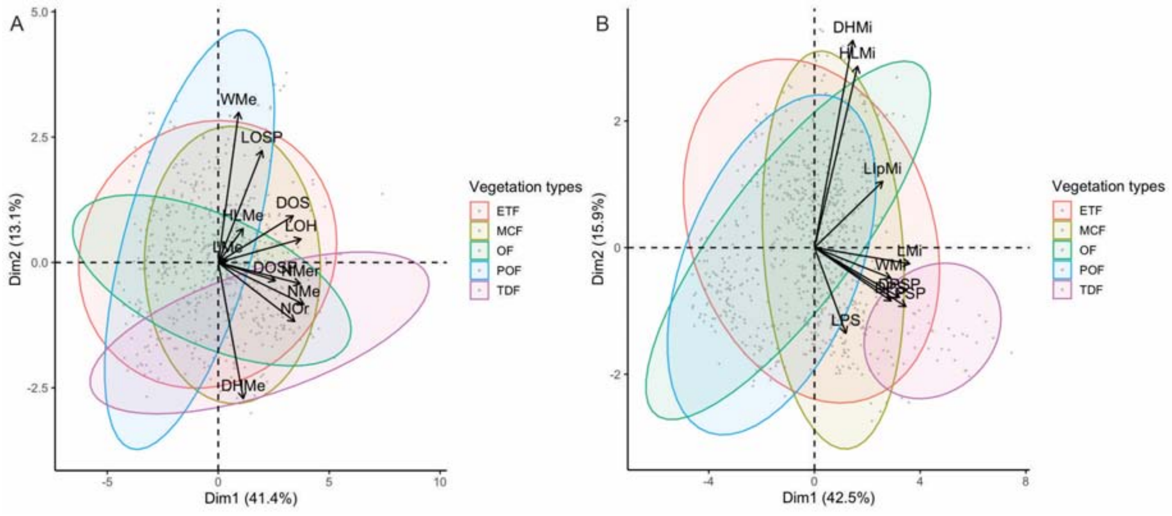

FIG. 6. Phenotypic variation of strobili for *Ceratozamia* species by vegetation types in which the populations occurred. (A) PCA of ovulate strobili. (B) PCA of pollen strobili.

Relative contribution of climatic variables and elevation to phenotype reproductive

Collectively, elevation and climatic variables explained 50.5% of the total variation in the ovulate strobilar characters among the sampled species (Fig. 8A). The results of RDA for ovulate strobili showed a relationship between climatic variables and elevation and phenotypic variation of *Ceratozamia* species across their distribution range ($F=1.9177$, $p < 0.001$, $N = 999$ permutations). This phenotype-climate interaction was not marked for a single species but rather for groups of species. The most important morphological variables were LOH and NMe (Fig. 8A); the most significant climate variables for the first axis were isothermality (BIO2) and maximum temperature of the warmest month (BIO5). For the second axis, it was temperature seasonality (BIO4) and temperature annual range (BIO7). While the largest ovulate strobili occurred in warmer areas, the variation of ovulate strobili size is homogeneously distributed across the climatic and elevational range of all species.

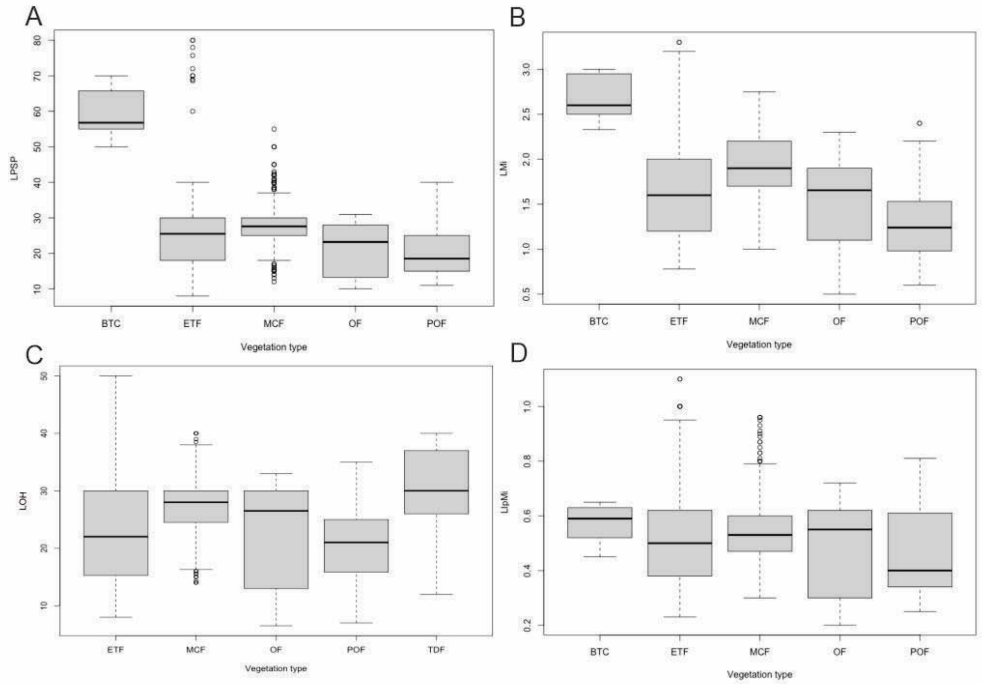

FIG. 7. Boxplots of the morphological characters by vegetation types. (A) Length of pollen strobilus peduncle. (B) Length of microsporophylls. (C) Length of the ovulate strobilus fertile portion. (D) Length of pollen strobilus fertile portion.

The results of the full redundancy analysis for pollen strobili indicated that climatic variables and elevation had a high impact on reproductive phenotype (F = 9.3961, $p < 0.001$, N = 999 permutations). This morphological variation could correspond to the amplitude of variation observed in the vegetation types (Fig. 6). The percentage of the variance explained with two axes was 80% (Fig. 8B). BIO4, BIO7 and BIO5 were the most significant variables for the first axis with annual precipitation (BIO12), precipitation seasonality (BIO15), precipitation of wettest quarter (BIO16) and precipitation of warmest quarter (BIO18) for the second axis. The LPSP was the only character highly correlated with BIO5 (Fig. 8B). Precipitation of the wettest month (BIO13) and annual precipitation (BIO12) were variables that contribute more to the explanation of the variation of LPS, where this character increased only in *Ceratozamia subroseophylla, C. leptoceras*, *C. mixeorum* and *C. robusta*.

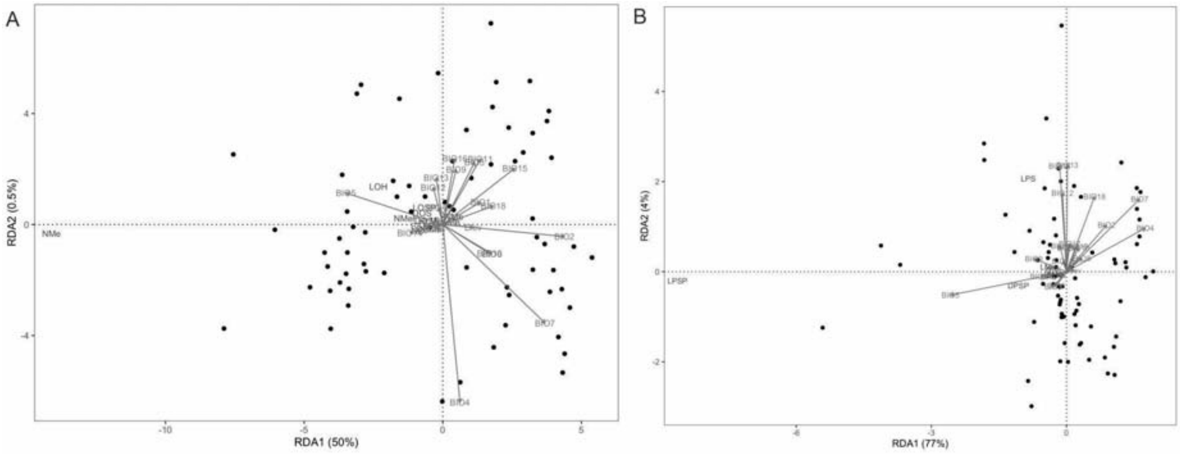

FIG. 8. Graph of the redundancy analysis that includes both climatic variables and elevation for *Ceratozamia* species. (A) RDA of ovulate strobili. (B) RDA of pollen strobili.

TABLE 3. *Parameter estimates and statistical support for the evolutionary models of pollen strobili size characters.*

Quantitative characters	Parameters			Models (AIC/ΔAIC/AICw)			
	α	σ^2	z_0	BM	EB	NW	OU
LPS	0.254	61.539	23.719	289.512/10.293/0.005	291.512/12.293/0.002	285.337/6.119/0.044	279.218/0/**0.947**
DPS	0.289	1.225	4.336	141.988/8.326/0.011	143.988/10.360/0.004	135.622/1.994/0.265	133.628/0/**0.719**
LPSP	0.176	3.635	8.765	192.677/1.722/0.010	194.677/5.683/0.003	190.716/1.722/0.266	188.994/0/**0.720**
DPSP	0.338	0.152	1.712	65.600/11.342/0.002	67.600/13.342/0.000	55.579/1.321/0.339	54.258/0/**0.612**
LMi	0.143	0.070	1.481	54.572/2.862/0.178	56.572/4.862/0.065	60.038/8.328/0.011	51.710/0/**0.744**
WMi	0.105	0.016	0.981	8.306/1.823/0.256	10.306/3.823/0.094	14.849/8.366/0.009	6.483/0/**0.638**
HLMi		0.008	0.272	-50.586/13.013/0.001	-48.586/15.013/0.000	-63.599/0/**0.730**	-61.599/2/0.268
DHMi		0.016	0.630	-36.494/3.402/0.095	-34.494/5.402/0.035	-39.896/0/**0.525**	-39.046/0.85/0.343
LIpMi	0.194	0.010	0.486	-20.001/4.578/0.064	-18.001/6.578/0.023	-22.858/1.721/0.270	-24.579/0/**0.640**

DISCUSSION

Phylogenetics relationships: towards synthesis

The overall topology of our phylogenetic tree mostly agrees with the last two previously published studies on *Ceratozamia* (Medina-Villarreal *et al.,* 2019; Habib *et al.,* 2023). We recovered the same two major clades as in Medina-Villarreal *et al.* (2019) with 28 taxa included, but species comprising each clade differ as well as most internal relationships. One of the causes of the differences could be related to the greater number of species included here (36 species vs 30 species in Habib *et al.,* 2023). Despite the low number of loci sampled here, the relationships were highly consistent with Habib *et al.* (2023), which included a broad sample of SCGs and 30 species. Our results were similar with respect to species relationships within subclades (Fig. 3). The primary difference was the 'Matudae Clade' comprised of *C. matudae* and *C. mixeorum* that was recovered by Habib *et al.* (2023). These two species were found as sister forming a subclade with *C. whitelockiana* within the 'Miqueliana Clade' in our analyses in contrast to the 'Matudae Clade' being sister to the rest of the genus in Habib *et al.* (2023) (Fig. 3). The 'Mexicana Clade' showed some nested positions that were moderately distinct, for example *C. decumbens* was recovered as sister by *Habib et al.* (2023) to the rest of species within this clade, whereas in our phylogeny this species was recovered within a subclade. However, both phylogenies found the similar relationships between species distributed in north of Sierra Madre Oriental province and species of *C. robusta* complex that occur in southern Mexico (Sierra Madre del Sur and Chiapas highlands) (Figs. 1, 3).

Incorporating fossils into a phylogenetic analysis is desirable (Donoghue *et al.,* 1989; Rothwell *et al.,* 2018). Nevertheless, for lineages such as *Ceratozamia*, which have a morphologically complex pattern and where extinct members are only known from characters of leaflets, it is a difficult task. This study is the first to include all *Ceratozamia* species, both extinct and extant, so that the resulting phylogenetic tree sheds light on character evolution and evolutionary trends. Despite the ambiguity in the phylogeny recovered by the analysis of combined morphological (with fossils included) and molecular characters (Fig. 2), the position of the two fossil taxa is a clade found to be a sister group to the rest of the extant *Ceratozamia* species (Fig. 2). These fossil species inhabited European during Cenozoic under climatic conditions of moderate to low humidity as compared to the extant species that occur in southeast Mexico (Kvacek, 2002, 2014) suggesting that the origin of the genus is likely to date back to the Oligocene. Currently, this disjunct distribution suggests that the genus was distributed in Europe at least until the lower Oligocene with its distribution becoming contracted during cool and arid periods. Considering the south region of the Trans-Mexican Volcanic Belt as an ancestral area for *Ceratozamia* and in a humid climate (Medina-Villarreal *et al.,* 2019; Habib *et al.,* 2023), this could have led to the genus finally becoming extinct by the Eocene–Oligocene cooling event of temperate regions in Europe.

The fossil record and ancestral characters reconstruction provided evidence of an intricate morphological evolution in *Ceratozamia* (Figs. 2, 3) with a re-diversification beginning in the Miocene and continuing into the Pleistocene (Condamine *et al.,* 2015; Habib *et al.,* 2023). The climatic and geographic conditions that characterized the Trans-Mexican Volcanic Belt could be representative of geographic and ecological barriers for *Ceratozamia* dispersal (Ferrari *et al.,* 2012). Moreover, the splits that occurred among species within *Ceratozamia* seem to correspond with a dynamic geological landscape from the Miocene to Pliocene to the North of Mexico and in Mesoamerican forests as in Isthmus of Tehuantepec or the emergence of isolated mountain systems in the plains such as in the

Sierra de Otontepec (Barrier *et al.,* 1998). Multiple vicariant events at different times could result in the isolation of *Ceratozamia* populations (Martínez-Domínguez *et al.,* 2022c). In this context, the elevated parts of the mountainous systems of Mexico could have acted as refugia similar to that of islands when Pleistocene climatic fluctuations occurred (González-Medrano, 1996; Graham, 1999).

The vegetative macromorphological characters of *Ceratozamia* exhibit a high degree of homoplasy (Figs. 2, 3). In fact, the morphology of fossil species is most similar to the 'Mexicana Clade' both in shape and size of leaflets (Fig. 2) with the lanceolate shape appearing independently in both clades; *C. vovidesii* is a clear example in the 'Miqueliana Clade' and *C. subroseophylla* in the 'Mexicana Clade' (Supplementary Data Figure S2D). Other types of characters, such as anatomical, seem to also show this pattern (c.f., Vovides *et al.,* 2012; 2022). A recent phylogenetic study of the cycad genus *Zamia* showed that anatomical characters are mostly homoplasious (Glos *et al.,* 2022). In addition, several morphological characters are highly polymorphic, which discourages their use in phylogenetic inference (Martínez-Domínguez *et al.,* 2016, 2017). In general, homoplasy in morphology is a characteristic of most cycads (Calonje *et al.,* 2019; Habib *et al.,* 2022).

Ovulate and pollen strobilus: diversity and evolutionary patterns

Unlike the patterns found in vegetative characters, reproductive characters have been shown to be more informative for diagnosing species (Martínez-Domínguez *et al.,* 2022 *b*). Our results showed that, although homoplasy was not absent in these characters, they were more phylogenetically structured where clades were characterized by unique combinations of several plesiomorphic and a few derived characters (Fig. 3; Supplementary Data Figure S2). For example, the elliptic microsporophylls shape is a novelty within the 'Miqueliana Clade' for *Ceratozamia alvarezii*, *C. whitelockiana*, *C. mirandae* and *C. chimalapensis*, whereas the discoid and obconic shape characterize both this clade and 'Mexicana Clade' and these two latter character states may have evolved independently at least three times in each clade (Supplementary Data Figure S2C). Thus, our findings indicated these characters across the tree could be due to both morphological stasis and to parallel evolution.

On the other hand, strobilar size is a question addressed from different approaches such as functionality in some gymnosperms such as *Pinus* and *Araucaria* (Leslie, 2011 *a*, *b*; Leslie *et al.,* 2014; Gleiser *et al.,* 2019). This has been poorly explored in cycads (Martínez-Domínguez *et al.,* 2022c). This is the first study in a cycad genus that has addressed this under an explicitly phylogenetic and evolutionary approach that includes a broad sampling of reproductive structures. The size of reproductive structures is especially relevant in evolution because this condition is directly related to the capacity of pollination and seed dispersal, a process that plays a basic role in evolutionary patterns (Contreras *et al.,* 2017; Zhang *et al.,* 2019).

In Araucariaceae, an evolutionary transition from small to large ovulate and pollen cones with a coexistence of species that had large and small cones for a brief time was proposed by using the fossil record across geological periods (Stults *et al.,* 2012; Gleiser *et al.,* 2019). Unfortunately, cycad strobili are scarcely represented in the fossil record and several of these records are doubtful or otherwise incomplete (Taylor, 1970; Klavins *et al.,* 2013). Recently, two species were described, from the middle Triassic, *Delemaya spinulosa* Klavins, Taylor, Krings & Taylor and from the Late Cretaceous, *Skyttegaardia nagalingumiae* Elgorriaga & Atkinson. These are the most complete cycad fossil pollen strobili preserved with microsporophylls helically arranged into a short pollen strobilus with a few pollen sacs per microsporophyll (Klavins *et al.,* 2013; Elgorriaga and Atkinson, 2023). No pollen or ovulate strobili have been described even though those bicornate sporophylls are unique and easily identified for extinct *Ceratozamia*. Pollen of *Ceratozamia* was found in cloud forest of northern Oaxaca (Mexico) from the Miocene, but the samples were not assigned to a species (Palacios and Rzedowski, 1993). Our results showed a trend to towards increased strobilus length in *Ceratozamia* (Supplementary Data Figure S2G-K), which was clearer in ovulate strobili, both in total length and sporophyll number per column as well as the number of orthostichies (Fig. 3). Nevertheless, the exceptional preservation of the pollen strobilus of *Skyttegaardia nagalingumiae* indicates that this structure was mature: therefore, this small, fossil pollen strobilus and our results in turn indicate an evolution towards larger strobili in cycads (Figs. 3-5; Supplementary Data Figure S2G-K).

The dioecy of cycads, as well as in other gymnosperms, influences the evolution of reproductive structures and can lead to uncoupled evolution between pollen and ovulate strobili by the separate functions of diaspores (Leslie, 2011 *a*, *b*; Gleiser *et al.,* 2019). Here, the exploration of size patterns between pollen and ovulate strobili for each extant species revealed coupling between the length of fertile portions for these structures in at least half of the *Ceratozamia* species (Fig. 4) and the uncoupled evolution was more marked with longer strobili. The rest of the size characters evaluated, except for infertile peduncle, showed a clear uncoupled pattern (Fig. 5). This independent path followed by reproductive structures, principally by sporophyll size in *Ceratozamia*, was consistent with the evolutionary trends described for the strobili in other gymnosperms (Gleiser *et al.,* 2019; Leslie, 2011 *a*). Thus, the patterns exhibited a different phylogenetic structure where ovulate strobili were more phylogenetically structured (Table 1).

The results indicated more phylogenetic signal for the size of ovulate strobili than for pollen strobili (Table 1), whose evolution was best described by the OU model (Tables

2, 3). All informative characters are related to changes in total volume of the ovulate strobilus (NOr, NMe, NMer and LOH). Under this model, the ovulate strobilus size follows stabilizing selection; their phenotype may have resulted from selective pressures moving lineages toward an optimum value with little variation (Butler and King, 2004; Ingram *et al.,* 2012). In contrast, the pollen strobilus size shows less phylogenetic signal than the ovulate strobilus size (Table 1). Only LPS and LMi had significant phylogenetic signal, and its model was OU (Table 3). We noticed that fewer variables related to volume of total length size were observed for the total number of microsporophylls during fieldwork. Because cycads are pollinated by insects, the relationships between reproductive structures depend on different signals that will undoubtedly give rise to numerous phenotypic responses where the ovulate strobilus has multifunctionality to ensure pollination and subsequently provide protection to ovules as compared to pollen strobili that expend more energy to avoid pollen predation (Terry *et al.,* 2007; Salzman *et al.,* 2021). Therefore, this could drive phenotypes that can develop independently.

Influence of environmental heterogeneity on ovulate and pollen strobilar size

Pollen and ovulate strobilar sizes of *Ceratozamia* have been considered less variable than vegetative characters such as width or length of leaves and leaflets (Whitelock, 2004). Here, we found high variability of pollen and ovulate strobilar size at intra- and interspecific levels for most *Ceratozamia* species (Supplementary Data Figure S2, S3). The variational properties of pollen and ovulate characters differed markedly, and characters of pollen strobili were more variable than those in ovulate strobili. Variation in pollen and ovulate strobili size by vegetation type cannot be explained by a direct correlation (Figs. 6, 7, 8). However, the patterns showed a tendency to more variability in strobilus size for heterogenous environments where the genus occurs such as ETF with higher temperatures and strong seasonal precipitation (Miranda and Hernández-X, 1963). Some characters such as LMi and LPSP showed atypical values and the widest range of variation. LOH included the shortest to longest strobili within the genus. Thus, the longest ovulate strobili were in regions with the highest maximum temperature during the warmest month (Figs. 6, 7).

Overall, the results obtained suggest an influence of climate on strobilus size, but the relationship was difficult to explain because we observed a mixed pattern without an apparent ecological pattern for all species (Fig. 8). The phenotype-climate interaction does not represent a generality for all *Ceratozamia* species. An increase in number of megasporophylls or length of ovulate strobilus is correlated with warmer regions, but only for some species (Fig. 8A). The shortest ovulate strobili occurred in OF, ETF and POF (Fig. 7C); the species inhabiting in MCF showed less variability (i.e., a moderate range with respect to the total variability recorded within this genus; Fig. 7). In contrast, pollen strobili variability was not directly related to climate and elevation for most species. The range of morphological variability was similar in different habitat types with a clear tendency to larger pollen strobili and microsporophylls in species that occurred in tropical sub-deciduous forest (Figs. 7, 8). Nevertheless, the amplitude of morphological variability in MCF is similar in both ovulate and pollen strobili, which was characterized by constant and similar range for each species. This habitat exhibits homogeneous levels of humidity that are more persistent despite the presence of a dry and cold period during the year that is maintained by the clouds that form in MCF (Williams-Linera *et al.,* 2013). The similar sizes in the different species could be related to the constant humidity, whereas the other habitats have regimes that change throughout the year.

The variability of environments could drive changes in reproductive phenotypes. In particular, the environmental heterogeneity where seasonal fluctuations operate would provide selective pressure (Schurr *et al.,* 2008; Fusco and Minelli, 2010; Pélabon *et al.,* 2013). In *Pinus*, the morphology associated with dispersal is related to the extremes of seasonal fluctuations such as temperature and precipitation (Salazar-Tortosa *et al.,* 2019). We found broader phenotypic reproductive variation for *Ceratozamia* in those environments with more fluctuations (Figs. 7, 8). Heterogeneous environments would have an indirect effect on reproductive phenotypes only by promoting or generating variation. On the other hand, a large strobilar size variation has been reported both within and among populations of *Ceratozamia* in species with narrow leaflets from Soconusco in Mexico (Martínez-Domínguez *et al.,* 2022 *c*), which was consistent with our current results. Another species with this variation, but only in ovulate strobili was *C. aurantiaca*, which has ovulate strobili from 11 to 40 cm long and 4 to 19 sporophylls per row (Fig. 6; Martínez-Domínguez *et al.,* 2022 *d*). This high variability in strobilar size could be a response mechanism to different environmental pressures. When plasticity of phenotype is adaptative, the plants are able to respond to the adverse effects of different environmental conditions (Chevin and Hoffmann, 2017).

Conclusions

The exploration of phenotypic reproductive structures in *Ceratozamia* presented in this study provides useful perspectives for better understanding the evolution of ovulate and pollen strobili. The findings provide insights into the evolutionary trajectories shaping strobili sizes that may have occurred by natural selection. The size characters with the most phylogenetic signal were related to strobili volume (Table 1). We did not directly assess the volume of these structures; therefore, future studies developing or applying methods to measure the total volume of structures could be relevant to address the still open questions of evolutionary trajectories. A total decoupling between pollen and ovulate strobili would be expected (Leslie *et al.,* 2011 *a*; Gleiser *et al.,* 2019); however, found that several species were the same in the relative length of the complete strobili (Fig. 4), but quite different in their ovulate and pollen reproductive units (i.e., characters of sporophylls Fig. 5). In addition, the exploration of shapes for these structures provides complementary evidence for characterizing the ancestral characters within this genus.

The larger and shorter strobili in *Ceratozamia* occur in habitats characterized by wider variation in climate conditions. The species with low intra-and interspecific variability with the mainly shorter strobili occur in drier habitats such as POF and OF, whereas longer strobili are found in species that occur in TDF. Conversely, species with high variability at intra-and interspecific level and with a broader climatic range occur in ETF. Thus, climate may act as a promoter in shaping variability in strobili size as has been reported for seed size in other gymnosperms (Leslie *et al.,* 2017). We consider that the strobili size could be the result of multiple ecological and developmental factors and not impacted by morphological stasis. The cycads have been considered as an evolutionary dead-end because they are strictly coevolved and highly specialized systems, and their event of genome duplication (Vamosi *et al.,* 2014; Roodt *et al.,* 2017); however, the higher levels of plasticity reported in this group might allow for preadaptations to extremes conditions at different spatial and temporal scales. Finally, these data and results have been obtained for *Ceratozamia* and the conclusions for cycads in general could also be tested in other genera such as *Dioon* and *Macrozamia* where we have a modern monographs and molecular data.

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CONFLICT OF INTEREST

We have no conflicts of interest to declare.

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SUPPLEMENTARY DATA

Supplementary Data Table S1. Sampled populations. The * indicates number of populations with pollen and/or ovulate strobili.

Species	Populations
C. alvarezii	3 (2*) Cintalapa, Chiapas, Mexico
C. aurantiaca	3 (2*) San Pedro Teutila, Oaxaca, Mexico
C. becerrae	1* Tacotalpa, Tabasco, Mexico; 1* Teapa, Tabasco, Mexico
C. brevifrons	2* Alto Lucero de Gutiérrez Barrios, Veracruz, Mexico; 2* Chiconquiaco, Veracruz, Mexico
C. chamberlainii	1* Xilitla, San Luis Potosí, Mexico; 1* Chapulhuacán, Hidalgo, Mexico; 2* Landa de Matamoros, Querétaro, Mexico
C. chimalapensis	1* Santa María Chimalapa, Oaxaca, Mexico
C. decumbens	1* Coetzala, Veracruz, Mexico; 1* Naranjal, Veracruz, Mexico
C. delucana	1* Atzalan, Veracruz, Mexico; 1* Landero y Coss, Veracruz, Mexico; 1* Xochitlán de Vicente Suárez, Puebla, Mexico
C. euryphyllidia	1 Uxpanapa, Veracruz, Mexico; 1* Santa María Chimalapa, Oaxaca, Mexico
C. fuscoviridis	1* Molando de Escamilla, Hidalgo, Mexico; 1 Tlanchinol, Hidalgo, Mexico; 1 *Zacualtipán de Ángeles, Veracruz, Mexico
C. hildae	1* Aquismón, San Luis Potosí, Mexico
C. hondurensis	1* La Ceiba, Atlántida, Honduras; 1* Tela, Atlántida, Honduras
C. huastecorum	2* Tepetzintla, Veracruz, Mexico
C. kuesteriana	2 (1*) Tula, Tamaulipas, Mexico

C. latifolia	1* Naranjo, San Luis Potosí, Mexico; 1* Rayón, San Luis Potosí, Mexico; 1* Tamasopo, San Luis Potosí, Mexico
C. leptoceras	2* Tlacoachistlahuaca, Guerrero, Mexico
C. matudae	1* Acacoyagua, Chiapas, Mexico
C. mexicana	1* Comapa, Veracruz, Mexico; 2* Teocelo, Veracruz, Mexico; 1* Tlaltetela, Veracruz, Mexico; 2* Totutla, Veracruz, Mexico; 1 Xico, Veracruz, Mexico
C. miqueliana	1 Huimanguillo, Tabasco, Mexico; 2 (1*) Ángel R. Cabada, Veracruz, Mexico; 2 (1*) Santiago Tuxtla, Veracruz, Mexico
C. mirandae	1 Jiquipilas, Chiapas, Mexico; 3* Villaflores, Chiapas, Mexico
C. mixeorum	1 Santa María Tepantlali, Oaxaca, Mexico; 1* San Juan Juquila Mixes, Oaxaca, Mexico
C. morettii	2* Chiconquiaco, Veracruz, Mexico; 1* Landero y Coss, Veracruz, Mexico
C. norstogii	1* Santo Domingo Zanatepec, Oaxaca, Mexico; 2* Cintalapa, Chiapas, Mexico
C. oliversacksii	1* Candelaria Loxicha, Oaxaca, Mexico; 1 San Agustín Loxicha, Oaxaca, Mexico; 1 San Miguel del puerto, Oaxaca, Mexico
C. osborneii	1* Cayo, Belize; 1* Toledo, Belize. From herbarium specimens and cultivated plants
C. robusta	1* Berriozábal, Chiapas, Mexico; 1* Tuxtla Gutiérrez, Chiapas, Mexico

C. sabatoi	1* Cadereyta de Montes, Querétaro, Mexico; 1* Pinal de Amoles, Querétaro, Mexico
C. sancheziae	1* Altamirano, Chiapas, Mexico; 1* Tenejapa, Chiapas, Mexico; 1
C. santillanii	1* Berriozábal, Chiapas, Mexico
C. subroseophylla	1* Uxpanapa, Veracruz, Mexico; 2* Santiago Tuxtla, Veracruz, Mexico
C. tenuis	1* Jilotepec, Veracruz, Mexico; 1 Xalapa, Veracruz, Mexico; 1* Coacoatzintla, Veracruz, Mexico; 1* Chiconquiaco, Veracruz, Mexico; 2* Tepetlán, Veracruz, Mexico
C. totonacorum	1 Cuetzalan del Progreso, Puebla, Mexico; 2* Jonotla, Puebla, Mexico
C. vovidesii	1* La Concordia, Chiapas, Mexico
C. whitelockiana	1* San Juan Bautista Valle Nacional, Oaxaca, Mexico; 1 Santiago Comaltepec, Oaxaca, Mexico
C. zaragozae	1* Río Verde, San Luis potosí, Mexico
C. zoquorum	3* Solosuchiapa, Chiapas, Mexico*; 1 Tila, Chiapas, Mexico

Supplementary Data Table S2. Qualitative morphological characters and their respective character states (vegetative and reproductive).

Character	Character state
Stem type	(0) hypogeous; (1) semi-hypogeous; (2) epigeous

Prickles on petiole	(0) absent; (1) present
Prickles on rachis	(0) absent; (1) present
Prickles type	(0) thin; (1) robust
Trichomes color	(0) whitish gray; (1) brown; (2) reddish-brown; (3) brownish yellow
Leaf color at emergence	(0) light-green; (1) dark-brown; (2) brown; (3) yellowish green; (4) reddish brown; (5) darkgreen; (6) green; (7) golden brown
Leaf position	(0) ascending; (1) descending
Insertion of leaflets on the rachis	(0) keeled; (1) plane
Arrangement of leaflets on the rachis	(0) opposite; (1) sub-opposite; (2) clustered
Arrangement of petiole	(0) straight; (1) twisted
Arrangement of rachis	(0) straight; (1) twisted
Leaflet shape	(0) obovate; (1) lanceolate; (2) oblong; (3) linear; (0) oblanceolate
Leaflet direction	(0) planar; (1) abaxially curved
Symmetry of leaflet lamina	(0) basally falcate; (1) not basally falcate
Leaflet consistency	(0) coriaceous; (1) papyraceous; (2) membranaceous
Symmetry of leaflet apex	(0) symmetric; (1) asymmetric

Venation appearance	(0) non-conspicuous; (1) conspicuous; (2) prominent
Color of leaflet base	(0) green; (1) yellow; (2) brown; (3) greenish
Leaflet apex shape	(0) acuminate; (1) acute; (2) rounded; (3) slight pungent
Leaflet base shape	(0) attenuate; (1) cuneate
Glaucous leaflet color (adaxial side)	(0) absent; (1) present
Glaucous leaflet color (abaxial side)	(0) absent; (1) present
Imbricate leaflets	(0) absent; (1) present
Involution of leaflet lamina	(0) flat; (1) canaliculate; (2) lightly involute; (3) strong canaliculate
Pollen strobilus position	(0) erect; (1) pendulous
Pollen strobilus shape	(0) angulate; (1) cylindrical
Pollen strobilus color	(0) greenish yellow; (1) greenish brown (2) reddish brown; (3) yellowish brown; (4) Wine; (5) beige; (6) white
Distal face of microsporophylls	(0) non-recurved; (1) recurved; (2) reflexed
Microsporophylls shape	(0) discoid; (1) obconic; (2) elliptic; (3) rhomboid
Microsporophylls horns type	(0) thin; (1) robust

Infertile portion shape of microsporophylls	(0) orbicular; (1) rounded; (2) linear; (3) triangular
Fertile portion shape of microsporophylls	(0) lobate; (1) deeply lobate; (2) straight
Direction of horns microsporophylls	(0) straight; (1) recurved
Angle between horns of microsporophylls	(0) acute; (1) obtuse; (2) right
Ovulate strobilus position	(0) erect; (1) pendulous
Ovulate strobilus color (trichomes)	(0) light brown; (1) blackish to dark; (2) brown; (3) reddish-brown; (4) greyish gray; (5) wine; (6) pink; (7) whitish gray
Ovulate strobilus apex	(0) acuminate; (1) acute; (2) mucronate; (3) apiculate; (4) aristate
Ovulate strobilus shape	(0) cylindrical; (1) globose; (2) ovoid
Angle between horns of megasporophylls	(0) acute; (1) right; (2) obtuse
Megasporophylls horns type	(0) thin; (1) robust
Direction of horns megasporophylls	(0) non-recurved; (1) recurved
Distal face of megasporophylls type between horns	(0) truncate; (1) prominent

Sarcotesta color	(0) whitish yellow; (1) whitish red; (2) red; (4) yellow
Seed shape	(0) ovate; (1) globose; (2) spherical; (3) ovoid
Distal end of megasporophylls type	(0) concave; (1) straight; (2) bicornate
Distal face of megasporophylls shape	(0) triangular; (1) hexagonal
Margin of leaflets type	(0) entire; (1) denticulate
Articulation of leaflets type	(0) plane; (1) prominent; (2) decurrent
Distal end of leaflet margin	(0) entire; (1) sinuate
Distal end of microsporophylls type	(0) concave; (1) straight; (2) bicornate
Visible veins on adaxial side of leaflets	(0) absent; (1) present
Trichomes on distal end of megasporophylls	(0) scarcely trichomes; (1) pubescent; (2) tomentose
Trichomes on distal end of microsporophylls	(0) scarcely trichomes; (1) pubescent; (2) tomentose
Direction of distal end of microsporophylls	(0) curved; (1) rounded; (2) right

Infertile portion on apex of ovulate strobili	(0) absent; (1) present
Infertile portion on apex of pollen strobili	(0) absent; (1) present

Supplementary Data Table S3. Quantitative morphological characters and abbreviations (vegetative and reproductive).

Character	Abbreviation
Number of leaves	NL
Pairs of leaflets	PL
Length of petiole	LP
Length of rachis	LR
Length of basal leaflet	LBL
Width of basal leaflets	WBL
Width of basal leaflet articulation	WBLa
Distance between basal leaflets	DbBL
Length of median leaflets	LML
Number of veins in median leaflet	NVML
Width of median leaflets	WML
Width of median leaflet articulation	WMLa
Distance between median leaflets	DbML
Length of apical leaflet	LAL
Width of apical leaflet	WAL
Width of apical leaflet articulation	WALa
Distance between of apical leaflets	DbAL
Length of pollen strobilus peduncle	LPSP
Diameter of pollen strobilus peduncle	DPSP
Length of pollen strobilus (fertile portion)	LPS

Diameter of pollen strobilus (fertile portion)	DPS
Length of microsporophylls	LMi
Width of microsporophylls	WMi
Horn length of microsporophylls	HLMi
Distance between horn of microsporophylls	DHMi
Length infertile portion of microsporophylls	LIpMi
Length of ovulate strobilus (fertile portion)	LOH
Diameter of ovulate strobilus (fertile portion)	DOS
Length of ovulate strobilus peduncle	LOSP
Diameter of ovulate strobilus peduncle	DOSP
Length of megasporophylls	LMe
Width of megasporophylls	WMe
Horn length of megasporophylls	HLMe
Distance between horn of megasporophylls	DHMe
Number of megasporophylls	NMe
Number of orthostichies	NOr
Number of megasporophylls per row	NMer
Length of seed	LS
Diameter of seed	DS

Supplementary Data Table S4. Primers used in this study and GenBank Accession Numbers.

Genome	Region	Forward primer	Reverse primer	Reference
nrDNA	ITS, nuclear ribosomal ITS region	5a CCTTATCATTTAGAGGAAGGAG3	4rev TCCTCCGCTTATTGATATGC3	Pennisi, 2007; Nicolalde-Morejón et al., 2011
ncDNA	GTP, GTP-binding protein Era mRNA	GTP_F2 TGATACWCCTGGTGTGAT	GTP_R2 CTCCATSTCCATATTTGGC	Salas-Leiva et al., 2013
ncDNA	CyAG, MADS-box transcription factor family AGAMOUS	CeAG_F7 CCATTTCAGAGTCCAATTCTCAG	AGM3596_R CTTAGTGCGAAGAAACTGATTCTC	Salas-Leiva et al., 2013

ncDNA	GroES, GroES-like zinc-binding alcohol dehydrogenase family protein	GroES_F1c CCAAGCTGATGATGGTAATTTC	GroES_R2 TACATGGTCWGCTCCTAA	Salas-Leiva et al., 2013
ncDNA	HTS, Histidyl-tRNA synthetase	HTS_F1a AACTTCWGATGCTGTTGG	HTS_R3 CAGCACCATGACGCTTRAA	Salas-Leiva et al., 2013
ncDNA	PEX4, peroxin4	PEX4_F1 TCCAGCTAGCCATGACTGTTC	PEX4_R1 GGTTTTGACCCCTATTCGGTA	Salas-Leiva et al., 2014
cpDNA	*matK*, maturase K	ATACCCCATTTTATTCATCC3	GTACTTTTATGTTTACGAGC	Nicolalde-Morejón et al., 2011
cpDNA	*psbK-psbI*, intergenic spacer	TTAGCCTTTGTTTGGCAA G	AGA GTTTGAGAGTAAGCAT	Pennisi, 2007; Nicolalde-

				Morejón et al., 2011
cpDNA	trnS-trnG, intergenic spacer	GCCGCTTAGTCCACTCAGC	GAACGAATCACACTTTTACCAC	Sangin et al., 2008; Xiao et al., 2020

Supplementary Data Table S5. Bioclimatic variables and abbreviations according to WorldClim.

Variable	Abbreviations
Annual Mean Temperature	BIO1
Mean Diurnal Range (Mean of monthly (max temp - min temp)	BIO2
Isothermality (BIO2/BIO7) (* 100)	BIO3
Temperature Seasonality (standard deviation ×100)	BIO4
Max Temperature of Warmest Month	BIO5
Min Temperature of Coldest Month	BIO6
Temperature Annual Range (BIO5-BIO6)	BIO7
Mean Temperature of Wettest Quarter	BIO8
Mean Temperature of Driest Quarter	BIO9
Mean Temperature of Warmest Quarter	BIO10
Mean Temperature of Coldest Quarter	BIO11
Annual Precipitation	BIO12
Precipitation of Wettest Month	BIO13
Precipitation of Driest Month	BIO14
Precipitation Seasonality (Coefficient of Variation)	BIO15
Precipitation of Wettest Quarter	BIO16
Precipitation of Driest Quarter	BIO17

Precipitation of Warmest Quarter	BIO18
Precipitation of Coldest Quarter	BIO19

Supplementary Data Table S6. Molecular markers best-fit evolutionary model.

Analysis	Marker	Model
Bayesian	ITS	HKY
	GTP	001202+F
	CyAG	HKY
	GroES	010110+G+F
	HTS	HKY
	PEX4	HYK
	matK	001202+F
	trnS-trnG	TPM3uf+G
	psbK/psbI	TPM1uf+G
Maximum Likelihood	ITS	HKY+F+R2
	GTP	TPM3+F+R2
	CyAG	HKY+F+R2
	GroES	TPM2+F+R2
	HTS	TPM3+F+R2
	PEX4	HKY+F+R2
	matK	K3Pu+F+G4
	trnS-trnG	HKY+F+R2
	psbK/psbI	K3Pu+F+G4

Supplementary Data Table S7. Principal Component Analysis for all quantitative characters of the ovulate strobili in *Ceratozamia* species.

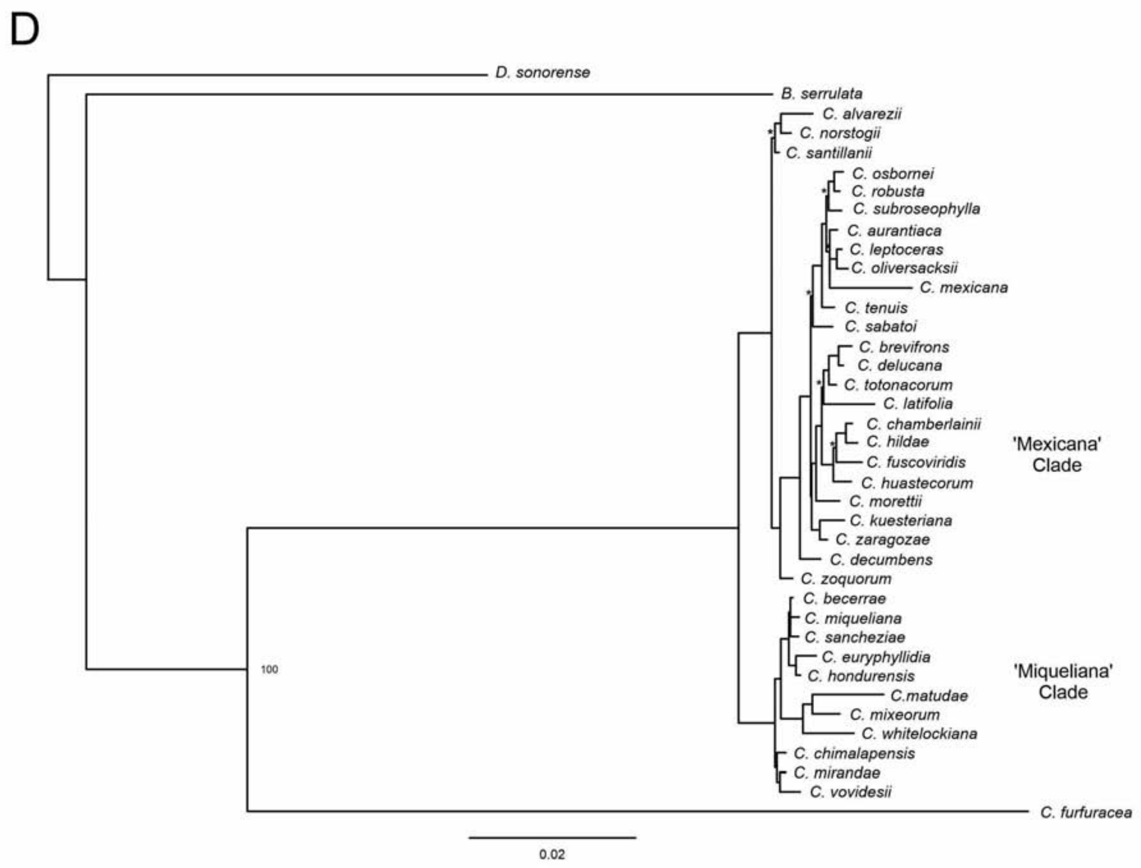
Contributions	Component 1	Component 2
LOH	17.418578	0.870317602
DOS	14.271350	3.427217939
LOSP	4.877078	19.734359530
DOSP	8.290013	0.515415752
LMe	0.232052	0.009606243
WMe	1.062801	35.511570877
HLMe	1.569909	1.816244133
DHMe	1.594525	9.167472732
NMe	1 8.654921	2.757906534
NOr	14.874029	5.480350009
NMer	17.154744	0.709538650
Eigen values	4.55868830	1.43918821
Percentage of variance	41.4426209	64.19088

Supplementary Data Table S8. Principal Component Analysis for all quantitative characters of the pollen strobili in *Ceratozamia* species.

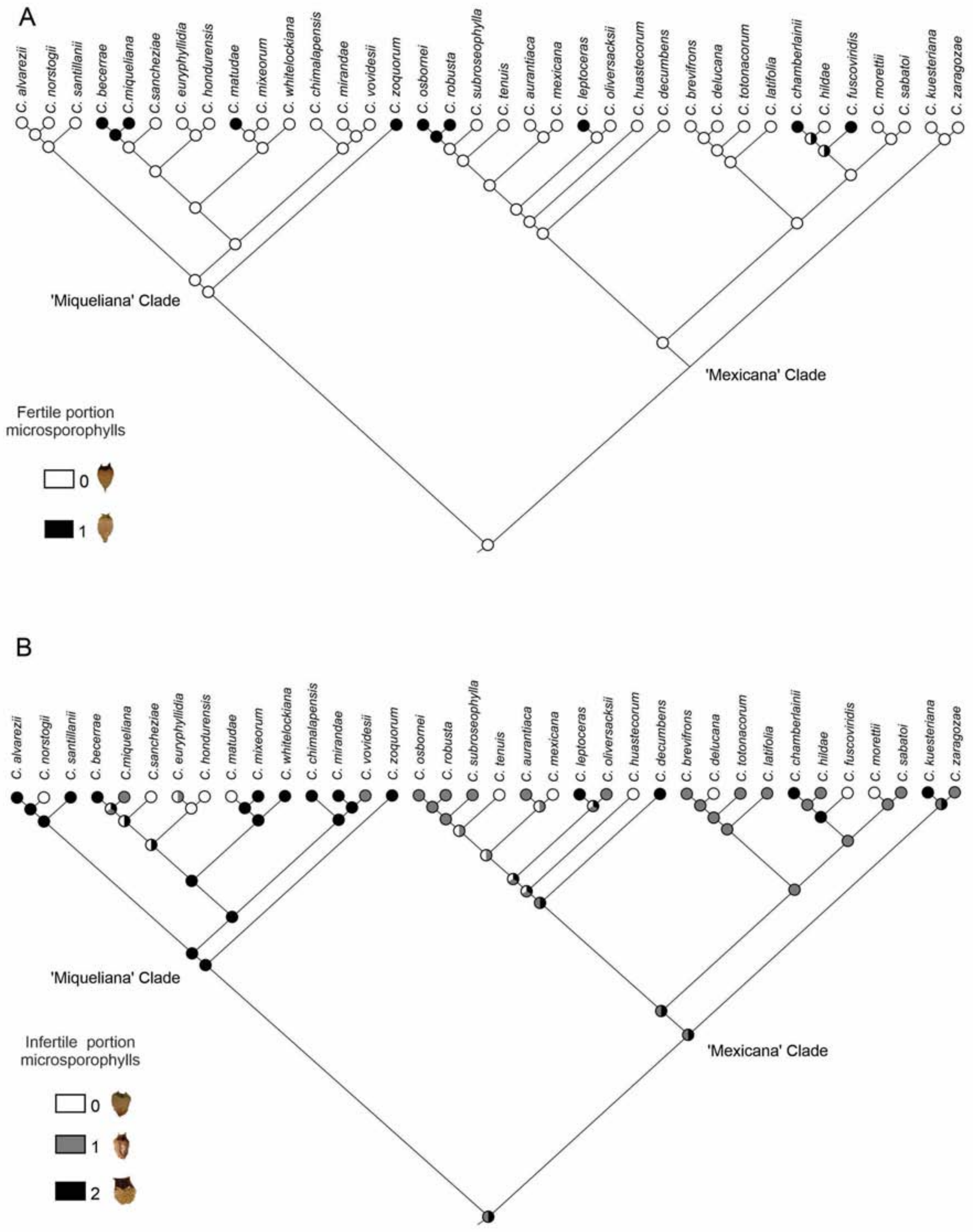
Contributions	Component 1	Component 2
LPSP	18.436858	3.5634537
DPSP	1 5.922663	2.5384213
LPS	2.187704	7.5284938
DPS	12.966079	2.9203498
LMi	20.040348	0.2674891
WMi	12.838005	0.9511844
DHMi	3.242524	44.0260060
HLMi	4.090176	33.7127076
LIpMi	10.275642	4.4918945
Eigen values	3.8240619	1.4337070
Percentage of variance	42.48958	58.41965

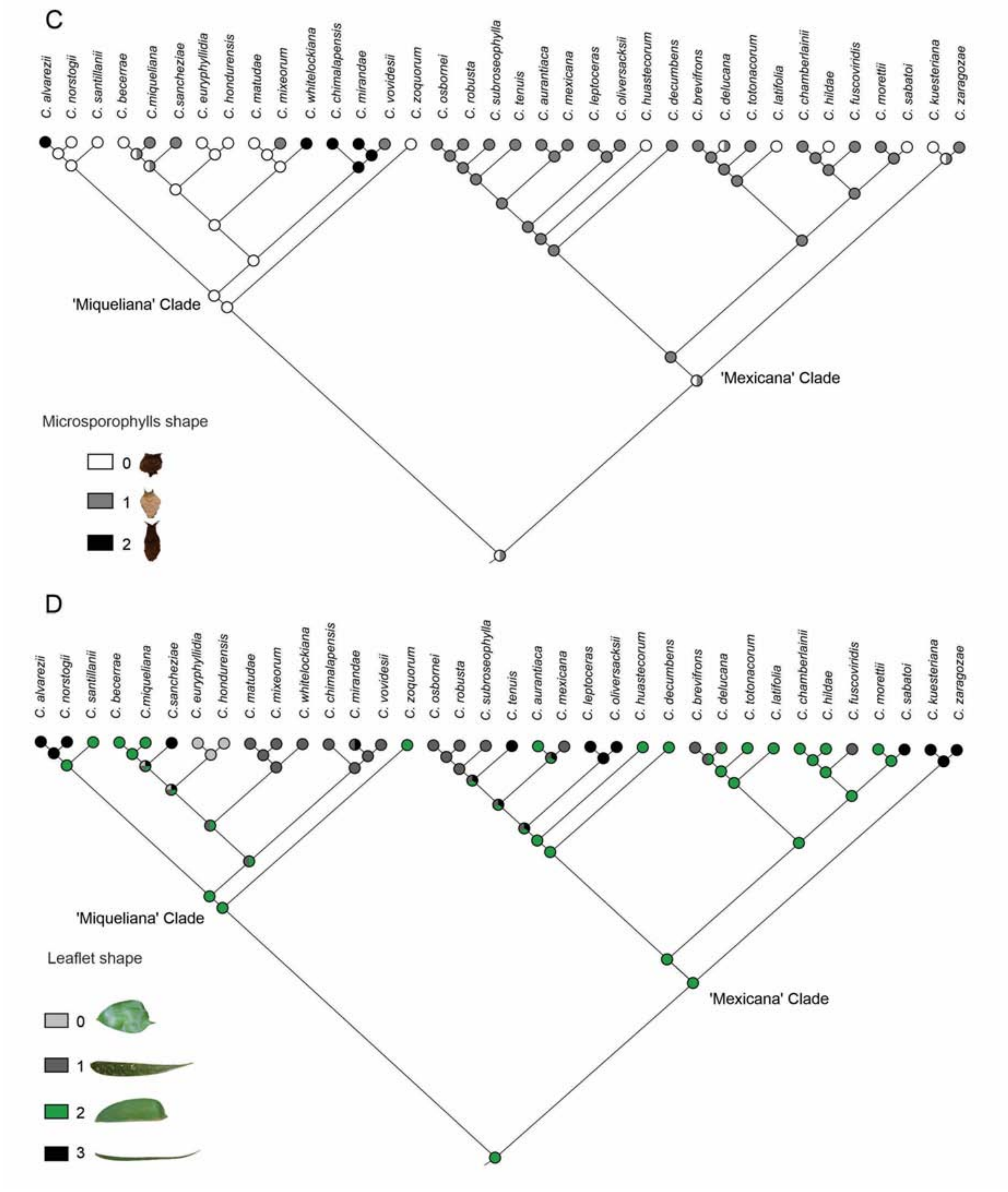
Supplementary Data Figure S1. A. The majority rule consensus tree of *Ceratozamia* in parsimony based on DNA data. B. Single most parsimonious tree based on all morphological data and recovered from parsimony analysis under *K*=25; low values for bootstrap and jackknife were indicated by asterisks (<80%). C. Single most parsimonious tree based on combined matrix with morphological and molecular data of only extant species and recovered from parsimony analysis under *K*=25; low values for bootstrap and jackknife were indicated by asterisks (<80%). D. Topology from Maximum Likelihood base on DNA data. Low values for ML bootstrap were indicated by asterisks (<80%).

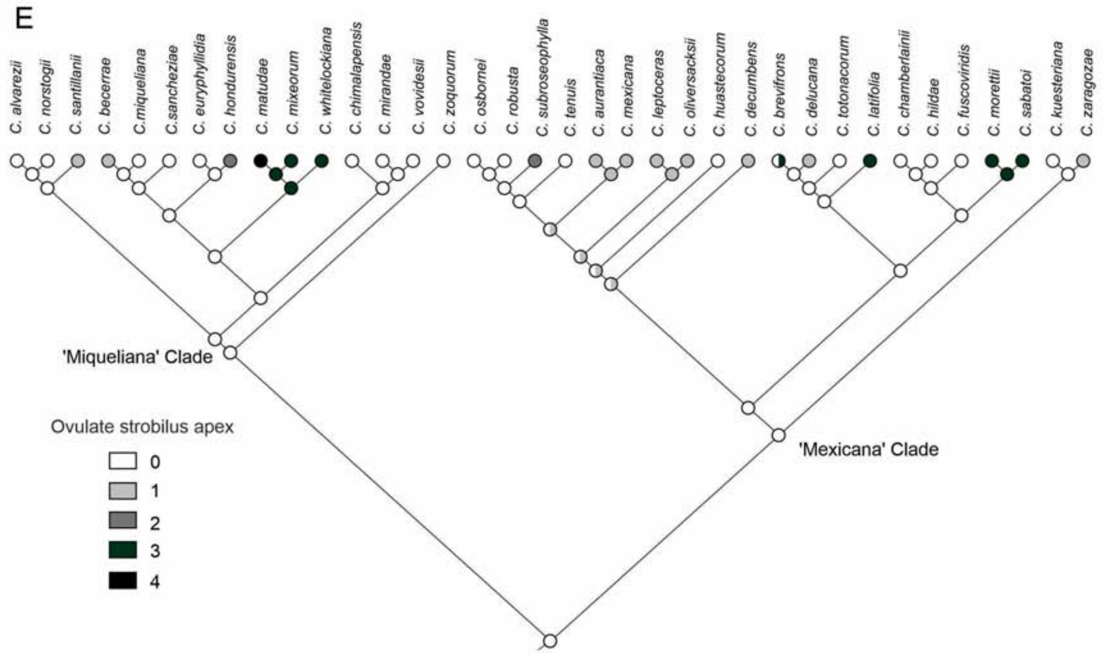

C
D. sonorense
B. serrulata
Z. furfuracea
C. latifolia
C. hildae
C. decumbens
C. morettii
C. zoquorum
C. santillanii
C. becerrae
C. miqueliana
C. hondurensis
C. euryphyllidia
C. sancheziae
C. mixeorum
C. whitelockiana
C. matudae
C. vovidesii
C. mirandae
C. chimalapensis
'Miqueliana' Clade
C. huastecorum
C. sabatoi
C. zaragozae
C. kuesteriana
C. norstogii
C. alvarezii
C. chamberlainii
C. tenuis
C. fuscoviridis
C. totonacorum
C. delucana
C. brevifrons
C. oliversacksii
C. leptoceras
C. mexicana
C. aurantiaca
C. subroseophylla
C. robusta
C. osbornei
'Mexicana' Clade
*


D
D. sonorense
B. serrulata
C. alvarezii
C. norstogii
C. santillanii
C. osbornei
C. robusta
C. subroseophylla
C. aurantiaca
C. leptoceras
C. oliversacksii
C. mexicana
C. tenuis
C. sabatoi
C. brevifrons
C. delucana
C. totonacorum
C. latifolia
C. chamberlainii
C. hildae
C. fuscoviridis
C. huastecorum
C. morettii
C. kuesteriana
C. zaragozae
C. decumbens
C. zoquorum
'Mexicana' Clade
C. becerrae
C. miqueliana
C. sancheziae
C. euryphyllidia
C. hondurensis
C.matudae
C. mixeorum
C. whitelockiana
C. chimalapensis
C. mirandae
C. vovidesii
'Miqueliana' Clade
C. furfuracea
100
*
*
*
*
*
0.02

Supplementary Data Figure S2. Ancestral character state reconstruction.




C
C. alvarezii
C. norstogii
C. santillanii
C. becerrae
C.miqueliana
C.sancheziae
C. euryphyllidia
C. hondurensis
C. matudae
C. mixeorum
C. whitelockiana
C. chimalapensis
C. mirandae
C. vovidesii
C. zoquorum
C. osbornei
C. robusta
C. subroseophylla
C. tenuis
C. aurantiaca
C. mexicana
C. leptoceras
C. oliversacksii
C. huastecorum
C. decumbens
C. brevifrons
C. delucana
C. totonacorum
C. latifolia
C. chamberlainii
C. hildae
C. fuscoviridis
C. morettii
C. sabatoi
C. kuesteriana
C. zaragozae
'Miqueliana' Clade
'Mexicana' Clade
Microsporophylls shape
0
1
2
D
C. alvarezii
C. norstogii
C. santillanii
C. becerrae
C.miqueliana
C.sancheziae
C. euryphyllidia
C. hondurensis
C. matudae
C. mixeorum
C. whitelockiana
C. chimalapensis
C. mirandae
C. vovidesii
C. zoquorum
C. osbornei
C. robusta
C. subroseophylla
C. tenuis
C. aurantiaca
C. mexicana
C. leptoceras
C. oliversacksii
C. huastecorum
C. decumbens
C. brevifrons
C. delucana
C. totonacorum
C. latifolia
C. chamberlainii
C. hildae
C. fuscoviridis
C. morettii
C. sabatoi
C. kuesteriana
C. zaragozae
'Miqueliana' Clade
'Mexicana' Clade
Leaflet shape
0
1
2
3


E
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C. norstogii
C. santillanii
C. becerrae
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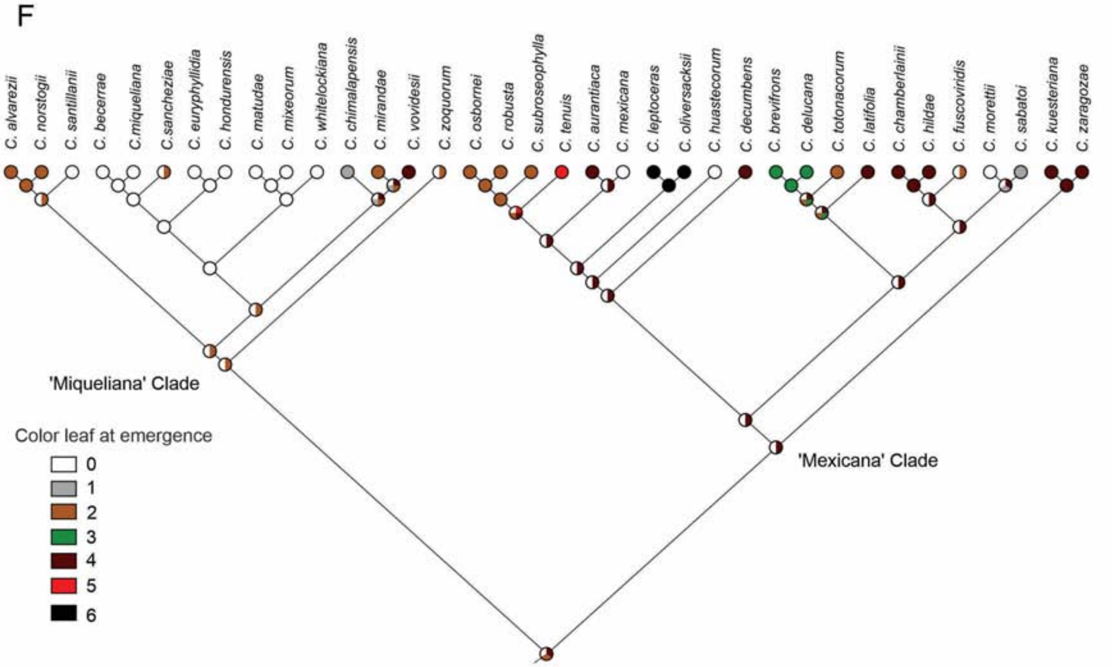
C. sancheziae
C. euryphyllidia
C. hondurensis
C. matudae
C. mixeorum
C. whitelockiana
C. chimalapensis
C. mirandae
C. vovidesii
C. zoquorum
C. osbornei
C. robusta
C. subroseophylla
C. tenuis
C. aurantiaca
C. mexicana
C. leptoceras
C. oliversacksii
C. huastecorum
C. decumbens
C. brevifrons
C. delucana
C. totonacorum
C. latifolia
C. chamberlainii
C. hildae
C. fuscoviridis
C. morettii
C. sabatoi
C. kuesteriana
C. zaragozae
'Miqueliana' Clade
'Mexicana' Clade
Ovulate strobilus apex
0
1
2
3
4


F
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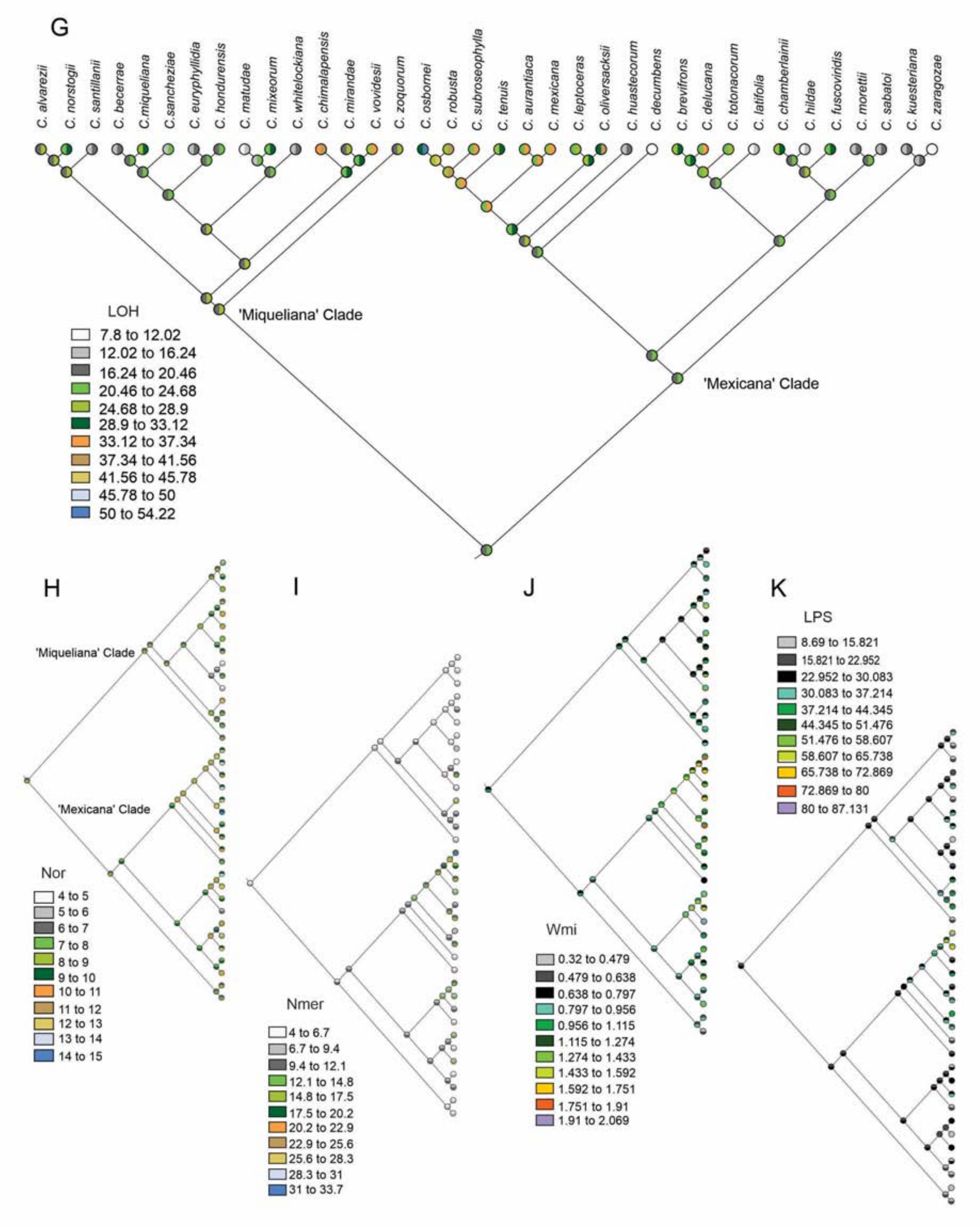
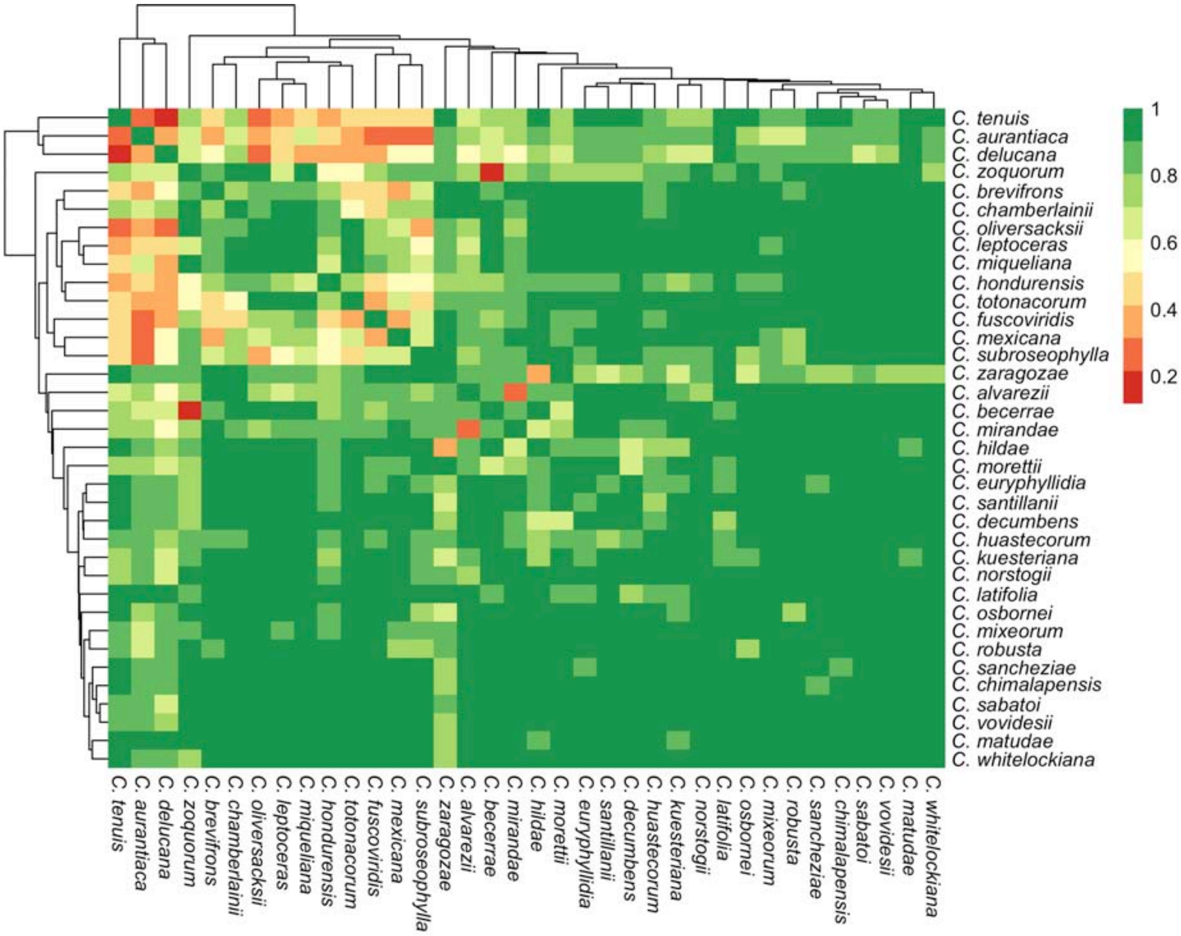
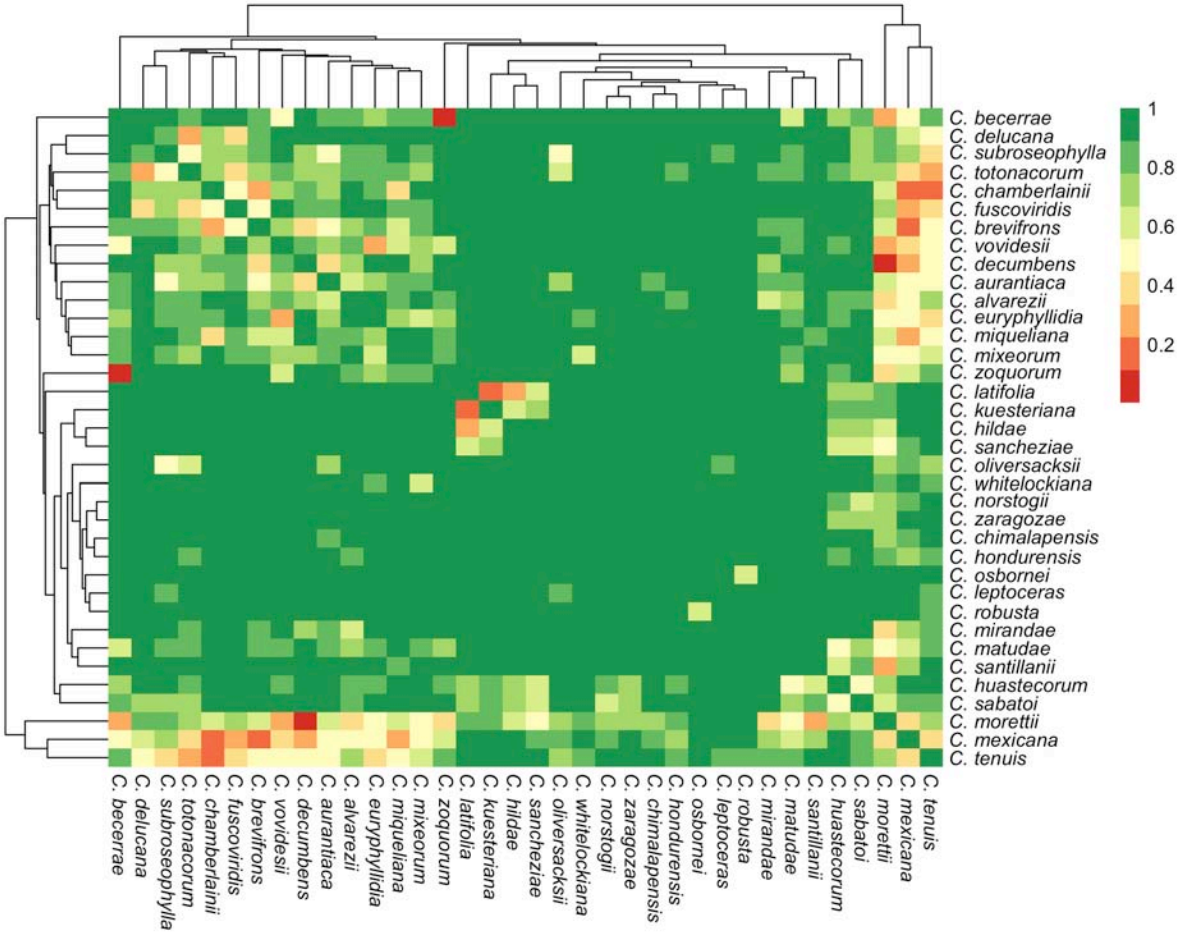
C. norstogii
C. santillanii
C. becerrae
C. miqueliana
C. sancheziae
C. euryphyllidia
C. hondurensis
C. matudae
C. mixeorum
C. whitelockiana
C. chimalapensis
C. mirandae
C. vovidesii
C. zoquorum
C. osbornei
C. robusta
C. subroseophylla
C. tenuis
C. aurantiaca
C. mexicana
C. leptoceras
C. oliversacksii
C. huastecorum
C. decumbens
C. brevifrons
C. delucana
C. totonacorum
C. latifolia
C. chamberlainii
C. hildae
C. fuscoviridis
C. morettii
C. sabatoi
C. kuesteriana
C. zaragozae
'Miqueliana' Clade
'Mexicana' Clade
Color leaf at emergence
0
1
2
3
4
5
6


G
C. alvarezii
C. norstogii
C. santillanii
C. becerrae
C. miqueliana
C. sanchezíae
C. euryphyllidia
C. hondurensis
C. matudae
C. mixeorum
C. whitelockiana
C. chimalapensis
C. mirandae
C. vovidesii
C. zoquorum
C. osbornei
C. robusta
C. subroseophylla
C. tenuis
C. aurantiaca
C. mexicana
C. leptoceras
C. oliversacksii
C. huastecorum
C. decumbens
C. brevifrons
C. delucana
C. totonacorum
C. latifolia
C. chamberlainii
C. hildae
C. fuscoviridis
C. morettii
C. sabatoi
C. kuesteriana
C. zaragozae
'Miqueliana' Clade
'Mexicana' Clade
LOH
7.8 to 12.02
12.02 to 16.24
16.24 to 20.46
20.46 to 24.68
24.68 to 28.9
28.9 to 33.12
33.12 to 37.34
37.34 to 41.56
41.56 to 45.78
45.78 to 50
50 to 54.22
H
'Miqueliana' Clade
'Mexicana' Clade
Nor
4 to 5
5 to 6
6 to 7
7 to 8
8 to 9
9 to 10
10 to 11
11 to 12
12 to 13
13 to 14
14 to 15
I
Nmer
4 to 6.7
6.7 to 9.4
9.4 to 12.1
12.1 to 14.8
14.8 to 17.5
17.5 to 20.2
20.2 to 22.9
22.9 to 25.6
25.6 to 28.3
28.3 to 31
31 to 33.7
J
Wmi
0.32 to 0.479
0.479 to 0.638
0.638 to 0.797
0.797 to 0.956
0.956 to 1.115
1.115 to 1.274
1.274 to 1.433
1.433 to 1.592
1.592 to 1.751
1.751 to 1.91
1.91 to 2.069
K
LPS
8.69 to 15.821
15.821 to 22.952
22.952 to 30.083
30.083 to 37.214
37.214 to 44.345
44.345 to 51.476
51.476 to 58.607
58.607 to 65.738
65.738 to 72.869
72.869 to 80
80 to 87.131

Supplementary Data Figure S3. Heatmap showing the results of analysis of similarity (ANOSIM) of quantitative characters in ovulate strobili for *Ceratozamia* species.

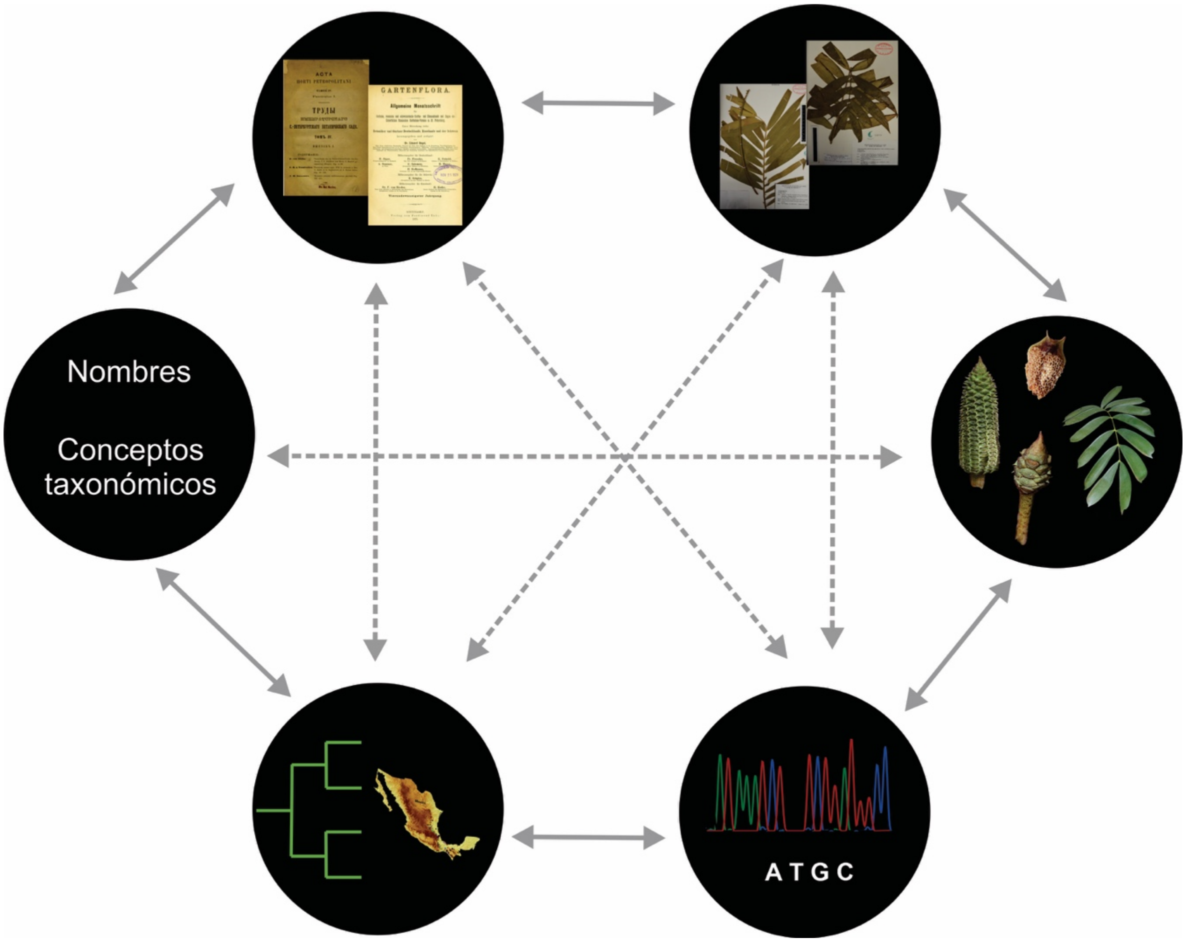


Supplementary Data Figure S4. Heatmap showing the results of analysis of similarity (ANOSIM) of quantitative characters in pollen strobili for *Ceratozamia* species.



II.V. Capítulo V.

Una revisión sistemática en el orden Cycadales: desde los nombres hasta las estrategias de delimitación



II.V.I. A review of taxonomic concepts and species delimitation in Cycadales. Artículo aceptado para publicación en la revista ‘The Botanical Review’ (BOTR-D-23-00012).

A review of taxonomic concepts and species delimitation in Cycadales

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Abstract

Taxonomic data is essential to advance the discovery and description of biodiversity, as well as the study of evolutionary processes. Emerging large-scale datasets and new methods of analysis have provided different approaches to describe biodiversity. Here, we present a review of the taxonomic history in Cycadales including an analysis of historical taxonomic concepts and approaches used for species delimitation. We examine the trends in the publication of new species following taxonomic works in books, journals and horticultural catalogues, monographic projects and floras where species treatments were published. In addition, we review the studies concerning species delimitations using the literature available in scientific journals appearing in the database ISI Web of Knowledge. The approaches used were discussed throughout all research focused on empirical and theoretical considerations in each study. We review the current state of the studies on causal processes that have given rise to the currently recognized diversity. The trend shows that taxonomic work on discovery and description of species has been intensive in the last 40 years culminating in 38.8% of binomials published. As a result, we consider the relevance of the monographs and floras for identification of species for other biological disciplines and the content of these contributions is compared and discussed. A total of six criteria (diagnosability, phenetic, phylogenetic, genotypic cluster, niche specialization and coalescent) were detected from the following three approaches to species delimitation within Cycadales: traditional, integrative taxonomy, and monophyletic. In all cases, the results from these species delimitations not only provided a taxonomic treatment or proposed a new species, but also supposedly clarified the other species involved as a result of the new taxonomic concept of the new species described. Most investigations of species delimitation used the traditional approach or a phenetic criteria. Finally, we discuss evolutionary studies on causal processes involved in cycad diversity. This is considered in the context of species delimitation as hypothesis testing for a successful evaluation of variation in both genetic and morphological understanding.

Keywords Cycad diversity, circumscription, Monograph, species concept, taxonomic treatment

Introduction

Scientific names are vital for accessing scientific information in all fields of biology. Over the centuries, the names have provided not only an effective means of communication and information transfer, but also conceptual and explanatory properties (Knapp, 2000; Valdecasas et al., 2014; Wheeler, 2023). However, there is a controversy concerning the actual existence of species (Slater, 2016; Mishler, 2021). There is an extensive literature on this subject (Dohzhansky, 1935; Cronquist, 1978; Zachos, 2016a,b,c; Sigwart, 2019; Wheeler, 2023; Wilkins, 2023). We are not discussing these arguments here because we have to use what has been used historically. We do suggest that Crowson (1970) provides an excellent summary of the types of species concepts that have been used historically. A name is a hypothesis applied to an evolutionary entity. Although the names are key resources for understanding global diversity, they are one of the greatest challenges in systematics (Knapp et al., 2004; Wheeler, 2023). Each name implies a taxonomic concept, which contains multiple sources of evidence associated that constitute the classified biological entity, such as geographic distribution, habitat, characters, among others (Franz et al., 2008). The taxonomic concepts are the result of a species concept applied to the circumscription of a species by an author and their meaning, both the species and the concept, can change through the time (Mallet & Willmott, 2003; Knapp et al., 2004; Wheeler, 2004). The different meanings that a same name acquires are due to redefinitions arising from new evidence and/or different interpretations by either the same author or others. This cumulative information produces a partial disconnection between the binomial and its conceptual definitions (Franz et al., 2008).

Monographs are comprehensive taxonomic treatments that systematize and synthesize the phenotypic and genotypic complexity of the organisms included (Grace et al., 2021). In these systematic treatments, the species hypotheses are tested, and the history of the taxonomic concepts of the family, genus and species are condensed (Marhold et al., 2013). A monograph is vital for the identification of species and the discovery of new taxa (Grace et al., 2021; Wheeler, 2023). Currently, new analytical tools have been developed and applied in systematics that have enhanced the discovery of new species (Wheeler et al., 2012; Zhang, 2020). However, monographs and taxonomic reviews have become neglected, which has resulted in either the over- or underestimation of diversity in several groups. The mismatched relationship between advances in the use of molecular evidence for taxonomic purposes and the limited development of monographs is now common. Under integrative approaches, monographs would provide key tools in studying the evolutionary history of species, the causes underlying phenotypic diversity, as well as developmental mechanisms and conservation (Grace et al., 2021; Wheeler, 2023).

Species delimitation is the process of identifying biological units through the recognition of evolutionary patterns (Carsten et al., 2013). In the delimitation of species, different methods are used to study population patterns, and propose classification systems (Sites & Marshall, 2003). These can be classified in non-tree and tree-based methods, where different approaches are involved with each having their own conceptual implications (Sites & Marshall, 2004). The traditional approach is based on strategies and methods historically applied in species delimitation and has been largely attributed to traditional morphological taxonomy (Sites & Marshall, 2003). The approach "integrative taxonomy" uses multiple sources of evidence and methods to infer boundaries between species by considering life history, phenology, reproduction, morphology, genetic diversity, ecological niche, and geographic distribution patterns (DeSalle et al., 2005; Goldstein & DeSalle, 2011). Because species are dynamic entities under different selective pressures, one of the main challenges of this approach is the integration of the results from these different methods and sources.

The limits within species complexes are particularly difficult. The study of these groups of species has been aided by new methods in the era of genomics (Knowles & Carstens, 2007). In the last decade, there has been a considerable increase in research aimed at inferring the boundaries between species in many plant groups (Prata et al., 2018). However, the synthesis of information and the recognition of taxonomic concepts are necessary to take advantage of the potential that these new methods can offer in our understanding of biological diversity and how it developed historically. Some efforts have recently emerged to integrate this taxonomic knowledge with new data through modern botanical monographs and highlighting the importance of these works, which are more pressing due to biodiversity loss (Marhold et al., 2013; Muñoz-Rodríguez et al., 2019). For most plant groups, the monographs date back more than a century, and groups with high species diversity have not been completely monographed (Grace et al., 2021). This lack shows the difficulty of making more efficient use of new data to propose robust species hypotheses, even in the cases with relatively low species diversity such as in Cycadales.

Cycadales, known as cycads, are an ancient group of gymnosperms with a high extinction risk (Brenner et al., 2003; Donaldson, 2003). Considering the unique morphological characters of cycads and their phylogenetic position as the earliest extant group of seed plants with a minimum age in the Early Permian, they have been relevant for the study of phenotypic evolution between gymnosperms and angiosperms, origin of the seed, neurotoxins, as well as studies on coevolution (Norstog & Nicholls, 1997; Salzman et al., 2021). Today, 10 genera with a total of 368 species are recognized within Cycadales (Calonje et al., 2013–2023). During the last decades, the recognized diversity in some genera has increased dramatically, even though monographs and similar taxonomic treatments are scarce. In this article, we discuss the history of species discovery in cycads, species concepts, and trends in species delimitation over time. Finally, the causal processes that operate in the phenotype and genotype are addressed, which are co-responsible for the direction, rate and origin of the variation in the species.

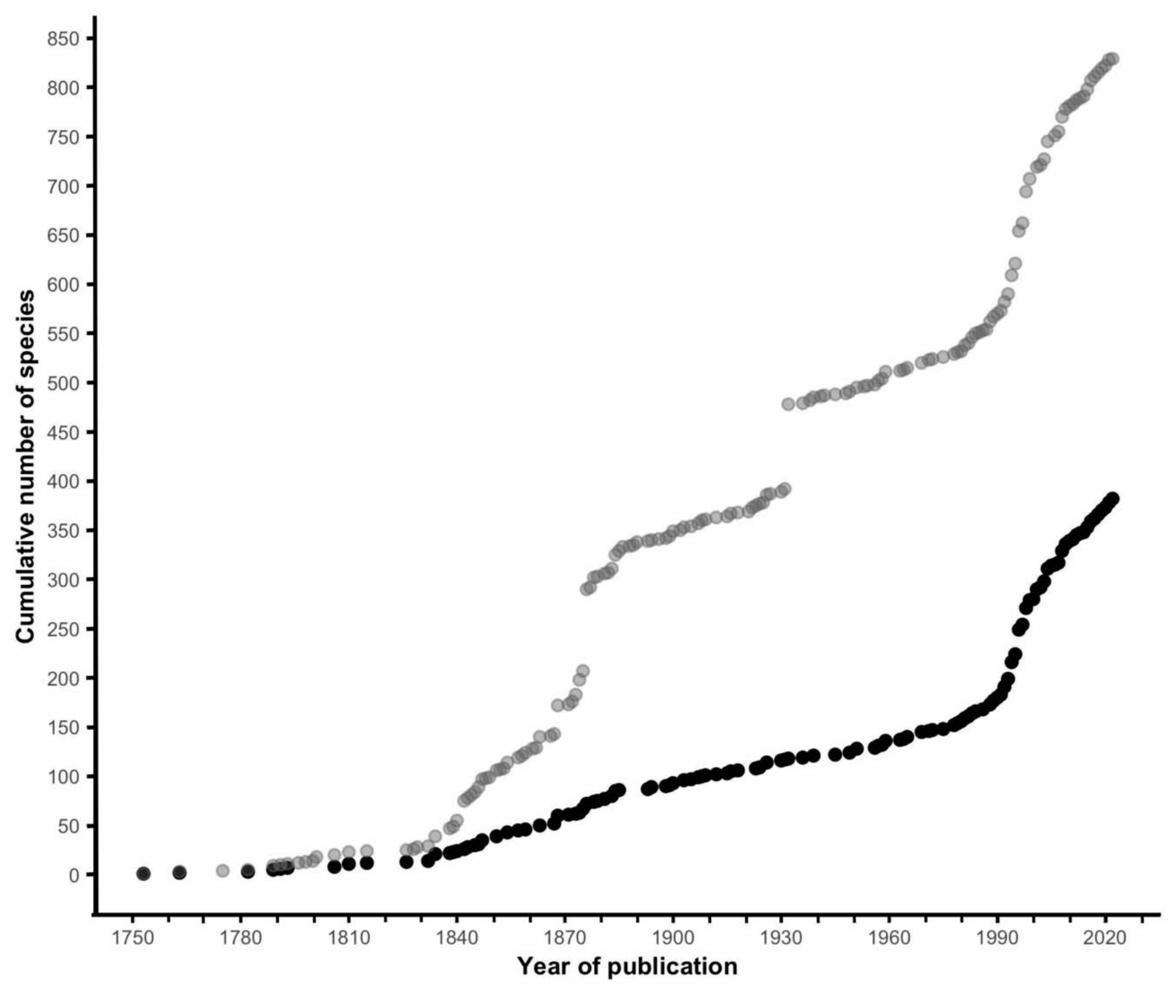
Survey Methodology

A useful guide to basic terms is in Supporting Information S1. We used the literature database from "The World List of Cycads" (https://www.cycadlist.org) and Read and Solt (1986) as primary resources for the publications of all species and selected all taxonomic publications including floras and monographs to describe the trend of publication and trace the changes in the taxonomic concepts within Cycadales through September 2022.

To analyze the strategies and methods of empirical research focused on species delimitation in cycads, we used the following terms to select articles through September 2022 in the ISI Web of Knowledge: "new species", "circumscription" "species delimitation" and "species complex" with "Cycadales" and each generic name in the order, i.e., *Aulacophyllum* Regel, *Bowenia* Hook. ex Hook.f., *Ceratozamia* Brongn., *Cycas* L., *Chigua* D.W.Stev, *Dioon* Lindl., *Dyerocycas* Nakai, *Encephalartos* Lehm., *Epicycas* deLaub, *Lepidozamia* Regel, *Macrozamia* Miq., *Microcycas* (Miq.)A.DC., *Stangeria* T.Moore and *Zamia* L. From this compilation, we selected the original studies of species delimitation published between January 1980 and August 2022 and conducted a systematic classification of the research for analytical criteria and methods of circumscription in Cycadales. We only selected the articles in which a method was explicitly included for sources of evidence as presented in each research presentation. In total, 41 articles have all the requirements for extensive analysis and those were reviewed in detail and classified according to the approach and criteria used by the authors (Supporting Information Table S2). The approach criteria and species concept were not explicitly indicated in most of these articles. For those cases, we classified according to the rationale used by the authors and issues of how they analyzed or integrated the results of sourced evidence obtained for the research. The articles were classified in three general approaches: i) traditional, ii) integrative taxonomy and iii) monophyletic. Historically, traditional taxonomy refers to studies that include only morphological data. However, molecular data analyzed independently is also part of this approach (Kotov & Gololobova, 2016). Basically, this approach has the descriptive perspective of defining features. We included all research in which the authors used at least two sources of evidence but without defined methods of analysis. Under the integrative taxonomy approach, we included articles with more than one source of evidence that was analyzed with the same or different methods for testing the species hypothesis. The monophyletic approach included studies based on tree methods because the species under this criterion are considered explicitly as historic lineages (Baum & Donoghue, 1995; Wiens & Penkrot, 2002).

To illustrate the importance of the morphological and molecular variation involved in taxa for species boundaries, we carried out a search for articles where the causes and processes involved at the level of the phenotype and genotype in cycads are addressed. In order to make recommendations and discuss future directions, we made a selection of these articles based on variety of methods and results that could be useful for new approaches in species delimitation.

Fig. 1. Total cumulative number of scientific names published (gray) and currently accepted taxa (black) each year in Cycadales, from 1753 up to September 2022.



Alpha taxonomy: from Linnaeus to phylogenomic

The formal taxonomic history of Cycadales began with the description of *Cycas circinalis* by Carl von Linnaeus (Linnaeus, 1763). The rate of species description in the order has not been constant. After a long period of 75 years, the publication of taxa currently accepted published and names published suddenly increased (Fig. 1). Overall, two peaks of descriptions were observed: (i) 1837 to 1880 and (ii) 1959 to 2004 (Fig. 1). The period of 1959 to 2004 is the historical maximum in which the number of described taxa were doubled compared to the period of 1900 to 1950. The current trend shows that taxa discovery has increased by nearly double over the last 50 years, which could indicate that almost all species of the order have been discovered and described (Fig. 1). Actually, several names published between 1875 and 1925 are currently considered synonyms or invalid names (Figs. 1, 2; Supporting Information Figure S3). The abrupt increase between 1925 and 1950 is derived from Schuster's (1932) monograph where 86 new varieties, subspecies and species were described, but none these have names were not accepted in subsequent works (Fig. 1). We noted that more than half of the scientific names in cycads were published between 1850 and 2004; thus, 85% of scientific names were described before 2005 (Fig. 1; Supporting Information Figure S3).

There are two periods (1837 to 1880 and 1959 to 2004) of increase in species descriptions that match the publications of monographic works at the genus level and broad geographic areas. The first period included the publication of six relevant monographic works prepared by Miquel (1842, 1861, 1863, 1869a,b, 1870), Regel (1875) and de Candolle (1868). The second period is characterized by the regional taxonomic treatments published for *Cycas* and *Macrozamia* (Johnson, 1959, 1961; Hill, 1994a,b, 1996; Wang, 1996; de Laubenfels & Adema, 1998; Hill & Yang, 1999). Most of the described species for these genera were published in these works. Half of the currently accepted taxa in *Cycas* and *Macrozamia* are synonymous or invalid and 50% and 44% of the names are accepted, respectively (Fig. 2c, g). In *Cycas,* several infraspecific categories such as varieties and subspecies have been described. However, most of the names described within this period were at the species level with very few at infraspecific levels (Supporting Information Figure S3). Identification of species in *Cycas* has been complex by the great variation in the characters usually used for species identification. In particular, *C. rumphii* Miq. and *C.*

circinalis L., have a long history of synonymy (Hill, 1994b; Hill, 1995a; Lindstrom, 2002; Hill et al., 2004). The publication pattern of names and taxa currently accepted in *Cycas* was regular, whereas in *Macrozamia* was irregular (Fig. 2; Supporting Information Figure S3). Until 1940 *Cycas* showed a trend of constant increase in currently accepted taxa with an abrupt increase during the last thirty years (Fig. 2c). The trend in *Macrozamia* shows three intervals without new names published during 1900 to 1990 (Fig. 2g; Supporting Information Figure S3). In this period of 90 years, Schuster (1932) introduced 26 scientific names. Most of these names were largely the cause of much of the confusion in this genus and overestimation of its diversity (Johnson, 1959). The number of currently accepted taxa published in this genus doubled in the last 40 years, and since 2000, only one taxon has been described.

The tendency in the first period (1837-1880) of cycad discovery was to recognize geographically localized species, particularly in *Encephalartos* and *Zamia.* This led to rapid increases in new taxa descriptions in these genera by incorporating the trends mentioned below (Figs. 1, 2). Both genera show two periods of growth during 19th (1860-1880) and 20th centuries (1980-1990) with more than half of the currently accepted taxa published before 1980 (Figs. 2e, j; Supporting Information Figure S3). Several names were published as varieties and currently half of those are considered synonyms (Supporting Information Figure S3). *Zamia* showed an upward trend in currently accepted taxa between 1763 and 1940 (Fig. 2j). The description of currently accepted taxa remained almost unchanged for 40 years with no new *Zamia* names published from 1960 to 1980, and 48 new names have been published in the last 40 years (Fig. 2j). However, the trend in number of names published was steady (Supporting Information Figure S3). The publication of scientific names from 2000 has been characterized by the explorations of new areas in the mountainous region of the countries of South and Central America that had remained relatively inaccessible until the 20th century (e.g. Calderón-Sáenz & Stevenson, 2003; Calonje et al., 2018; Segalla et al., 2023). In general, a minority of less that 10% of the species have been described based on a reassessment of morphological characters or populations previously considered part of other species (e.g. Calonje et al., 2010; Lindstrom et al., 2013; Nicolalde-Morejón et al., 2019). In relation to *Encephalartos,* most (96%) of the currently recognized diversity was described by about 2000. After this year, only 5 currently accepted names were published (Fig. 2e) and two that are currently synonyms were published (Supporting Information Figure S3).

Dioon and *Ceratozamia* have a similar trend that shows a gradual increase of currently accepted taxa published from the description of the genera with an interval where no species were described (Fig. 2b, d). Two inactive periods of 50 and 40 years were registered for *Ceratozamia* with no new taxa described. The first period occurred between 1880 and 1930 and the second occurred from 1940 to 1980 in which only two species were described (Fig. 2b). The publication rate of taxa in these genera has accelerated in the last two decades, in which 50% of new taxa have been described. The descriptions of these species were based on re-circumscriptions of some taxa and/or reassessments of the range extension of widely distributed species. On the other hand, new taxa publications in *Dioon* increased in the decade of 1980 to 1990 where 30% of the total species were described (Fig. 2d). Unlike *Ceratozamia*, most names published in *Dioon* up to 1900 are now synonyms (Supporting Information Figure S3). Similarly, as in *Cycas,* there was a steadily increasing trend in the number of taxa currently accepted published in *Ceratozamia* and *Dioon* with the greatest increase in the 1980-2020 period with a lull during a short period of 20 years during 1950 to 1970 (Fig. 1). The rest of the genera are monotypic or currently have only two currently taxa accepted. The publication of names in *Stangeria, Lepidozamia* and *Microcycas* has remained unchanged from the nineteenth century (Fig. 2 f, h, i).

By 2000, 88% of species in Cycadales had been discovered and described (Fig. 1). The more recent descriptions were derived from reassessments of known taxa and/or populations that have been identified and segregated as new species (e.g. Gutiérrez-Ortega et al., 2020b; Martínez-Domínguez et al., 2022). By 1999, 78% of the cycad species had been described with several of these species described in regional taxonomic treatments and Floras (Vovides et al., 1983; Stevenson, 1993, 2001, 2004).

In Cycadales taxonomy, the works of Miquel and Regel have been among the most historically relevant. Miquel published 61 binomials under his authorship, of which 15 correspond to currently accepted species. In 1842, in the Miquel's "Monographia Cycadearum", four new species and 15 new varieties in four genera were described. He continued to publish new species and varieties in subsequent years, particularly in 1847 when he described eight species and one variety. After 1842, the recognition of varieties decreased considerably and by 1868, Miquel's species concepts were more inclusive. Miquel in his Prodromus systematis Cycadearum proposed a classification with four tribes and eight genera in 1861 and later also added an infrageneric classification for *Macrozamia* (Miquel, 1868). Regel published 39 names of which five correspond to binomials of currently accepted species. Most of the names published throughout his taxonomic works were varieties. However, Regel described two genera *Aulacophyllum* by transferring species from *Zamia* while describing a new species and *Lepidozamia* as a segregate from *Macrozamia* (Regel 1857a,b, 1876a,b). All species in the former are now considered a synonym of *Zamia* and the latter is currently accepted. In general, Regel's early work had a focus on horticulture that included a list of species and notes on those species. Regel's later works were more extensive and included taxonomic details and keys. In 1876, he published parallel taxonomic works in Horti Petro and Gartenflora in which he recognized varieties by other authors and proposed 15 new varieties of his own.

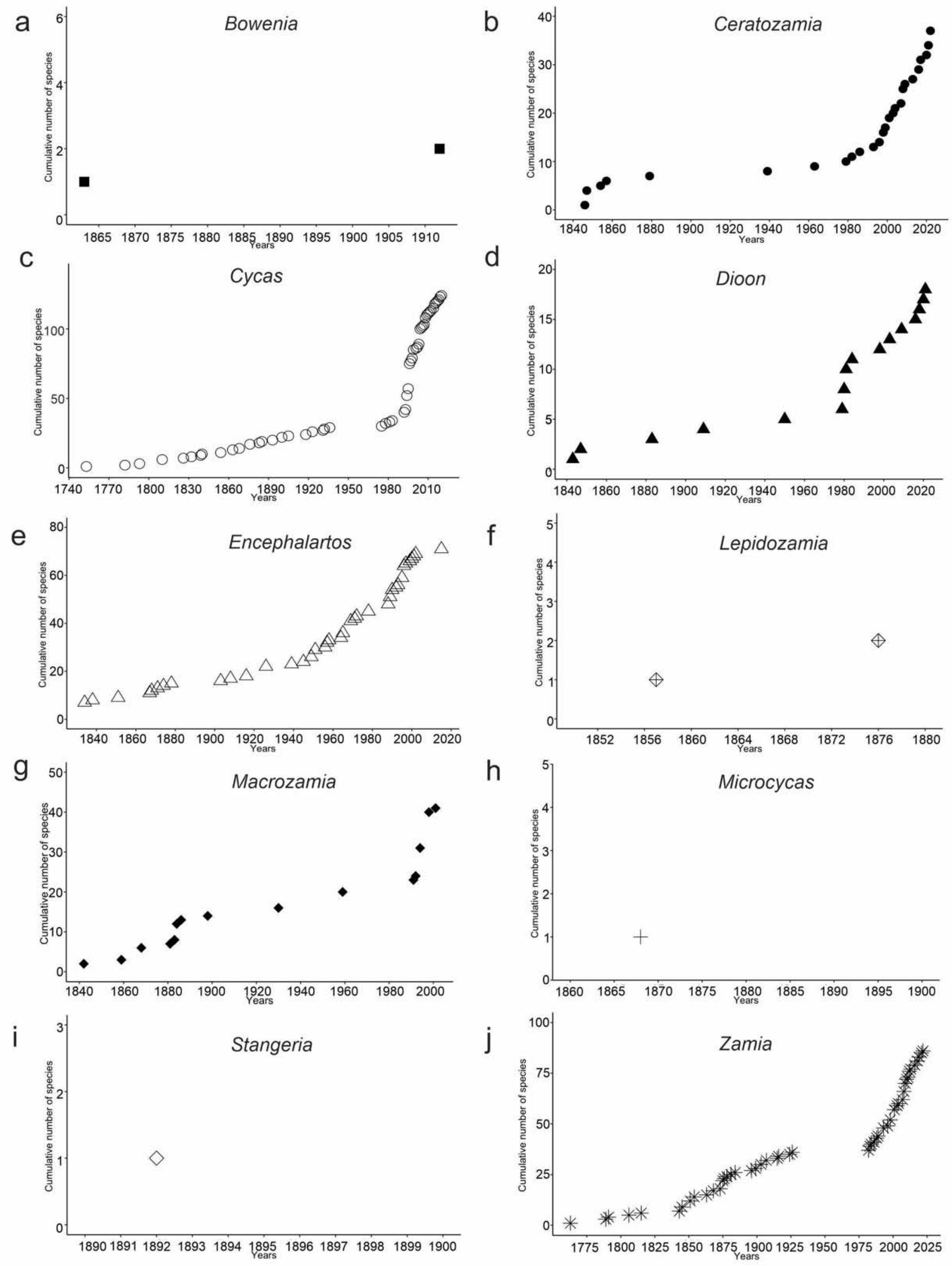
The works of Miquel and Regel show differences in some concepts and circumscriptions. In particular, *Encephalartos* in Miquel's "Monographia Cycadearum" (1842) and Regel's "Cycadearum generum specierumque revisio" (1876a), 11 scientific names were addressed in each of these monographs. These taxonomic works are characterized by the proposal of several infraspecific categories at the variety level. The varieties proposed between both treatments show conspicuous differences to each other. The same species addressed in these treatments have a different circumscription. For example, Miquel (1842) recognized *E. altensteinii* Lehm., with three varieties, whereas Regel (1876b) recognized five different varieties from which three were new proposals, and the "Miquelian names" were placed as synonyms. Under those outlines, the taxonomic concepts for the subspecific categories are incongruent with each other. Additionally, the number of recognized species differed in the final publications of Miquel (1868) and Regel (1876a,b) on cycads, 68 and 57 species, respectively.

The taxonomic concepts of the species have been subject to significant changes derived from the reevaluation of morphological characters and the relevance of these to designate categories (Miquel, 1842, 1848; Schuster, 1932). This has led to subsequent reassignments of the relationships among the historical taxonomic concepts of some binomials. In *Macrozamia,* the taxonomic concepts have been relatively stable and consistent with each other; however, due to its morphological similarity to *Encephalartos*, several transfers took place between these genera particularly by von Mueller (1858, 1859) where he lumped *Macrozamia* into *Encephalartos*, which was not accepted by others

(Miquel 1861, 1868; Regel, 1876a,b; de Candolle, 1868; Schuster, 1932). Another genus with a taxonomic history with few changes and misconceptions is *Dioon*. This genus is one of the few that has been fairly clearly understood from its beginning with little misapplication of names.

Overall, the taxonomic concepts of species have been narrower such as *Dioon tomasellii* de Luca, Sabato & Vázq.Torres, *D. sonorense* (De Luca, Sabato & Vázq.Torres) Chemnick, T.J.Greg. & Salas-Mor. and *D. merolae* de Luca, Sabato & Vázq.Torres. *Dioon merolae* was proposed as a narrow concept; however, that taxonomic concept was extended during a revaluation of populations discovered during the late 1990s. Recently, two new species, *D. oaxacensis* Gut.Ortega, Pérez-Farr. & Vovides and *D. salas-moralesiae* Gut.Ortega & Pérez-Farr., were segregated from the broad concept of *D. merolae* (Gutiérrez-Ortega et al., 2020b, 2021). Similarly, *Dioon tomasellii* de Luca, Sabato & Vázq.Torres was proposed with two varieties from western Mexico. These varieties were described as *D. tomasellii* var. *tomasellii* and *D. tomasellii* var. *sonorense*, posteriorly these varieties were associated to the taxonomic concept of *D. edule* not without some ambiguity (McVaugh & Pérez de la Rosa, 1992). Both varieties of *D. tomasellii*, following these authors, were conceptually part of a broad concept proposed for *D. edule* that included *D. tomasellii* and their varieties. Thus, species concepts in *Dioon* were aggregated to form a more inclusive taxonomic concept. However, the taxonomic concepts were again reduced by the subsequent authors for which *D. sonorense* (De Luca, Sabato & Vázq.Torres) Chemnick, T.J.Greg. & Salas-Mor. and *D. tomasellii* are congruent with the originally described varieties. More recently, *D. stevensonii* Nic.-Mor & Vovides was segregated and described from populations previously considered to be *D. tomasellii* (Nicolalde-Morejón et al., 2009b).

Fig. 2. Total cumulative number of currently accepted taxa described each year in (**a**) *Bowenia*, (**b**) *Ceratozamia*, (**c**) *Cycas*, (**d**) *Dioon*, (**e**) *Encephalartos* and (**f**) *Lepidozamia*, (**g**) *Macrozamia*, (**h**) *Microcycas*, (**i**) *Stangeria* and (**j**) *Zamia*, up to September 2022.



Taxonomy in Cycadales has a complex history marked by the multiple synonyms that show a clear taxonomic disagreement at the species level (Supporting Information Figure S3). For example, *Epicycas* and *Dyerocycas* were separated from *Cycas* and subsequently synonymized because the characters using to segregate these genera after a closer examination were found to be inconsistent (Chen et al., 2004). On the other hand, the taxonomic history within most genera is characterized by mutually independent species descriptions and the recognition of numerous infraspecific categories (sub-species, varieties, among others). This is clearly seen when looking at the number of published species names when compared to currently accepted taxa (Fig. 1). *Encephalartos*, *Cycas* and *Zamia* have the highest number of synonyms (Hill, 1995b; Hill, 1998; Hill & Yang, 1999; Hill, 2008; Nicolalde-Morejón et al., 2009a; Calonje et al., 2013–2023). Currently, 65 species and six infraspecific categories (subspecies) are recognized in *Encephalartos* and there are 85 synonyms of which 41 are varieties, two subspecies and six forms. *Cycas* is the largest genus with 120 species and six subspecies and 99 synonyms. *Zamia* is the second most speciose genus with 83 species and five varieties and 84 synonyms. One of the major issues in the early cycad treatments was the lack of a type specimen concept as well as the lack of specimen citations. Johnson (1959) established a turning point for Australian taxa followed by Stevenson and Sabato (1986a,b) for the typification of neotropical cycad names. Similarly, this was done for African taxa by Vorster (2004) and the genus *Cycas* by Hill (1995a,b) and Hill et al. (2004).

The designation of several varieties, subspecies and forms is mainly due to the morphological similarity between species and the lack of vegetative morphological characters that could be used as diagnostic. Some of the long history of synonymy within the order are in genera with little diversity, as is the case of *Lepidozamia*, *Stangeria* and

Bowenia. In the latter, two specific epithets with three varieties are recognized as synonyms and even with changes in category recognition, all the taxonomic concepts since 1912 are consistent with each other (Table 1). This taxonomic history shows synonyms of the same entity under the concepts of variety and species. In this case, the changes have been raised into a more conservative approach discarding the variations in some characters (Table 1) within individuals and populations.

Table 1. Graphic diagram showing relationships among historical taxonomic concepts in *Bowenia* species. The correspondence between the concepts is shown by the alignment between the boxes. Parentheses indicate taxonomic concepts in synonymy.

Author	**Taxonomic concept**	
Hooker, 1863	*B. spectabilis*	
Regel, 1876	*B. spectabilis*	
Bailey, 1883	*B. spectabilis*	*B. spectabilis* var. *serrata*
Warburg, 1900	*B. spectabilis*	
Chamberlain, 1912	*B. spectabilis* (*B. spectabilis* var. *spectabilis*)	*B. serrata* (*B. spectabilis* var. *serrata*)
Schuster, 1932	*B. spectabilis* (*B. spectabilis* var. *spectabilis*)	*B. spectabilis* var. *serrulata* *B. serrulata* (*B. spectabilis* var. *serrata*)
Johnson, 1959	*B. spectabilis* (*B. spectabilis* var. *spectabilis*)	*B. serrulata* (*B. spectabilis* var. *serrulata*) (*B. spectabilis* var. *serrata*)

Ceratozamia has a complex taxonomic history in which *C. mexicana* Brongn., was one of the most difficult taxonomic concepts to clarify (Stevenson & Sabato, 1986b; Vovides et al., 2016; Martínez-Domínguez et al., 2018a,b, 2022). All species in this genus that were described up to 2004 were added within one of the seven species complexes proposed by Vovides et al. (2004). Recently, the taxonomic concept of *C. robusta* Miq. has been meaningfully changed from a broad concept employed by Miquel (1847) into a narrower concept by Martínez-Domínguez et al. (2022). Thus, this name is only partially congruent with its use in all previous taxonomic treatments (e.g., de Candolle, 1868; Miquel, 1868; Stevenson et al., 1986; Martínez-Domínguez et al., 2016; Gutiérrez-Ortega et al., 2021). This species had a wide range of distribution with several populations that have been reduced in the last 10 years. Considering the recent description of new species previously considered as part of *C. robusta*, this taxonomic concept could be confusing, but these changes have been described in a recent monograph (Martínez-Domínguez et al., 2022). Other species previously considered as varieties or forms were transferred to the rank of species. In most cases, the taxonomic concepts within *Ceratozamia* have been narrowed. In contrast, *Zamia* showed a tendency to a broad taxonomic concept. *Zamia loddigesii* Miq., is a species with a wide distribution range through which it presents morphological and molecular differences. From its description by Miquel (1843) to the 20th century, 12 new names related to *Z. loddigesii* have been published. Regel (1857a,b; 1876) published 4 names under the variety rank using the names of species previously published by Miquel (1843, 1847). Later, in Flora Centrali-Americana (Thiselton-Dyer, 1884) two new species were proposed and the synonyms under *Z. loddigesii* were rearranged and Schuster (1932) described one new species and designated several new varieties. Evaluation of morphological variation and geographic discontinuities in populations similar to *Z. loddigesii* led to the clarification in its taxonomic concept including all variations (Nicolalde-Morejón et al., 2009a).

Currently, infraspecific taxa are not used in most genera. Only *Cycas*, *Zamia* and *Encephalartos* have a classification that includes infraspecific ranks such as subspecies and varieties. After an assessment of intra- and inter-population variation, most of those varieties and subspecies described were not supported. The classification based on these ranks was discounted for lack of clarity as in *Macrozamia* where Schuster (1932) and Johnson (1959) used infraspecific ranks. Schuster (1932) proposed ranks of varieties and forms in his taxonomic that caused considerable confusion. In contrast, Johnson (1959) used subspecies that are now unequivocally recognized at the species level (Hill, 1998). Johnson (1959) provided thorough descriptions of morphological characters for identified variations and diagnoses and these concepts were adopted by Hill (1998) in his treatment of the genus in the Flora of Australia.

The definitions of variety and subspecies are still controversial and criticized for their apparently arbitrary nature. The use of subspecies is based on the slight differences that are present in at least one character among populations (Zachos, 2016c); however, subspecies and variety show no discernible clear differences. Beginning in the latter part of the 20th century, variety is usually used for one population and subspecies includes more than one population (Hamilton & Reichard, 1992). These arbitrary classifications could be useful for identifying and studying discontinuities in those described biological entities. One problem with the use of these ranks is that splitting is based on different criteria being used with the most common being allopatric distributions. The uncritical acceptance of these infraspecific categories is an unnecessary burden when based purely on phytogeography. In cycads, variety was commonly used early and later replaced by subspecies. This was prompted because of limited sampling of a few plants in living botanical collections and gardens without herbarium vouchers. It is possible that some of these plants were actually not a healthy representation of wild populations. Some recent species discoveries have even been made from cultivated plants that had been introduced into horticulture in the USA such as *Zamia splendens* Schutzman. The critical issue in using these discontinuities in some cycad genera has led to species recognitions by changing the rank and/or without using more detailed information on morphological variation and other sources of evidence. The excessive use of this approach historically has resulted in problems in understanding the group and contributed to taxonomic inflation of infraspecific nomenclature. The scattered distribution of many cycad species could lead to taxonomic inflation if differences in populations are raised to species level without an integrated approach to data analyses.

Monographing the cycad diversity

Most taxonomic treatments at the ordinal or familial level were published during the 19th century (Regel, 1857a,b, 1876a,b; Miquel, 1861, 1868, 1869a,b; de Candolle, 1868; Thiselton-Dyer, 1884). All these works described and tested species hypotheses of the group, they also showed the difficulties related to vegetative similarities among and between species. The most inclusive monograph was published by Schuster (1932) in the Pflanzenreich series. This monograph overestimated species diversity because it described any morphological character as a different taxonomic entity. Some sort of difficulty in interpreting Schuster's taxonomic treatment stems from his reliance of material housed in the Berlin Herbarium which cannot be consulted due to the destruction during the bombing of Berlin in 1943 during World War II. The use of photographs in species publications and digitization of type specimens at herbaria as is now being done would overcome these drawbacks. One major problem with his approach was the recognition of a large number of varieties and forms under each species. This led to establish vague species circumscriptions, lack of nomenclatural priorities and typification as thoroughly reviewed by Laurie Johnson (1959). That and the fact that Schuster did not use or designate types made the work even more enigmatic. Progress was made with the typification of New

World taxa by Stevenson and Sabato (1986a,b) and Australian taxa by Johnson (1959). Since then, regional taxonomic treatments and individual species description papers have predominated in cycad taxonomic literature. In particular, Flora of Australia (Johnson, 1959; Hill, 1998) is a contribution that included all species described for *Macrozamia, Lepidozamia* and *Cycas* species in Australia. In the early treatment by Johnson (1959), nine new species were described in *Macrozamia* based upon careful evaluation of all available data and numerous herbarium and living collections. This work in particular has served to establish the evaluation of detailed comparative morphological data as well as distributional and ecological data.

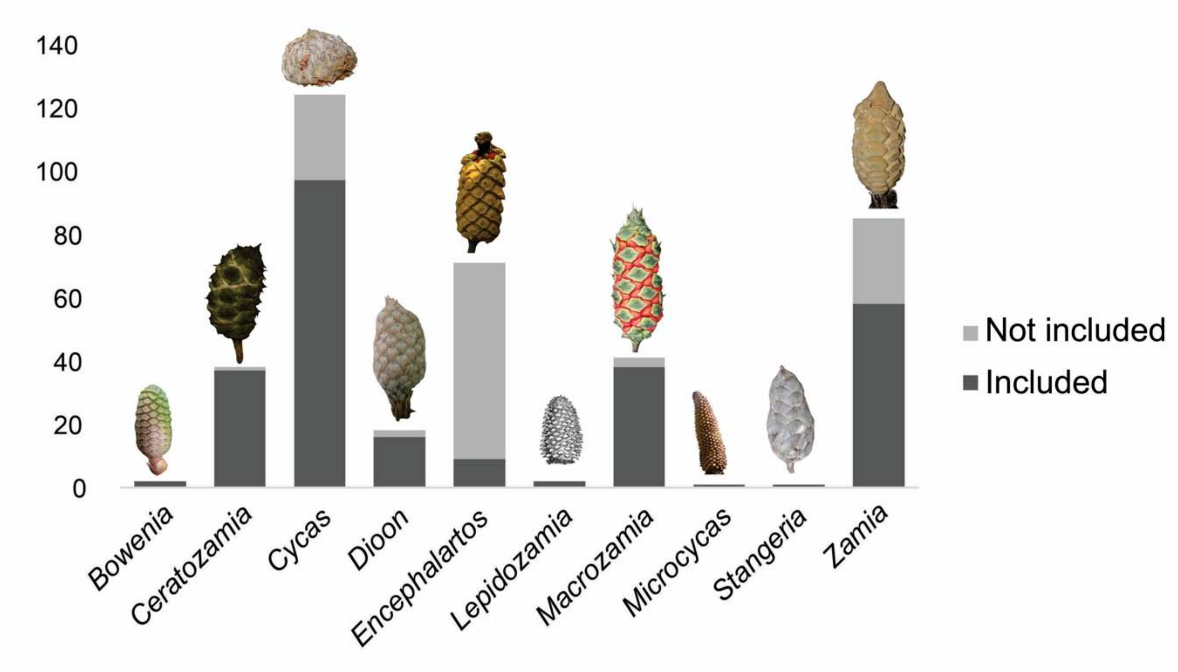
Some regional Flora projects such as Flora del Bajío (Vovides, 1999), Flora del Valle de Tehuacán-Cuicatlán (Medina & Dávila, 1997), Flora Novo-Galiciana (McVaugh, 1992), Flora de Veracruz (Vovides et al., 1983) for Mexico, for the Guianas and Venezuelan Guayana (Stevenson, 1991b,c, 2006) and Flora of North America (Landry, 1993; Stevenson, 1991a) have contributed with descriptions, illustrations and distributional ranges of cycad species that occur in these areas. Other relevant flora treatments and that are more extensive in number such as Flora of Australia (Hill, 1998), Flora of China (Chen & Stevenson, 1999), Flora de Colombia (Stevenson, 2001), Panama (Stevenson, 1993) and the countries Bolivia, Ecuador, and Peru (Stevenson, 2004) contributed to the recognition of new species and tested several species hypotheses in *Cycas* and *Zamia*.

The regional taxonomic revisions (i.e., taxonomic treatments) contributed significantly to clarifying the identity of many described species from 1753, the starting date for nomenclature, and transferred some varieties, subspecies and forms to ranks of species (Fig. 3). *Cycas* is the genus with the highest number of taxonomic revisions because of its high diversity. It was mostly during the 20th century that the diversity of

Cycas was discovered and described (de Laubenfels & Adema, 1998). However, the taxonomic revisions from Vietnam, Philippines and China led to a 40% increase in known species within this genus during the 21th century (Lindstrom & Hill, 2007; Hill, 2008; Lindstrom et al., 2008). Although revisionary work is still needed in this diverse genus, all these works plus Flora of China provide a solid base for a future monograph of the genus.

Zamia and *Encephalartos* have been scarcely addressed through comprehensive taxonomic treatments. *Zamia* has had a slow but steady increase of new species since 1842 with several species published from different countries by various authors. The most inclusive treatments focused on broad geographic regions such as Colombia, Panama and Mega-Mexico as well as Bolivia, Ecuador and Peru were particularly relevant in clarifying the historical species concepts, providing dichotomous keys as an effective means to identify species, and data to recognize hitherto undescribed taxa as well as more extensive specimen citations (Stevenson, 1993, 2001, 2004; Nicolalde-Morejón et al., 2009a). This encouraged the discovery and description of some new species from Mexico and South America during the last decade, which significantly increased the diversity of *Zamia* (e.g. Calonje et al., 2011; Nicolalde-Morejón et al., 2019). In contrast, a modern complete taxonomic revision is lacking for *Encephalartos*, which is essential to test the species hypotheses raised in the genus over the last century (Fig. 3).

Fig. 3. Number of currently accepted taxa in Cycadales included in studies categorized as monographs (taxonomic treatments and regional Flora projects, and species delimitation studies with taxonomic treatments).



Dioon has remained without significant taxonomic rearrangements in the 20th century. Most species were discovered and described through botanical explorations in Mexico. These individual descriptions provided an understanding of morphological variation that raised good foundations for the taxonomy within the group. Recently, a taxonomic revision of *Dioon* was published using the examination of herbarium specimens (Hernández-Tapia et al., 2020), which has provided a reference of integrated taxonomic information from several previous taxonomic works within this genus by different authors. This treatment was revisited with a focus on some inconsistencies related to the diagnostic characters and nomenclature (Haynes, 2020). However, in general, these works did not change the taxonomic concepts of *Dioon* species.

In *Ceratozamia*, the early names published by Miquel (1847) remained synonyms for decades including in the later works of Miquel (1868-1869). Several varieties and forms were proposed in the infraspecific level taxonomy in the monograph of Schuster (1932) and

Flora de Veracruz by Vovides et al. (1983). The recent regional taxonomic treatments from Sierra Madre Oriental (Mexico) and its circumscriptions aided in the discovery of new species and clarified the use of names that were typified in 1986b by Stevenson and Sabato (Martínez-Domínguez et al., 2016, 2017, 2018a; Vovides et al., 2016). More recently, the monograph published for this genus is the first comprehensive treatment for this genus in the last century and doubled the number of species during the last forty years (Martínez-Domínguez et al., 2022; Fig. 3).

Regional taxonomic treatments have treated more than 80% of the species described to date in most of the world's genera (Fig. 3). Together these efforts have demonstrably aided progress monographing the diversity of cycads. The combination of these projects with taxonomic reviews and monographs at the genus level will be the cornerstone for future research on cycads. Some Flora projects were published when the diversity of regions and genera were poorly explored and that could now be updated and revised such as Flora de Veracruz (Vovides et al., 1983). In this floristic treatment, only one *Ceratozamia* species with three varieties were recognized based upon available data, specimens and knowledge at that time. Now, based upon using this treatment and subsequent data and collections, there are currently six different species described and recognized for Veracruz (Martínez-Domínguez et al., 2022; Fig. 3). In contrast, considering that *Macrozamia* is a genus with few taxonomic modifications and only one new species described since the 2000, the Flora Australia remains up to date (Hill, 1998). Monographs and flora treatments provide the framework for facilitating future knowledge and new species discovery when they cannot be identified in those works. These projects lead to evaluation and eventually updated treatments when the data and collections have been increased significantly.

Species delimitation: discovery strategies and description of diversity

Species delimitation studies were only found in *Ceratozamia, Cycas, Dioon*, *Macrozamia* and *Zamia*. The most used approach was the traditional, which was used in these five genera (Table 2). From all approaches six species delimitation criteria were detected (Table 2). Even so, a species concept was not explicitly stated in the taxonomic works. The criteria and methods used imply that the researchers have used an ontological definition of species (Sites & Marshall, 2004). From non-tree-based methods, diagnosability, phenetic, genetic and niche specialization criteria were detected. Historically, the diagnosability criterion has been related to the use of morphological characters to recognize species. This criterion is based on the patterns of discontinuities in observable characters to establish limits between species (Sites & Marshall, 2004). From these considerations, both diagnosability and phenetic criteria share a common history. However, the diagnosability criterion recognizes the presence of both morphological and molecular character states to delimit a species (Cracraft, 1983). This criterion has predominated in the discovery and description of cycad species. The morphological evidence has been applied only descriptively, whereas the molecular data have been analyzed through DNA barcoding (e.g. Little & Stevenson, 2007; Nicolalde-Morejón et al., 2009b; Calonje et al., 2018; Martínez-Domínguez et al., 2020) and molecular phylogenetic approaches at the genus level (Liu et al., 2018; Habib et al., 2022).

The phenetic criterion has been used in all the genera of Cycadales (Table 2). This criterion is based on quantifying shared similarities between individuals by assigning a numeric value that allows to recognize similarities or dissimilarities among species (Sokal & Crovello, 1970). Several statistical methods are used in this criterion with the clustering of individuals and populations according to similarities through distance analyses. Some examples are sorting methods such as Principal Component Analysis (PCA) and canonical correlation analysis (Sites & Marshal, 2004). This criterion is applied to both morphological and molecular data and but is most commonly used with vegetative morphological characters in cycads (Table 2; Supporting Information Table S2). Only in *Zamia* has this criterion been used with qualitative evidence discretized using PCA (Nicolalde-Morejón et al., 2008). Also, UPGMA is a widely used phenetic method in which through Euclidean distances the differences between groups are calculated and visualized in similarity phenograms (Saitou & Nei, 1987). Generally, molecular data have been analyzed using this method from one individual per population of each species under circumscription in *Ceratozamia* (Pérez-Farrera et al., 2017) up to multiple individuals per population of each species as in *Macrozamia* (Sharma et al., 1998).

The genotypic cluster criterion is based on the degree of mixture among populations. Thus, this method evaluates the genetic subdivision and analyzes the absence of genetic intermediates (Mallet, 1995; Sites & Marshal, 2004). The PCO-MC method was one of the first proposed approaches (Mallet, 1995). Recently, other methods have been implemented using the genetic information to analyze potential contact between putative populations or groups such as in Hardy-Weinberg equilibrium (Huelsenbeck et al., 2011; Carstens et al., 2013). Here, we considered the methods based on these genetic intermediates such as those that describe the genetic structure of populations (e.g. Radha & Singh, 2011). Overall, this method in Cycadales has been used as a descriptor of genetic variation and to propose putative genetic groups that are later tested by other methods like Bayesian or coalescent (Table 2).

All the previous methods have in common an orientation towards the variation of characters, whereas the niche specialization criterion is the interaction between the environmental variables in the species habitat. This criterion consists of modeling the ecological niche of the species based on the geography and habitat description of the species (Wiens, 2007). Thus, the method analyzes whether the ecological niches of the species overlap or diverge (Schoener, 1968; Warren et al., 2008). This criterion has been applied in *Dioon* and *Cycas* as complementary evidence (Mudannayake et al., 2019; Gutierrez-Ortega et al., 2021) and the ecological data were used with other evidence sources for testing species hypotheses (Table 2; Supporting Information Table S2).

The phylogenetic and coalescent criteria are based on tree methods and are oriented to the study of patterns between species (Table 2). In the first criterion, there are phylogenies based on maximum parsimony, maximum likelihood and Bayesian inference (Goloboff, 2003). The method more commonly used in cycads is the Bayesian method (Supporting Information Table S2). Also, methods as proposed by Brower (1999) are included in this criterion, which implies reconstructing a phylogeny of haplotypes looking for parsimonious patterns to test the hypotheses of species recognized *a priori*. Their principles of phylogenetic reconstruction are relations between species, whereas in the coalescent criterion is tokogenetic relations (Knowles & Carstens, 2007; Degnan & Rosenberg, 2009). The latter criterion has been hardly applied in Cycadales, within *Cycas* and *Dioon* (Table 2).

In our review of literature, only 17% of species delimitation studies in Cycadales presented a taxonomic treatment (Table 2). Of this total, 34% presented typification of the names, but did not provide a botanical description for any of the species addressed. Most studies made taxonomic recommendations as synonyms or new species, but not a formal proposal (Supporting Information Table S2). These results are congruent with the prevailing trend in species delimitation studies (Carstens et al., 2013). Our content analysis suggests two potential explanations for this situation: (i) limited support for the species hypotheses and (ii) the lack of morphological evidence for describing the species. This last point is related with the lack of an evaluation of reproductive morphological evidence at population level (Supporting Information Table S2). In general, the morphological data and the review of herbarium specimens are basic in carrying out taxonomic revisions. The taxonomic proposals as descriptions of new species or synonymy were made in fewer than 30% of studies in Cycadales (Table 2).

Also, we found species delimitation studies that only discussed the possibility of more or fewer species in a species complex but without confidence in the data and methods used because the taxa were not clearly recircumscribed species (e.g. Medina-Villarreal & González-Astorga, 2016). Two species delimitation studies were found in which new species were suggested, but the taxa were described in another journal (Gutiérrez-Ortega et al., 2018a,b; Gutiérrez-Ortega et al., 2020a,b). This is consistent with observations of several authors who have suggested a crisis in the value that the scientific community gives on traditional descriptions of new species (Wheeler et al., 2004; Carstens et al., 2013; Grace et al., 2021; Wheeler, 2023). The taxonomic treatments and species descriptions based on circumscription are time consuming and most are published in journals with a low impact factor (Carsten et al., 2013). Unfortunately, this is due, in part, to the fact that the papers are cited in the actual nomenclatural treatment but not in the literature cited. Thus, they have low citation statistics. This could lead, in turn, to practices that waste valuable research time, sources and new methods of interest for circumscription, and without clarifying many of the species included in species complexes. Conversely, it is important that evolutionary biology research papers who discover the impact of their results on species circumscription publish formal taxonomic proposals according to the International Code of Nomenclature for Algae, Fungi, and Plants (Turland et al., 2018). Moreover, synonymizations should be formally proposed by including them and their types under a proposed accepted name.

Species Concepts: Meaning and Change

The literature of species concepts is vast and several species concepts have been proposed (Zachos, 2016a). Each species concept is a definition of the species category with implied different levels of inclusiveness (Zachos, 2016c). According to the species concept adopted, the biological entities that the authors are studying should be more or less inclusive. In Cycadales, there is a plurality of species concepts some of which are not compatible in terms of their definitions and scopes. This situation has led to the described biological entities being inconsistent with each other, for example, the species included within the *Ceratozamia miqueliana* complex (c.f. Martínez-Domínguez et al., 2017; Vovides et al., 2020) and *Zamia splendens* vs Z. *katzeriana* (c.f. Nicolalde-Morejón et al., 2009a; Pérez-Farrera et al., 2006). These concerns were pointed out by cycad botanists as the need to incorporate the new tools available within the classification at the species level and avoid taxonomic inflation (Norstog & Nicholls, 1999). Historically, the term "morphogeographic" has been proposed as a species concept in cycads (see Schutzman, 2004; Whitelock, 2004; Pérez-Farrera et al., 2021). It is defined as a population or group of geographically isolated individuals with morphological characters that allow them to be differentiated from other individuals (Schutzman, 2004). Overall, it assumes a geographic component but excludes sympatric speciation as a process. According to theoretical reviews and debates on the concepts of species and their definitions, it was found that the definition and interpretation of this term in taxonomic studies in cycads corresponds with the biological or phenetic concept of species (De Queiroz, 2007; Zachos, 2016a,b).

Table 2. Strategies implemented by species delimitation studies in each genus in Cycadales. The reference is an example of each criteria applied. We show the properties of strategies: general approach, evidence sources, number of assumed species prior to analysis, number of inferred species after the delimitation analysis, and the type of taxonomic decisions proposed.

Genus	**Criteria**	**Approach**	**Source evidence**	**Existing species**	**Species after delimitation**	**Taxonomic proposals**	**References**
Ceratozamia	Diagnosability, Phenetic (PCA)	Integrative taxonomy	Qualitative and quantitative morphology (vegetative and reproductive), molecular data and geography	6	5	TT	Martínez-Domínguez et al., 2017
	Phenetic (PCA, frequencies)	Traditional	Qualitative and quantitative morphology (vegetative and reproductive)	6	2	None	Medina-Villarreal & González-Astorga, 2016

	Phenetic (UPGMA), genotypic cluster	Traditional	Molecular data (RAPDs)	4	4	None	Pérez-Farrera et al., 2017
	Phenetic (PCA)	Traditional	Leaf anatomy	4	5	TT	Vovides et al., 2020
	Diagnosability	Integrative taxonomy	Molecular data, phenology, geography, qualitative and quantitative morphology (vegetative and reproductive)	2	3	NS	Martínez-Domínguez et al., 2020
	Phenetic (PCA)	Traditional	Quantitative morphology (vegetative), leaf anatomy	1	2	NS	Vovides et al., 2012

	Phylogenetic (Bayesian method)	Monophyletic	Molecular data	26 species from 31 of genus	2 new species	None	Medina-Villarreal et al., 2019
Cycas	Genotypic cluster, phylogenetic (CHA)	Monophyletic	Molecular data	3	1	TT (only typification)	Liu et al., 2015
	Phylogenetic (Bayesian method, maximum-likelihood), phenetic (Neighbor-joining), genotypic cluster	Monophyletic	Molecular data	8	2	TT (only typification)	Feng et al., 2016
	Coalescent	Monophyletic	Molecular data	6	2	None	Wang et al., 2019

	Phenetic (UPGMA), genotypic cluster	Traditional	Molecular data	5	5	None	Xiao & Gong, 2006
	Phenetic (PCA), phylogenetic (Bayesian method, maximum-likelihood), niche specialization	Integrative taxonomy	Qualitative and quantitative morphology (vegetative and reproductive), molecular data, ecological niche models	Subsection Rumphiae	1 new species complex with 6 groups	None	Mudannayake et al., 2019
	Phylogenetic (parsimony method, Bayesian method)	Monophyletic	Molecular data, qualitative and quantitative morphology	16 species from Stangerioides, 2 from Indosinenses, 1 from	Proposal one new species from Stangerioides section	NS	Zhou et al., 2015

				Panzhihuanses, 1 Asiorientalis			
Dioon	Phenetic (distances)	Traditional	Quantitative morphology (vegetative), geography	1	2 (subspecies)	None	González-Astorga et al., 2003
	Phylogenetic (parsimony method)	Monophyletic	Molecular data	13 species of the genus	Proposal one new species	NS	Nicolalde-Morejón et al., 2009b
	Phenetic (PCA), coalescent, genotypic cluster, niche specialization	Integrative taxonomy	Quantitative morphology (vegetative), molecular data, ecological niche models (climatic variables)	1	2	NS	Gutiérrez-Ortega et al., 2020a,b*

	Coalescent, genotypic cluster, niche specialization	Integrative taxonomy	Molecular data, ecological niche models (climatic variables)	2	2	None	Gutiérrez-Ortega et al., 2021
Macrozamia	Phenetic (UPGMA), genotypic cluster	Traditional	Molecular data (Alloenzymes)	2	2	None	Sharma et al., 1998
Zamia	Phenetic (PCA)	Traditional	Quantitative morphology (vegetative), leaf anatomy	2	3	TT	Pérez-Farrera et al., 2016
	Phenetic (PCA)	Traditional	Quantitative and Qualitative morphology (vegetative)	5	4	TT (partial)	Nicolalde-Morejón et al., 2008

*Two articles of the same species group (one of the delimitation species and the other of the formal description); TT=taxonomic treatment; NS=new species description only.

Most descriptions of new species in *Ceratozamia* suggest geographic isolation, a property of the biological species concept (e.g. Pérez-Farrera et al., 1999, 2009; Vovides et al., 2004). This concept is based on species as individuals that are interbreeding and reproductively isolated from other groups (Sokal & Crovello, 1970; De Queiroz, 2007). Recently, Pérez-Farrera et al. (2021, p. 243, 252) highlighted the use of the morphogeographic concept. Nonetheless, these authors focused on methods from numerical taxonomy for discovery that coincide with the phenetic species concept (Pérez-Farrera et al., 2021). The phenetic concept is defined as groups of individuals with quantifiable differences that allow them to be recognized as a biological entity (Sneath, 1976). Also, apparently, this concept was applied by Medina-Villarreal & González-Astorga (2016); however, the authors point out "taxonomic species concept" (Medina-Villarreal & González-Astorga (2016, p. 213), which was defined as a population(s) similar in morphological characters within a delimited geographic area that differ(s) from other species. Based on these properties and the methodological procedure through numerical methods using quantitative and qualitative morphological characters analyzed for 13 populations of 4 species by uni- and multivariate techniques, we could infer the phenetic species concept in this study (Medina-Villarreal & González-Astorga, 2016).

Recently, the phylogenetic concept of species has been used in *Ceratozamia.* This species concept has been defined from different perspectives; however, all show greater or lesser degrees of concordance in considerer a species as an aggregation of populations or lineages diagnosable by an exclusive combination of character states (Cracraft, 1983; Baum & Shaw, 1995; Meier & Willmann, 2000; Wheeler & Platnick, 2000). Some traditional and integrative taxonomy approaches have a theoretical framework and inferential procedure of the phylogenetic species concept (e.g. Stevenson et al., 1986; Martínez-Domínguez et al., 2016). On the other hand, the evolutionary concept considers a species as a lineage with its own evolutionary tendencies and historical fate (Wiley & Mayden, 2000; de Queiroz, 2007). A recent phylogenetic study based in six loci with one individual per species analyzed by Bayesian Inference proposed a circumscription for the genus of 30 species, of which there would be two new species, seems to be framed in the evolutionary concept (see Medina-Villarreal et al., 2019). However, the authors stated a history-based approach where species are defined from coalescent theory and recognized as independent evolutionary lineages (Vovides et al., 2020, p. 12).

The biological species concept has been the most commonly one applied in Cycadales. This species concept has been used in most species of *Zamia*, with few exceptions where the phenetic species concept was applied (e.g. Pérez-Farrera et al., 2016). Generally, the phylogenetic species concept and the biological species concept have been applied in *Bowenia, Encephalartos, Lepidozamia, Macrozamia, Microcycas,* and *Stangeria.* In *Cycas* and *Dioon,* the ecological species concept has been explored in which a species is defined as biological entity that possesses and evolves to the same niche or adaptive zone (Van Valen, 1976; e.g. Gutiérrez-Ortega et al., 2020a). More recently, the phylogenetic species concept under genealogical criteria has been raised in these genera (Table 2).

In the species problem, the unified species concept and the hierarchical species concept have been postulated as approaches that reconcile the dispute between species concepts (de Queiroz, 1998, 2007). These species concepts do not introduce a conceptualization of species in nature. Conversely, they are models based on the main principles of the evolutionary species concept and converge in their vision of species as evolutionary lineages (Mayden, 1999; Naomi, 2011). In practice, these concepts are commonly used for studies of delimitation or description of new species under integrative approach, but in the circumscriptions under this approach in cycads, the species concept has not been clarified or discussed. The unified species concept and hierarchy species concept could be alternatives to the species problem because they represent integrated frameworks that conceptualize species in the evolutionary context (Zachos, 2016b). It is imperative on researchers to clearly state species concept of their investigations. Thus, statements can also establish the appropriate level of inclusiveness in delimitating species. Under these considerations, integrative taxonomy would provide a robust epistemological framework for delimitation.

In some genera in Cycadales, the use of species concepts has been *ad hoc*. There is no consensus on which species concept clearly describes the biological entities, but the consistency and use of species concepts according to their conceptualizations will allow a robust framework in the species hypothesis (Rieppel, 2009; Zachos, 2016a,b). Although the species delimitation implies a degree of arbitrariness, a solid understanding of the species concepts and the methods would allow to conduct a species delimitation investigation consistent across the results. This is necessary to avoid artificial conservation proposals or evolutionary inferences (Zachos, 2016c).

Phenotypic and genotypic variation: implications for species delimitation

Species are spatio-temporally located lineages that evolve at different scales and times through different evolutionary processes (Rieppel, 2009; Goldstein & DeSalle, 2011; Zachos, 2016b). Therefore, species delimitation is a complex systematic task. The detection of patterns at the level of the phenotype or genotype of individuals is crucial in species boundaries. In turn, these patterns are essential to study and understand the evolutionary processes underlying to the current diversity of species (Wake et al., 2011).

The phenotypic patterns detected in Cycadales have been convergent in vegetative morphological characters, morphological polymorphisms and morphological stasis including the retention of ancestral characters (Brenner et al., 2003; Calonje et al., 2019). At the genotypic level, different studies have highlighted low levels of genetic divergence between species (Brenner et al., 2003). The patterns and inference of causal processes that interact at the phenotypic and genotypic levels have been approached from different angles of research (Supporting Information Table S4). Evolutionary processes directly change phenotype and genotype in generating variation or modifying the frequencies of heritable variation (Laland et al., 2015). Evolutionary processes such as genetic drift, gene flow and natural selection are powerful agents on organisms at population level, which leads to modifying potentially heritable variation (Supporting Information Table S4). Additionally, heritable epigenetic variation induced by environmental changes has potential influence on adaptation and, thus, speciation.

The current diversity of cycads is the result of a recent adaptive radiation process during Miocene-Pleistocene in which vicariance played a relevant role (Nagalingum et al., 2011; Liu et al., 2018). The long-range dispersal events are rare, but have occurred and the climatic conditions appear to have limited its expansion due to conservation of ecologically suitable conditions (Liu et al., 2018; Gutiérrez-Ortega et al., 2021). The processes directly related to the origin of variation at the genetic level are recombination and mutation (Supporting Information Table S4). The correlation of chromosomal fission with the environment and morphology has been studied in *Zamia* (Jones, 1998; Moretti & Sabato, 1984; Olson & Gorelick, 2011). Most of the genera have conservative morphological evolution (Brenner et al., 2003); however, there is high variation of vegetative characters within *Zamia*, which has been attributed to this process. Recently, genome duplication was proposed as a process in gymnosperms that is linked to the generation of innovation in the phenotype (Stull et al., 2021).

Because cycads are characterized by slow growth and a long life cycle, they are not a model group for this type of evo-devo studies. Nevertheless, recent approaches have explored the processes at this level and their regulatory networks during cycad development, such as expression of MADS-box and KNOX1 genes involved in the control of reproductive growth and in the control/regulation of cell identity in the shoot apical meristem, respectively (Supporting Information Table S4). On the other hand, epigenetic factors could play a relevant role on the phenotype and genotype (Laland et al., 2015). Accordingly, the influence of DNA methylation on the cycad phenotype have been studied from pioneering approaches in *Cycas* (Sae-Eung et al., 2012).

Studies on environmental changes in Cycadales found that the aridification process, and the climatic and environmental changes temporarily (on a geological scale) could promote phenotypic variation in both the macromorphological and micromorphological levels (Supporting Information Table S4). In particular, the interspecific variation detected in anatomical characters in *Dioon* has allowed us to infer how environmental changes such as volcanism and water stress might have driven phenotypic expression (Barone-Lugama et al., 2015; Gutiérrez-Ortega et al., 2018b). Also, the high intraspecific variation among populations of *Zamia* species were related to climatic conditions in the area where those species occur (Limón et al., 2016).

A relevant issue in cycads is reproductive phenotype, which has attracted considerable attention due to several characters such as the sarcotesta of seeds and simple strobili (Brenner et al., 2003). All genera have sporophylls arranged in a determinate central axis, i.e., a simple strobilus. The exception is the ovulate plants of *Cycas* where there is a spirally arranged overlapping indeterminate set of megasporophylls (Brenner et al., 2003). Although the reproductive structures occupy a key position in the phenotype, few studies have examined the intra and interspecific variation within genera as well as the role of the causal processes that have influenced the current variety of sporophyll shape. Even though phenotypic plasticity has been omitted from studies on vegetative morphology, it could a key on the divergence and origin of the phenotype (c.f. Medina-Villarreal et al., 2019; Vovides et al., 2020). Recent studies proposed niche conservatism as a process that can promote speciation in cycads (Gutiérrez-Ortega et al., 2021). However, this tendency of closely related species to occupy similar niches represents a pattern (Crisp & Cook, 2012). New approaches that enable investigation of this pattern in *Dioon* could be focused on exploring the causality of the underlying processes between climate pattern and morphology. For instance, geographical and/or ecological allopatry is a driver of speciation at the genotypic and/or phenotypic level (Supporting Information Table S4). The response of populations to new habitats implies a multidimensional model.

In addition to the above, mutualistic pollination systems could contribute to species diversification. Pollination is mediated by highly specialized pollinating insects where chemical communication is a mechanism in this coevolution (Salzman et al., 2021). New inferences on morphological convergence of diaspores in gymnosperms as a result of the dispersal process show how multiple processes interact at different times during the evolution of species (Contreras et al., 2017).

The developmental processes such as neoteny, progenesis, peramorphosis and paedomorphosis were proposed in Cycadales (Carpenter, 1991; Coiro et al., 2021). The first two processes were studied using the morphological comparison among species of *Macrozamia* and *Bowenia* (Supporting Information Table S4) with respect to peramorphosis and paedomorphosis as addressed in *Ceratozamia* (Medina-Villarreal et al., 2019) where the research focused on the approach of ultimate and proximate causes according to Mayr's proposal in 1961 (Supporting Information Table S4). This approach is on the rise in current debates because separation among proximate causes with ontogeny and ultimate causes with phylogeny that arise from evo-devo, developmental plasticity, inclusive inheritance and niche construction as part of the extended evolutionary synthesis (Laland et al., 2011). The duality of phenotype and genotype converges in the ontogenetic processes of individuals and have an impact on the divergence of species. In particular, the environment has a direct and indirect effect on the observable characters above the molecular level and the genes of the species. This includes both the physical environment (soil, temperature, etc.) and biotic interactions (Laland et al., 2011, 2015).

An alternative to avoid one-way bias is reciprocal causality where there is no dichotomy between ultimate and proximate causes (Laland et al., 2011). This approach requires an understanding of preexisting developmental processes and assumes that these are interacting with genetic, environmental, and epigenetic factors. Additionally, the characters that are acquired, including phenotypic plasticity, are not the direct result of the proximate causes of an individual, but rather the origin of selection and variation (Laland et al., 2011, 2015). Our understanding of mechanisms underlying the evolution in cycads has increased with genomics and new approaches in the study of the phenotype. Integrate and synthesize the actual phenotypic and genotypic knowledge of cycads could lead to benefits and provide information relevant for the species delimitation using modern approaches with a solid base in taxonomy.

Conclusions and perspectives

Accurate species delimitation integrating multiple methods and data sources will establish a solid taxonomy. Under this concept, modern monographs in Cycadales should and could be developed and set a standard for the disseminate of scientific information from taxonomic data, for example, such as descriptions, distributions, ecological data and even photographs. Such monographs will impact other fields such ecology, genetics, conservation and natural resource management in this group, which is considered to be at a high risk of extinction.

We emphasize the importance of species delimitation with taxonomic treatments based on an examination of a consistent number of specimens and exhaustive evaluation of characters variation within and between species. We particularly discourage species splitting based on only in quantitative characters and one population without both morphological and geographical circumscriptions because the species are highly variable at intrapopulation level. Ideally, we recommend the approach that integrates information from different sources including rigorously handling data from prior taxonomic research and nomenclature. The explanation of cumulative information on a scientific name is necessary for species subject to significant changes in past so that the taxonomic legacy associated with that name is not lost.

We further stress the value of the identification of voucher specimens that are deposited in public institutions provided by the authors of the monographs. As such, these results make the information accessible to other biological disciplines. Finally, our review suggests several approaches for further research to address causal processes in the evolution within Cycadales. In particular, more focus is needed on reproductive structures in the evolutionary trends of cycads. We advocate those approaches that enable studies across the different scales from genes to ecology.

Data Availability

All data is available either in the article or as online supplementary information.

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Supporting Information S1. Some useful definitions, which are based on the studies cited in this manuscript. We provide definitions short and self-explanatory that summary the ideas of this terms.

Biological species concept. A group of organisms that can successfully interbreed and produce fertile offspring.

Coalescent criterium. Lineages evolving independently that do not require reciprocal monophyly of alleles or fixed differences.

Ecological species concept. Group of organisms with the same niche or adaptative zone.

Evolutionary species concept. A set of organisms with a unique historical tendency, independent evolutionary fate and own identity.

Genealogical species concept. Organisms sharing exclusive genealogical relationship (i.e., multiple gene genealogies).

Hierarchical species concept. Integrated framework of species concepts based on primary and secondary concepts of species proposed by R. L. Mayden (1999).

Morphogeographic species concept. Groups of organisms correlated by a geographical distribution pattern and characterized by a morphological gap from others.

Phenetic species concept. Assemblage of organisms that share similarities (phenetic cluster).

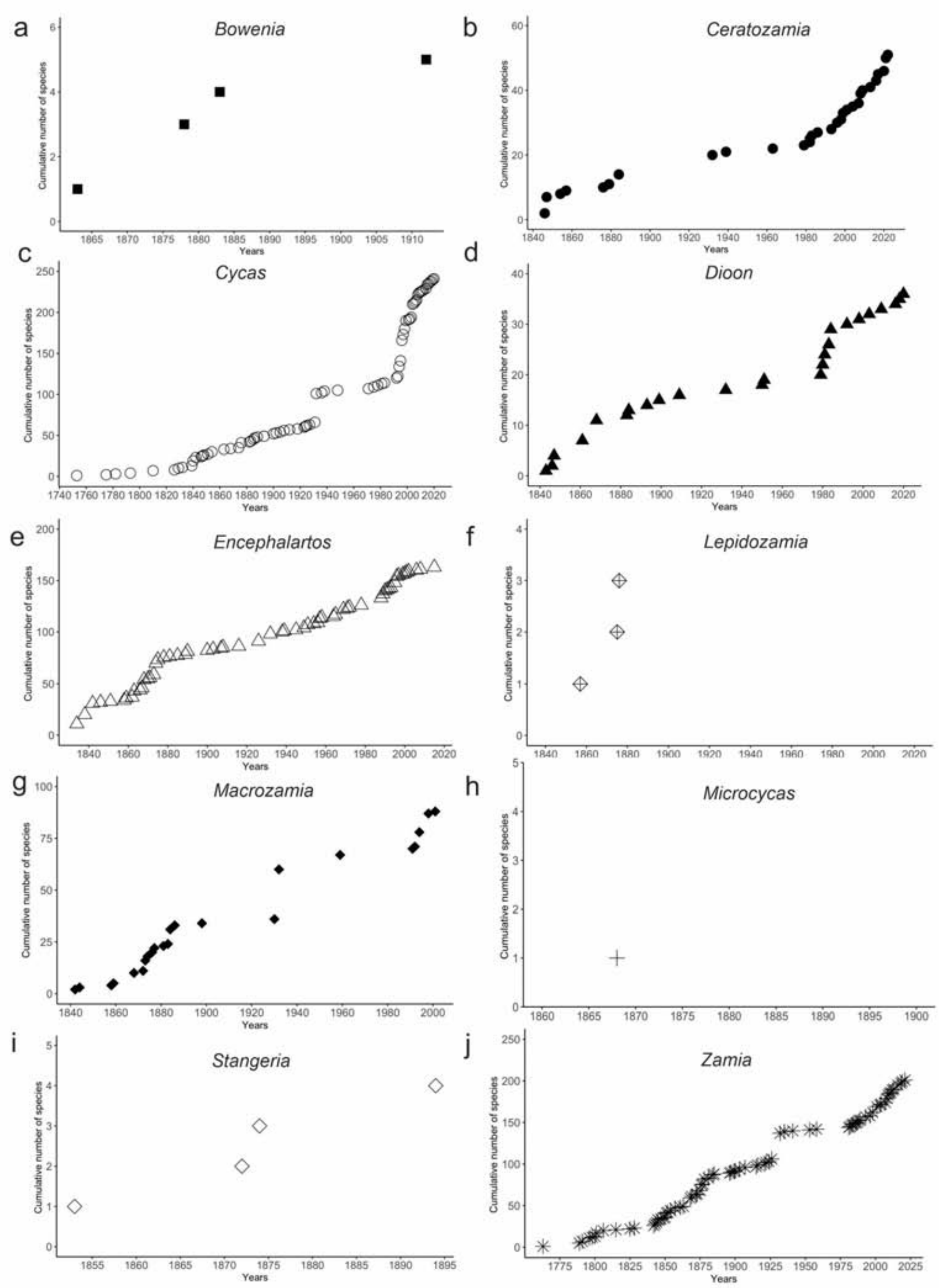
Phylogenetic species concept. Aggregation of populations or lineages diagnosable by an exclusive combination of character states. This species concept has several variants such as Hennigian, monophyletic, genealogical and diagnosable.

Taxonomic concept. Underlying meaning of a specific scientific name defined by the author, the circumscription and a species concept applied.

Unified species concept. Framework of species concepts to unifying other species concepts where species are metapopulation-level evolutionary lineages. This concept was proposed by de Queiroz (2007).

Supporting Information Table S2. References complete of strategies implemented by species delimitation studies in each genus in Cycadales. This table is in a excel database.

Supporting Information Figure S3. Total cumulative number of scientific names published each year in (**a**) *Bowenia*, (**b**) *Ceratozamia*, (**c**) *Cycas*, (**d**) *Dioon*, (**e**) *Encephalartos* and (**f**) *Lepidozamia*, (**g**) *Macrozamia*, (**h**) *Microcycas*, (**i**) *Stangeria* and (**j**) *Zamia*, from 1753 up to September 2022.



Supporting Information Table S4. Evolutionary processes at the phenotypic and genotypic level proposed in Cycadales. The general conclusions of each study are described in the description column, and species or genera in which it was carried out are indicated within parentheses.

Type process	**Process**	**References**	**Description**
Variation causes	Aridification	Gutiérrez-Ortega et al., 2018b; Habib et al., 2022	The epidermal anatomy of leaflets is related to aridity (*Dioon*); this suggests a potential morphological response to water stress (*Macrozamia*)
	Glacial cycles	Feng et al., 2016; Dorsey et al., 2018; Nolasco-Soto et al., 2015	Climatic fluctuations occurred during the Pleistocene affected the genetic structure promoting a rapid adaptation (*C. guizhouensis, Dioon* and *Z. paucijuga*)
	Climate and environmental changes	Lopez-Gallego & O'Neil, 2014; Barone-Lugama et al., 2015; Limón et al., 2016; Vovides et al., 2018	(i) Environmental change as a driver of strong directional selection (divergence at the genetic level that will be expressed in the phenotype, *Z. fairchildiana*); (ii) micromorphological characters of the epidermis in leaflets showed adaptation to climatic conditions derived from volcanism (*Dioon*); (iii) local

		ecological adaptations of the phenotype to different environmental conditions (*Z. prasina* and *Z. loddigesii*); (iv) incidence of the climate on cuticular and stomatal characters (*Dioon*)
Niche conservatism	Gutiérrez-Ortega et al., 2021	Retention of ecological niche through allopatry facilitates allele accumulation and morphological variation (*D. merolae*)
Chromosome fission	Vovides & Olivares, 1996; Olson & Gorelick, 2011	Fission allows directional selection on unlinked genes, thus a high number of chromosomes correlated with morphological variability and habitats could influence small-scale radiation (adaptation; *Z. loddigesii* and *Zamia*).
Whole-genome duplication (WGD)	Stull et al., 2021	Positive relationship between levels of phenotypic variation and gene duplication in gymnosperms *C. hildae*)
Dispersion	Contreras et al., 2017	Convergence in the morphology of diaspores and function (gymnosperms including *Cycas, Zamia* and *Encephalartos*)

Polinization	Salzman et al., 2021	Mutualism between Caribbean *Zamia* clade and their pollinators under a chemical communication interaction; this could influence the evolution of morphological characters (*Z. integrifolia*)
Neoteny	Carpenter, 1991	Leaf cuticle anatomy suggests neotenous features (*Macrozamia* section Parazamia)
Progenesis	Coiro et al., 2021	Leaflets of juvenile individuals with some characteristics present in juvenile individuals of other genera (*Bowenia*)
Peramorphosis and paedomorphosis	Medina-Villarreal et al., 2019	Juveniles with low morphological differentiation compared to adults; juveniles of species that inhabit areas with low humidity show greater ontogenetic transformation than those from humid areas (*Ceratozamia*)
Gene expression MADS-box	Brenner et al., 2003; Chen et al., 2017	Genes of ABC lineage were relatively conserved in gymnosperms, which are related to shape and function of reproductive structures (*Cycas*)

	Gene expression KNOX-1	Bharathan et al., 2002	Control of cell identity in shoot apical meristem (*Z. floridana*=*Z. integrifolia*)
	DNA methylation	Sae-Eung et al., 2012	Variation in methylation patterns across species, which determinate levels of gene expression that may have effects on phenotypes (10 species of *Cycas*)
Variation frequencies	Vicariance (allopatry) – gene flow	González-Astorga et al., 2008	Genetic divergence corresponds to the Trans-Mexican Volcanic Belt (*D. sonorense, D. tomasellii* and *D. holmgrenii*)
	Genetic drift	González-Astorga et al., 2006	Alleles loss and clinal variation in loci (*Z. loddigesii*)
	Adaptive radiation – natural selection	Nagalingum et al., 2011; Calonje et al., 2019	Rapid speciation promoted by ecological opportunity or available niches (Cycadales, *Zamia*)

III. DISCUSIÓN GENERAL

Los enfoques centrales de esta tesis fueron la monografía y los patrones de evolución en el género *Ceratozamia*. Este género es un linaje de plantas en peligro de extinción considerado como *fósil viviente* (Brenner et al., 2003), el cual está incluido en el apéndice I de CITES y la mayoría de sus especies están bajo alguna categoría de riesgo en la lista roja de especies de la Unión Internacional para la Conservación de la Naturaleza (IUCN, 2022). A pesar de los esfuerzos de conservación, la vulnerabilidad de numerosas especies ha aumentado debido principalmente a la pérdida de hábitat (Donaldson, 2003). Un estudio reciente que evaluó el grado de vulnerabilidad de las especies de *Ceratozamia* que habitan en la Sierra Madre Oriental en México bajo un enfoque multidisciplinario con modelado de nicho ecológico, taxonomía y antropología sociocultural, señaló que la mayoría de las especies han pérdido más de la mitad de su área potencial de distribución derivado de la transformación de hábitat y de aplicaciones de estrategias de conservación a escala local fallidas (Martínez-Domínguez et al., 2021).

Uno de los ejes centrales de este proyecto de investigación fue la morfología que es una de las disciplinas biológicas clásicas. La morfología ha contribuido al conocimiento de la forma de los organismos, pero ha sido relegada desde la década de 1980 debido a los grandes y rápidos avances en técnicas moleculares (Wanninger, 2015). No obstante, la morfología es una herramienta valiosa tanto para describir la forma de un organismo como para estudiar los patrones fenotípicos en un contexto evolutivo (Wanninger, 2015; Anest et al., 2021). El estudio y análisis de la morfología de plantas integrado dentro de un marco filogenético permite abordar de manera más concisa el conocimiento de los eventos que han moldeado los fenotipos (Dkhar & Pareek, 2014; Chomicki et al., 2017; Coudert et al., 2017). Por tanto, en esta tesis, la morfología de *Ceratozamia* se analizó bajo un enfoque integrador que incorpora el conocimiento desde otras áreas como la información ecológica, fenológica y molecular para estudiar la diversidad de sus fenotipos. Esto se registró en la monografía y se analizó para estudiar la evolución dentro del género.

Monografiando la diversidad de *Ceratozamia*

Las monografías botánicas son recursos que presentan una clasificación completa y una serie de datos, principalmente, caracteres comparativos (Wheeler, 2014; Borsch et al., 2015). Esto conlleva a que sean clave para que los taxónomos faciliten e incrementen el descubrimiento de especies nuevas (Marhold et al., 2013; Grace et al., 2021). Además, estos trabajos son ampliamente utilizados en diversas áreas como la ecología, la genética, la biología evolutiva, entre otros, incluso en áreas aplicadas entre las que destacan la conservación, el fitomejoramiento y la horticultura (Grace et al., 2021). Una monografía aborda tanto al fenotipo como a los conceptos taxonómicos del grupo bajo estudio y la historia taxonómica de cada binomio (Wheeler, 2014; Borsch et al., 2015). En las monografías se condensan los datos propios de la taxonomía y de la transmisión del conocimiento biológico como son los nombres, e incluye datos asociados como observaciones fenológicas, hábitat e incluso datos genéticos (Borsch et al., 2015; Grace et al., 2021).

La monografía realizada en esta tesis sintetiza el conocimiento acumulado en *Ceratozamia* durante casi dos siglos desde su descubrimiento formal en 1846 por Brongniart (ver capítulo dos). El género ha presentado dos auges taxonómicos. El primer impulso en descubrimiento y descripción de especies sucedió inmediatamente después de la descripción del género y continuó durante aproximadamente 25 años derivado de los trabajos taxonómicos principalmente realizados por Miquel y Regel (Miquel, 1869; Regel, 1876). El segundo auge en el descubrimiento de su diversidad inició alrededor del año 1980, cuando en un período relativamente corto de tiempo (aproximadamente 30 años) la diversidad del género se duplicó de manera ininterrumpida como producto de las exploraciones botánicas realizadas a zonas poco conocidas de Oaxaca, Veracruz y Chiapas en México por colectores mexicanos y extranjeros.

Esta monografía de *Ceratozamia* se basa en trabajos de circunscripción de grupos de especies con afinidades morfológicas y geográficas inciertas, los cuales están incluidos dentro del primer capítulo de la tesis. Estos casos particulares de delimitación de especies se abordaron bajo la aproximación de taxonomía integrativa, la cual se centra en conjugar diversos conjuntos de datos para someter a prueba hipótesis de especies (DeSalle et al., 2005). Se aplicaron diferentes métodos para las fuentes de evidencia dentro de las cuales la fenología representó una parte novedosa, pese a su limitado conocimiento dentro de *Ceratozamia* (Martínez-Domínguez et al., 2018b). Dentro del segundo capítulo, que representa en sí mismo la monografía, se planteó un último caso de delimitación que fue detectado dentro del complejo de especies *Ceratozamia robusta*. De esta manera, la monografía articula todos los datos previos y los nuevos generados como evidencias dentro del grupo para difundir este conocimiento de las especies.

Los tratamientos de Flora regional y monográficos no se terminan complementamente, por el contrario, estos trabajos integrales promueven el descubriento de nuevas especies, ya que representan un recurso primario para identificar material recolectado (Wheeler, 2014; Grace et al., 2021). Además, en estos trabajos se resaltan las áreas o poblaciones que aún requieren atención. En este caso, las poblaciones de *Ceratozamia* desde la región del sureste de México y Guatemala, aún podrían albergar alguna especie nueva debido a que los patrones de variación morfológica reproductiva no se conocen para todas las poblaciones de las especies que habitan en esta zona, e incluso algunos sitios de Guatemala frontera con Belize han sido poco explorados en términos florísticos (Capítulo dos).

Un punto crucial para la monografía fueron los especímenes de herbario. Históricamente, los herbarios han sido los recursos primarios de investigación taxonómica, al ser fuentes masivas de datos sobre los caracteres que están contenidos en los ejemplares botánicos que resguardan (Müller-Wille, 2006; Heberling, 2022). En esta investigación, los herbarios desempeñaron un rol relavante para proveer datos geográficos y de juego de caracteres. También permitieron detectar aquéllas zonas que requerían más exploración como ocurrió con algunas áreas del norte de Oaxaca para construir el mosaico de información, en primera instancia, fenotípica. Incluso permitieron el descubrimiento de una nueva especie desde el estado de Guerrero donde no se tenía conocimiento de la presencia de *Ceratozamia* (Vovides et al., 2004; Nicolalde-Morejón et al., 2014). En este contexto, se encontró un ejemplar dentro de material no procesado formalmente en herbarios que era evidencia de la distribución del género en este estado y culminó con el descubrimiento de una nueva especie, *C. leptoceras* Mart.-Domínguez, Nic.-Mor., D.W.Stev. & Lorea-Hern. Este descubrimiento fue posible gracias a la combinación de los recursos de herbario con nuevo trabajo de campo para colectar y registrar caracteres reproductivos.

Los herbarios no sólo conservan los datos evidentes contenidos en las etiquetas de los ejemplares como la localidad, el hábitat, la elevación y el fenotipo, también proveen caracteres funcionales (Heberling, 2022). En este caso, uno de los datos alternos que resultó valioso fue la información fenológica. En el orden Cycadales se han utilizado datos de herbario para inferir patrones fenológicos en algunas especies del género *Zamia* L. (Clugston et al., 2016). No obstante, previamente en *Ceratozamia,* se carecía de una revisión sobre esta información. Se encontró que los datos fenológicos son insuficientes para aproximadamente un 80% de las especies del género. Esto se debe a que la mayoría del material botánico, ha sido colectado sin incluir en los datos de etiqueta la información sobre el estadío de desarrollo de las estructuras reproductivas como tamaños y coloraciones. Registrar color y tamaño es relevante porque estos dos caracteres se pierden durante el secado, principalmente en el caso de estructuras reproductivas en desarrollo e incluso en aquéllas que están próximas a la fase de maduración. Al perder estos datos se dificulta el uso del estróbilo para describir patrones fenológicos. Además, gran cantidad de material colectado es infértil, lo cual en algunos casos se debe a que la estructura se extravío durante el proceso de ingreso del material a la colección científica. En este contexto, la revisión de los herbarios de manera indirecta mostró que aún hacen falta registros botánicos.

En el último capítulo de este proyecto de investigación se abordaron las tendencias de publicación y los conceptos taxonómicos así como la comprensión de los mismos tanto en *Ceratozamia* como en el resto de géneros en el orden Cycadales. Se describieron las tendencias de descubrimiento de especies nuevas en cada uno de los géneros. Después de los avances logrados con esta tesis, aunque *Ceratozamia* está dentro de los géneros más diversos se convierte en uno de los más completos en términos taxonómicos. Los resultados mostraron que *Encephalartos* Lehm. y *Zamia* requieren de trabajos monográficos. Además, en esta parte de la tesis se analizaron los conceptos taxonómicos de las especies y la comprensión que se tiene de los mismos.

Patrones evolutivos de *Ceratozamia*

Las inferencias de las relaciones filogenéticas en *Ceratozamia* han sido controversiales. Las hipótesis planteadas han recuperado topologías con marcadas diferencias (c.f. González & Vovides, 2002; Nagalingum et al., 2011; Medina-Villarreal et al., 2019). Recientemente, el uso de datos moleculares a gran escala ha impulsado el desarrollo de nuevas reconstrucciones filogenéticas en este género (Habib et al., 2023). A pesar de las diferencias encontradas, estas topologías exhiben una gran complejidad filogenética. Este género de reciente especiación no muestra un claro patrón morfológico ni geográfico (Medina-Villarreal et al., 2019; Habib et al., 2023). Aquí se aplicó una combinación de fuentes de datos incluyendo morfológicos y moleculares para realizar una reconstrucción que incluyera a las especies extantes y extintas del género. Los patrones filogenéticos encontrados son muy similares a los de las últimas dos filogenias publicadas (Medina-Villarreal et al., 2019; Habib et al., 2023). Esto muestra que estamos aproximándonos a una comprensión más detallada de las relaciones filogenéticas del género y apoya la idea de patrones de diversificación intrincados.

Ceratozamia posee una serie de características como la conservación morfológica y un rango geográfico limitado que han llevado a considerarlo, al igual que el resto de géneros en el orden Cycadales, como un *fósil viviente*. Este término fue utilizado en el ámbito evolutivo por primera vez por Darwin y es común en la literatura actual, pero no ha estado exento de arduos debates (Werth & Shear, 2014; Cavin & Alvarez, 2022). No existe una definición precisa de este concepto, lo cual ha dificultado aún más su uso (c.f. Turner, 2019; Lidgart & Love, 2021). En la última década, este concepto ha sido cuestionado debido a que especies extantes con caracteres morfológicos similares a las de sus especies fósiles, pero con diversificación reciente, como ha sido mostrado a través de estudios de datación, han puesto en entredicho este término. Esto se ha encontrado en las cícadas cuyas especies extantes derivan de una radiación reciente que tuvo lugar hace aproximadamente 12 m.a. (Nagalingum et al., 2011; Renner, 2011). Algunos autores coinciden en que el uso de este término no sólo implica estas características sino que incluye baja diversidad actual, estasis morfológica, tasas de cambio evolutivo lentas, entre otros (Turner, 2019; Lidgart & Love, 2021).

Si bien el concepto de fósil viviente es controvertido, es evidente que *Ceratozamia*, al igual que otros linajes considerados bajo ese concepto, poseen características que no son comunes en la mayoría de otros grupos evolutivos, principalmente angiospermas, que surgieron en épocas más recientes. Este género posee una distribución actual limitada y restringida a condiciones climáticas particulares, conservadurismo morfológico y diversidad críptica (Moretti et al., 1980; Martínez-Domínguez et al., 2017). Estas particularidades hacen a este género complejo y se requiere de aproximaciones que integren diferentes datos y métodos para su estudio. Esto no implica que el género no ha evolucionado sino que sus respuestas a las presiones por selección no son típicas o bien difíciles de dilucidar.

En esta investigación, el estudio de los estróbilos, incluido en el capítulo cuatro, mostró que estas estructuras reproductivas no son tan simples. En particular el estudio de los tamaños de los estróbilos arrojó caracteres notablemente relevantes que están asociados a unidades de volumen. En general, los patrones morfológicos entre estróbilos ovulíferos y poliníferos exhibieron una disparidad morfológica. Además, la evaluación de las trayectorias evolutivas de los tamaños de los estróbilos mediante el ajuste de modelos macroevolutivos a la filogenia encontró que estas estructuras siguen el modelo OU. Por tanto, estos caracteres son atraídos a un óptimo fenotípico mediante selección estabilizadora que tiende a una restricción de la variación (Ingram et al., 2012). En relación a caracteres vegetativos, los resultados sugieren convergencia evolutiva y estasis morfológica. Esto último es consistente con resultados planteados de manera generalizada, tanto en este género, bajo otras aproximaciones metodológicas, como en otros géneros de Cycadales (Calonje et al., 2019; Medina-Villarreal et al., 2019; Glos et al., 2022; Habib et al., 2022).

Este trabajo es el primero que aborda el estudio detallado de los estróbilos en un contexto evolutivo en el orden Cycadales. El ambiente puede ser un impulsor relevante del fenotipo de los organismos y por ende, conductor de la especiación (Laland et al., 2015; Chevin & Hoffman, 2017). En cícadas hay estudios puntuales sobre los efectos de condiciones ambientales sobre características morfológicas particulares como el caso de *Dioon* Lindl. y *Ceratozamia*, donde se propuso que las características epidérmicas y el largo-ancho de los folíolos, respectivamente, responden al estrés hídrico (Gutiérrez-Ortega et al., 2018; Medina-Villarreal et al., 2019). Aquí, se abordó la incidencia de las variables ambientales en el fenotipo reproductivo. Se encontró que las variables ambientales evaluadas (ver capítulo cuatro) inciden de manera indirecta sobre el fenotipo que expresan las estructuras reproductivas; sin embargo, esta relación no es lineal. La incidencia más clara se detectó sobre las estructuras reproductivas ovulíferas, lo cual podría estar relacionado con la menor variabilidad de tamaños encontrada al interior de las poblaciones. En contraste, las variables ambientales parecen poseer menor incidencia sobre los estróbilos poliníferos cuyos tamaños varían considerablemente dentro de las poblaciones. En general, las variables ambientales promueven la variación pero no mantienen una incidencia constante sobre las estructuras reproductivas ovulíferas y poliníferas.

Por otra parte, la fenología estudia eventos biológicos recurrentes a lo largo del ciclo de vida de los organismos y su relación con las condiciones climáticas prevalecientes en el área de distribución de los organismos (Morellato et al., 2010, 2013). Esta área requiere más exploración en un contexto evolutivo, tanto en *Ceratozamia* como en el resto de géneros de Cycadales. Las aproximaciones detalladas son escasas siendo el género *Zamia* donde los resultados se basan en un monitoreo intensivo, pero de baja duración en relación al largo ciclo de vida de las cícadas (Clark & Clark, 1987, 1988; Tang, 1990; Segalla et al., 2021). En esta tesis se realizaron esfuerzos mediante el registro detallado de datos/caracteres fenológicos en las etiquetas de los ejemplares de herbario colectados durante este proyecto, como en el monitoreo intensivo en campo para un grupo de especies modelo (ver capítulo tres). No obstante, es evidente que se requiere de un muestreo más intensivo para la mayoría de las especies.

En el capítulo tres de esta tesis se presentan los resultados del monitoreo intensivo de poblaciones de tres especies *Ceratozamia brevifrons* Miq., *C. delucana* Vázq.Torres, A.Moretti & Carv.-Hern. y *C. morettii* Vázq.Torres & Vovides. Estas especies están cercanas geográficamente entre sí, lo que facilitó su monitoreo de manera periódica. Los resultados indicaron que el momento de receptividad en las tres especies fue altamente sincrónico, con un ligero desfase en la especie *C. morettii*, la cual inició antes que el resto de especies. El sobrelapamiento entre las fases de receptividad y liberación de polen entre las especies podría indicar un potencial flujo de genes entre poblaciones de diferentes especies. Esta inferencia preliminar se apoya en los datos aquí obtenidos y en los estudios de polinizadores que se han realizado en los últimos años, donde han reportado que hay más de una especie de insecto potencialmente polizadora para una especie de cícada (Tang et al., 2018; Figura 3). En contraste, se han encontrado especies de géneros distintos habitando en un estróbilo polinífero (Santiago-Jiménez et al., 2019). Además, parece existir una movilidad de insectos en relativamente largas distancias como muestran estudios indirectos en zonas urbanas (Martínez-Domínguez et al., 2020).

Estudios puntuales que registren valores directamente en los sitios de muestreo podrían contribuir a una evaluación más precisa de la fenología. Un muestreo puntual en habitat permitiría detectar potenciales cambios en los puntos de inicio y termino en particular de la fase de receptividad. Esta fase fenológica permite abordar las preguntas relacionadas con el potencial movimiento de polen entre poblaciones de especies diferentes. En las observaciones indirectas realizadas durante las diferentes visitas a campo, se encontró que al final de una temporada reproductiva de estróbilos poliníferos en una población, los escasos estróbilos que permanecen poseen una cantidad excesiva de insectos, los cuales prácticamente destruyen toda la estructura (Figura 3). Este escenario no se observó cuando hay abundantes estróbilos poliníferos en una población. En conjunto, esto nos abre a nuevos estudios integrales de fenología y dinámica poblacional que permitirían responder nuevos cuestionamientos sobre la interacción entre cícada-insecto y si esto ha moldeado, al menos en parte, su intricado patrón filogenético.

Figura 3. Interacción insectos polinizadores y *Ceratozamia tenuis* (Dyer) D.W.Stev. & Vovides en hábitat. A. Inicio de temporada reproductiva con numerosos estróbilos poliníferos en la población. B. Final de temporada reproductiva con sólo tres estróbilos registrados en la población.

Perspectivas en *Ceratozamia*

Las causas de la diversidad en el orden Cycadales han sido abordadas desde diferentes enfoques (Capítulo cinco). A través del análisis del contenido de los principales trabajos publicados que abordan la diversificación y especiación dentro de Cycadales, se detectaron las áreas hacia las cuales se podrían dirigir los esfuerzos para comprender el patrón filogenéticamente aleatorio que sigue la mayoría de la variación morfológica en muchos de los géneros (Gutiérrez-Ortega et al., 208; Habib et al., 2022). En algunos de estos géneros, los avances en términos evolutivos han esclarecido en gran medida las causas de diversificación, como es el caso de *Dioon* y *Cycas* (Gutiérrez-Ortega et al., 2018; Mankga et al., 2020); sin embargo, géneros como *Encephalartos* y

Macrozamia han sido poco estudiados. Debido, en parte a que las características biológicas de las cícadas no hacen de éstas un grupo modelo, no se han abordado este tipo de preguntas desde una perspectiva de la biología evolutiva del desarrollo (evo-devo).

En particular, la evolución de los estróbilos es uno de los desafíos más interesantes, pero complejo de explorar (Brenner et al., 2003). En esta tesis, los resultados sobre la evolución de la diversidad morfológica de los estróbilos de *Ceratozamia* en combinación con los estadíos ontogenéticos evaluados a través de fenología, plantean nuevas interrogantes relacionadas con los impulsores de la evolución de esta diversidad. Comprender la evolución de los estróbilos y su asociación con cambios tanto ecológicos como bióticos requiere, en última instancia, de estudios sobre ecología evolutiva del desarrollo (eco-evo-devo). Esto podría contribuir a esclarecer el papel multifacético que parece desempeñar el medio ambiente en la determinación del desarrollo del fenotipo en *Ceratozamia* (capítulo cuatro). Algunas direcciones de investigación están en el sistema de dispersión donde los colores de la sarcotesta y los tamaños de semillas son relevantes en términos evolutivos (Figura 4), así como la relación entre tamaño de semilla y morfología del estróbilo.

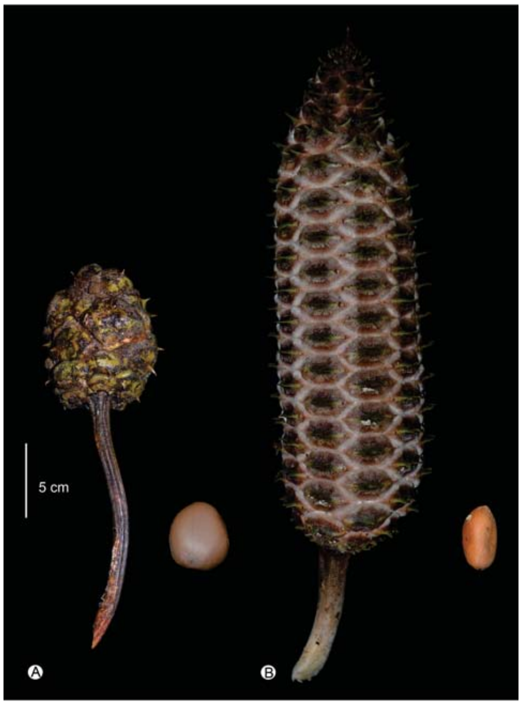


Figura 4. Comparación entre tamaños de estróbilos ovulíferos y semillas en *Ceratozamia*. A. *Ceratozamia matudae* con estróbilos cortos y semillas grandes en proporción al tamaño del estróbilo. B. *Ceratozamia subroseophylla* con estróbilos largos y semillas proporcionalmente pequeñas.

El tamaño de la semilla es un carácter relevante en la historia de vida que influye directamente sobre el éxito reproductivo (Leslie et al., 2017), pero que su variación en Cycadales no se ha documentado de manera sistemática. Esto a pesar de que el origen de la semilla representa una radiación adaptativa en la evolución de las plantas que surgió durante el Devónico y se diversificó en el Paleozoico (Sims et al., 2012). Aquí se detectó de manera indirecta que estróbilos generalmente pequeños desarrollan semillas más grandes o de mayor diámetro en comparación con los estróbilos más largos donde las semillas son pequeñas o proporcionalmente más largas que anchas (Figura 4).

Aparentemente, *Ceratozamia* posee poca variabilidad en diversidad de caracteres reproductivos; sin embargo, la sarcotesta que recubre el óvulo va de tonalidades amarillo-verdosas a blanco-rosáceas (Capítulo dos). Esto llama la atención, ya que las coloraciones son indicativos de la dispersión por animales (Di Stilio & Ickert-Bond, 2021; Nigris et al., 2021), aún cuando en este grupo la dispersión se considera limitada, ocurriendo principalmente por gravedad (Pérez-Farrera et al., 2000). Además, todas las especies muestran un espectro de color rojizo durante la receptividad en los estróbilos ovulíferos que podría atraer a los polinizadores (Martínez-Domínguez et al., 2018b). Aunque, la temperatura y los compuestos volátiles parecen ser las principales señales para la polinización en las cícadas (Terry et al., 2007); una comprensión detallada de como se logran las pigmentaciones a nivel celular, bioquímico y genético podría contribuir a dilucidar los mecanismos evolutivos y de desarrollo que subyacen a la evolución del color y como se ha relacionado de manera indirecta con su éxito reproductivo. En las coníferas, los colores en estróbilos resultan de la acumulación de antocianina donde el rol del color como una señal visual para atracción es un efecto secundario de la protección (Rudall, 2020).

En este contexto, esfuerzos en algunos géneros como *Ephedra* Tourn. ex L., han iniciado los estudios de evo-devo en gimnospermas (San Martin et al., 2022). Por lo que, en las cícadas, nuevos enfoques desde un campo interdisciplanario que reúna eviencia desde la genómica, morfometría y ecología con una perspectiva filogenética podrían mejorar nuestra comprensión de cómo los cambios en el desarrollo moldean la diversidad de los fenotipos en las cícadas y en particular, en *Ceratozamia*. Por tanto, los estróbilos ovulíferos y poliníferos en Cycadales, los cuales pasan por diferentes etapas de desarrollo donde cambian tanto sus coloraciones como su morfología (Figura 5), son órganos que requieren atención bajo estos nuevos enfoques donde se reconoce a los fenotipos como mosaicos dinámicos cuya interacción y adaptación ecológica están estrechamente relacionadas con el fenotipo (Harder, 2019).

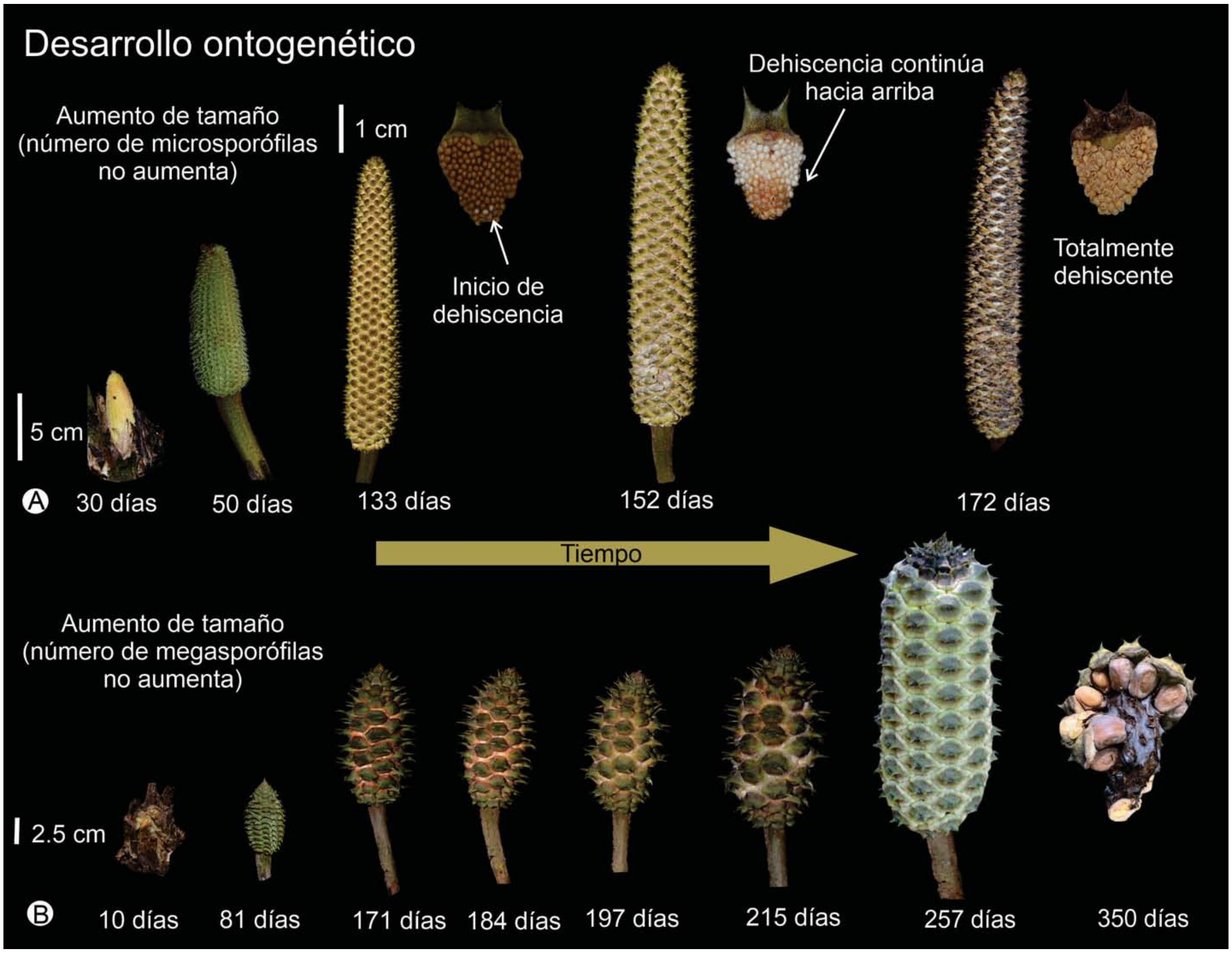


Figura 5. Cambios morfológicos y pigmentación durante el desarrollo ontogenético de las estructuras reproductivas en *Ceratozamia*. A. Estróbilo polinífero de *C. tenuis* desde la emergencia (estróbilo emerge de entre las catáfilas) hasta la senescencia. Se muestra la maduración del polen en las esporófilas. B. Estróbilo ovulífero de *C. tenuis* desde la emergencia (estróbilo emerge de entre las catáfilas) hasta el desgrane (liberación de las semillas).

IV. CONCLUSIONES GENERALES

Los hallazgos de esta tesis se sustentan en la integración de diversas fuentes de evidencia, mismos que permiten abordar aspectos taxonómicos y evolutivos. Se resalta la importancia de un conocimiento taxónomico sólido, que incluye el conocimiento sobre la identidad de las especies y su historia taxonómica, para estudios evolutivos. A continuación se destacan las principales conclusiones resultado de la interacción entre los dos aspectos centrales de la tesis.

- Las exploraciones botánicas permitieron encontrar nuevos registros de poblaciones para *Ceratozamia*, los cuales amplían su rango de distribución. Dentro de estos destacan las poblaciones en el estado de Guerrero, mismas que representan un nuevo registro para el estado y la distribución más norte del género en el occidente de México.

- La diversidad de *Ceratozamia* fue mayor de la esperada. Se describieron 4 especies nuevas para la ciencia: *C. leptoceras*, *C. martinezii*, *C. oliversacksii* y *C. osbornei*. Estas contribuciones ascienden la diversidad del género a 36 especies.

- La monografía presentada es la base para futuros estudios en *Ceratozamia,* incluyendo: aspectos taxonómicos, evolutivos, ecológicos y de conservación. Además, se clarificaron varios conceptos taxonómicos ambiguos de algunas especies e incluso definiciones de caracteres que se usaban de manera indistinta entre diferentes autores. Dentro de los caracteres morfológicos relevantes que se propusieron y son útiles para la diagnosticabilidad de las especies están la forma de la parte infértil y fértil de la microsporófila, la forma de los cuernos de las microsporófilas, el ángulo y dirección de los cuernos de la microsporófila, y tipo de cuernos de las megasporófilas. Esta monografía representa la primera en casi un siglo desde la última monografía del género que incluyó únicamente 2 especies, 4 variedades y dos formas, y colocó dos nombres más como sinonimos dentro de éstas.

- Este trabajo es el primero en evaluar la variación de los patrones de estróbilos ovulíferos y poliníferos en *Ceratozamia* a nivel intra e interpoblacional. Debido a que se encontró que los tamaños de los estróbilos son altamente variables en algunas especies, pero consistente en otras y que algunos caracteres de las esporófilas pueden ser útiles en la identificación de especies, se recomienda registrar datos de caracteres a nivel poblacional para una determinación precisa de las especies.

- Se detectó discordancia entre la evidencia genética y la fenotípica, por lo que es importante integrar diferentes fuentes de evidencia para la propuesta de nuevas especies.

Se plantean las siguientes recomendaciones: 1) evitar describir especies nuevas utilizando únicamente caracteres morfológicos cuantitativos; 2) considerar las especies afines geográficamente y morfológicamente al taxón putativo; 3) realizar un muestreo amplio para registrar la variación evitando así describir variaciones presentes de una misma especie como algo diferente y 4) usar caracteres reproductivos debido a que estos resultaron más informativos para delimitación y diagnosticabilidad.

- Las redes de haplotipos mostraron retención de polimorfismos ancestrales entre las especies analizadas e incluso se detectaron diferencias moleculares entre individuos de una misma población y especie. Esto aunado a la alta similitud morfológica entre grupos de especies y considerando que se ha reportado a más de una especie de insecto potencialmente polinizadora para una misma especie de *Ceratozamia*, se abre la puerta a escenarios evolutivos que consideran potencial flujo de genes como eventos históricos de hibridación.

- Se encontró que los patrones fenológicos en *Ceratozamia* pueden ser datos útiles y relevantes para la delimitación de especies.

- Se detectó una marcada sincronía de patrones fenológicos en especies cercanas geográficamente como es el caso de *Ceratozamia brevifrons*, *C. delucana* y *C. morettii*, las cuales están estrechamente relacionadas en términos filogenéticos, incluso las dos primeras se recuperaron como hermanas. Este sobrelape durante las fases de receptividad y de liberación de polen a nivel poblacional sugiere que existe posibilidad de flujo génico y/o eventos de hibridación que podrían ser conductores de la especiación en el género.

- Los estróbilos ovulíferos son receptivos en un período de tiempo muy corto, pero la disponibilidad de polen en las poblaciones dura en promedio seis meses, aunque con picos en períodos cortos. Esto último se debe a que los estróbilos poliníferos emergen y alcanzan la maduración en diferentes momentos (asincronía poblacional). En conjunto esto sugiere que el género tiene una estrategia para mantener las poblaciones de insectos polinizadores con alimento hasta la siguiente temporada reproductiva; sin embargo, este patrón podría ser una estrategia evolutiva para garantizar la disponibilidad de polen potencialmente viable para la fecundación a pesar de que eventos externos alteren el inicio de la receptividad.

- La temperatura y precipitación son factores ambientales que influyen en el ciclo reproductivo de *Ceratozamia.* La sincronización entre los picos de receptividad y de liberación de polen a nivel de las especies ocurre durante la temporada seca, mientras que la liberación de las semillas en la temporada lluviosa. Los resultados permiten inferir un efecto de las condiciones climáticas sobre los patrones fenológicos.

- Los análisis filogenéticos son congruentes con las filogenias previas para *Ceratozamia*. Se encontró fuerte soporte para clados previamente reconocidos. Sin embargo, la mayoría de los caracteres resultaron homoplasicos siendo los reproductivos los que expresan mayor señal filogenética. En particular, los caracteres reproductivos de tamaño de estróbilos ovulíferos estuvieron más estructurados filogenéticamente.

- Se detectó disparidad entre los tamaños de los estróbilos ovulíferos y poliníferos aunque este patrón no es una generalidad en el grupo. Entre más largas las estructuras reproductivas mayor disparidad. En contraste, la disparidad fue marcada y generalizada dentro de *Ceratozamia* en los caracteres de las esporófilas. Además, los modelos evolutivos sugieren que estas estructuras siguen un modelo de selección estabilizadora.

- El clima puede desempeñar un rol relevante para impulsar los cambios iniciales en el fenotipo de *Ceratozamia*. Los resultados indican una incidencia indirecta de las variables ambientales sobre las estructuras reproductivas siendo los ambientes extremos (las combinaciones: 1) muy fríos y secos, y 2) más cálidos y húmedos), así como los más heterogéneos los que promueven la variabilidad fenotípica.

- La historia de diversificación reciente de *Ceratozamia* ocurrió durante el Mioceno tardío. Las relaciones filogenéticas no se corresponden con un claro patrón biogeográfico, lo cual podría deberse a las múltiples aparentes rutas migratorias del género que moldearon su diversificación.

- La disyunción entre los fósiles de *Ceratozamia* en relación con su distribución actual sugieren que el género habitó Europa y América durante el Paleógeno. Su distribución actual, es producto de los eventos climáticos que condujeron su desplazamiento hacia el sur y de manera vertical en las partes elevadas de los sistemas montañosos de México. Algunas poblaciones sobrevivieron aísladas (como islas) y conforme la temperatura se modificó ocurrieron nuevos eventos de migración hacia el norte de la Sierra Madre Oriental desde el sureste de México.

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ANEXO I

Desde el Herbario CICY 12: 228–233 (22/Octubre/2020)
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Tesoros naturales ocultos en los herbarios: el caso del insólito descubrimiento de una nueva cícada en México

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Los herbarios son sitios de gran valor para el estudio y conservación de la biodiversidad. Son de particular relevancia para recabar información de especies raras con alto riesgo de extinción, como es el caso de las cícadas. Este grupo de plantas, cuyo origen se remonta a más de 250 millones de años atrás, es uno de los más amenazados a nivel mundial. Aquí describimos el caso del reciente redescubrimiento de una cícada colectada hace 36 años y su descripción como especie nueva. Esta historia resalta la importancia de los herbarios como guardianes de la diversidad que aún permanece oculta en estos sitios.

Palabras clave: Biodiversidad, *Ceratozamia*, Cycadales, taxonomía, Zamiaceae.

Un herbario es una colección científica que documenta la diversidad biológica de plantas a nivel regional o global. Estos sitios preservan especímenes disecados de plantas, científicamente catalogados y archivados en gabinetes. Cada ejemplar cuenta con información relevante del lugar geográfico de colecta, la fecha, el colector y las características morfológicas relevantes, entre otros datos (Fosberg y Sachet 1965, Besnard *et al*. 2018). El naturalista Carl von Linneo proporcionó minuciosos detalles de la recolecta de plantas, el secado, los materiales para su montaje y su almacenamiento. Si bien el procesamiento básico de las muestras se conocía desde mucho antes (Camus 1895), estas aportaciones son la base de la estandarización de las técnicas en los herbarios y el surgimiento de estos sitios físicos para guardar ejemplares botánicos (Müller-Wille 2006).

Permítanos reiterar que los herbarios son importantes por toda la información útil que se puede obtener del estudio de sus ejemplares; de ella es posible reconstruir el inventario de la flora de un lugar determinado, averiguar las condiciones ecológicas donde viven las especies, sus interacciones, morfología, abundancia, rareza o endemismo y elaborar hipótesis sobre las

ANEXO II

Cycad Focus

Ceratozamia osbornei D.W.Stev., Mart.-Domínguez & Nic.-Mor.

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Introduction

Past copies of the Journal of the Cycad Society of South Africa, "Encephalartos" have featured "Focus on ... " articles on a selected number of *Ceratozamia* species, i.e. *C. euryphyllidia, C. mexicana, C. norstogii* and *C. tenuis* (see references below). This New World genus, now comprising 36 extant species (Calonje et al. 2022; Martínez-Domínguez et al. 2022), is characterized by the morphological character of having two apical horns on the microsporophylls and megasporophylls. The various species of *Ceratozamia* occur in oak forest, cloud forest and tropical rain forest, generally on karstic rock geology, at elevations from near coastal to just above 2000 m, in Mexico, Guatemala and Belize (Norstog & Nicholls 1997).

Although *Ceratozamia* was first described by Brongniart in 1846, the last three decades have shown a resurgence of interest in the genus, with revisions of many of the older descriptions and publications of a significant number of new species, all of which has been comprehensively treated in a new monograph by Lilí Martínez-Domínguez et al. (2022).

The monograph is also the vehicle for the publication of a new species, *Ceratozamia osbornei*, which the authors have named for Roy Osborne, Founder and first President of the Cycad Society of South Africa, "in recognition of his remarkable contributions to the knowledge of the cycad diversity in the world". His work on cycads demonstrates excellence and diversity ranging from, but not limited to, tissue culture, various aspects of phytochemistry, palynology, floristic treatments, extensive field work, conservation activities and much more. He serves as an exemplar for us and the future. In honouring him, this article summarises the known data about the new species.

Discovery

The relatively small central American country of Belize (previously British Honduras) is a land of mountains, swamps and tropical jungles. It is bounded by Mexico to the north, Guatemala to the west and the Caribbean Sea to the east. Offshore, the massive Barrier Reef, dotted with hundreds of low-lying islands,

ANEXO III

CAMOTILLO HONDUREÑO: LA PRESUNTA PLANTA DE LA VENGANZA

LILÍ MARTÍNEZ DOMÍNGUEZ, FERNANDO NICOLALDE MOREJÓN, OLVIN OYUELA ANDINO Y FRANCISCO VERGARA SILVA

Debido a su toxicidad, el camotillo es muy temido en algunas comunidades; sin embargo, esta cícada no es tan dañina como se piensa, incluso puede tener algunos beneficios.

Las cícadas son las plantas con semilla más antiguas, sus fósiles datan de la era de los dinosaurios.

Durante una reciente exploración botánica en Honduras registramos una historia que forma parte del acervo cultural de las comunidades locales asentadas en el área de distribución del camotillo, la historia narrada por uno de nuestros guías es la siguiente: "*Cierto conquistador español le envió cartas al Virrey de España en las cuales le comentó que los nativos eliminaban soldados con la planta llamada camotillo. El tiempo que se deja la raíz o tallo cortado antes de elaborar la infusión es el tiempo que le tomará a la persona fallecer*".

En los recorridos realizados en varios asentamientos humanos cercanos a donde habita el camotillo se documentaron más historias similares, las cuales están extendidas en varias regiones de Honduras. En algunos sitios se conoce como la planta de los infieles porque puede ser utilizada como venganza. Incluso se piensa que al estar cerca o tocarlas puedes intoxicarte y morir, por lo que las personas recomiendan mantenerse lejos de estas plantas y cortarlas. Debido a estas historias, los camotillos son muy temidos, esto podría tener graves consecuencias en la conservación de esta planta milenaria.

Los camotillos pertenecen al grupo de las cícadas que están incluidas en el orden Cycadales y son los representantes vivos más antiguos de las plantas con semilla, cuyos primeros fósiles datan de hace 290 millones de años. Estas plantas fueron dominantes durante la era Mesozoica, llamada la "era de los dinosaurios", pero que sin duda podría denominarse la "era de las cícadas". En Honduras habitan seis especies de cícadas de tres géneros *Dioon*, *Zamia* y *Ceratozamia*, a estas dos últimas se les denomina camotillos.

Ceratozamia hondurensis es endémica, muy poco conocida y la única especie del género en Honduras. Debido a que esta especie se encuentra en peligro de extinción está incluida en el apéndice I de la "Convención sobre el Comercio Internacional de Especies Amenazadas de Fauna y Flora Silvestres". Esto indica que su comercio está regulado por leyes internacionales para evitar saqueos, actividad común en la mayoría de las cícadas por la belleza de sus