Received: 25 September 2023 | Revised: 19 March 2025 | Accepted: 22 March 2025

DOI: 10.1111/1442-1984.70008

EDITOR'S CHOICE PLANT SPECIES BIOLOGY WILEY

SPECIAL FEATURE

Current topics on cycad biology: Deciphering the Rosetta Stone of plant evolution

Integrative taxonomy of the *Ceratozamia mexicana* Brongn. (Zamiaceae) species complex from a phylogeographic perspective

Anwar Medina-Villarreal[1] | **Jorge González-Astorga**[2] | **Alejandro Espinosa de los Monteros**[3]

[1]Herbario BCMEX, Facultad de Ciencias, Universidad Autónoma de Baja California, Carretera Transpeninsular Ensenada-Tijuana No. 3917, Ensenada, Baja California, México

[2]Laboratorio de Genética de Poblaciones, Red de Biología Evolutiva, Instituto de Ecología, A.C. Carretera Antigua a Coatepec No. 351, Xalapa, Veracruz, México

[3]Laboratorio de Sistemática Filogenética, Red de Biología Evolutiva, Instituto de Ecología, A.C. Carretera Antigua a Coatepec No. 351, Xalapa, Veracruz, México

Correspondence
Jorge González-Astorga, Laboratorio de Genética de Poblaciones, Red de Biología Evolutiva, Instituto de Ecología, A.C. Carretera Antigua a Coatepec No. 351, Xalapa, Veracruz, 91070, México.
Email: jorge.gonzalez@inecol.mx

Funding information
Instituto de Ecología, A.C., Grant/Award Number: 2003/10776; Consejo Nacional de Humanidades, Ciencias y Tecnologías, Grant/Award Number: 282059

Abstract

In recent years, there has been a growing interest in research on *Ceratozamia* cycads, which has resulted in a significant increase in our understanding of their diversity. However, species complexes that have been tackled through integrative methodologies have shown a bias toward morphology over genealogy. Thus, there arises the necessity to re-evaluate the *Ceratozamia mexicana* species complex at the inter-population level using phylogeographic tools. For this purpose, we have employed an integrative taxonomy protocol. This protocol delineates species as testable hypotheses, incorporating lines of evidence within a reproducible and falsifiable framework. The evaluated data encompassed (i) phylogenetic analyses, (ii) multivariate morphometric analyses, and (iii) climatic niche analyses. Out of the 196 individuals from 13 studied populations, seven monophyletic groups were recovered. These groups were substantiated by multivariate morphometric analysis, revealing significant differences, and were further validated by niche divergence. These results underscore a "whole agreement" across the three disciplines, thus providing robust support for acknowledging the species as distinct lineages. The species delimited by these lineages, encompassing phylogenetic, morphological, and environmental factors, correspond to *C. brevifrons*, *C. delucana*, *C. decumbens*, *C. mexicana*, *C. morettii*, *C. tenuis*, and a novel cryptic species, sister to the latter. Finally, this genealogical characterization will offer utility in the future for uncovering the genetic identity of the holotype of the type species within the *Ceratozamia* genus.

KEYWORDS

cycad, *ITS*, Mexico, phylogeny, *trnL-trnF*

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2025 The Author(s). *Plant Species Biology* published by John Wiley & Sons Australia, Ltd on behalf of The Ecological Society of Japan.

1 | INTRODUCTION

"Things rely on their names, and vice versa" (Paz, 1972). Taxonomy serves as the foundation for exploring and comprehending biodiversity, as it is the discipline responsible for characterizing, classifying, and naming taxa (Wilson, 2004). The species is the fundamental unit in biology, as it provides a direct link to understanding the organism under study (Mace, 2004). Consequently, the demand for robust alpha taxonomy has intensified due to the biodiversity crisis, both for documenting species richness before potential losses occur and for underpinning conservation efforts (Wilson, 2005). An approach through multiple sources harnesses the complementarity among disciplines or fields of study (Schlick-Steiner et al., 2010). This approach has been labeled in various ways: "combined" (Malhotra & Thorpe, 2004), "multidisciplinary" (Schlick-Steiner et al., 2006), "multidimensional" (Yoder et al., 2005), "collaborative" (Fisher & Smith, 2008), or "integrative" (Dayrat, 2005; De Queiroz, 2007; DeSalle et al., 2005). While their treatments are not equivalent due to the diverse operationalization used, all these approaches share similar goals: the delimitation, recognition, and discovery of species (Yeates et al., 2011). Among these terms, "integrative" is the concept that has persisted since its inception (approximately 20 years ago) to better describe the complementary nature of different sources of evidence (Dayrat, 2005; DeSalle et al., 2005).

Integrative taxonomy (IT) has recently been defined as a "species delimitation process, in which species are proposed as hypotheses based on the integration of multiple lines of evidence within a reproducible and falsifiable framework" (Pante et al., 2015). IT does not replace traditional taxonomy. Rather, it combines the findings from different disciplines into a coordinated process, leading to an enhanced understanding of biodiversity (Schlick-Steiner et al., 2010). Several concepts for governing IT praxis have emerged. These range from abstract ideas like the "taxonomic circle" (DeSalle et al., 2005) or "taxonomic loop" (Pante et al., 2015) to sequential rounds of exploration in flowcharts (Schlick-Steiner et al., 2010), as well as series of guidelines (Dayrat, 2005) and iterative steps (Yeates et al., 2011). All these methodologies place species within a hypothetical-deductive framework, where information generated by different disciplines is independently analyzed and subsequently integrated.

A robust species delimitation hypothesis hinges upon the number and demarcation of species within a set of specimens. In this regard, Schlick-Steiner et al. (2010) proposed the "hypothesis-driven approach" and the "discovery approach" (or "hypothesis building"). Under the former approach, a set of specimens is evaluated based on the a priori hypothesis of species existence. Conversely, the latter approach involves analyzing specimens without a preconceived hypothesis regarding the number of species.

In congruence-based integration, "total congruence" is considered when the same groups of specimens are recognized across all sources of evidence; "partial congruence" when not all sources of evidence align; and "total incongruence" when the groups delimited by each discipline are entirely distinct (Schlick-Steiner et al., 2010). Alternatively, accumulation-based integration assumes that the divergence of any differentiable attribute among groups of specimens constitutes a taxonomic character that may provide evidence for the existence of distinct species (De Queiroz, 2007).

In this regard, it is emphasized that there exists a trade-off between both integration approaches. On the one hand, there is an overestimation through accumulation (i.e., alpha error) and an underestimation through congruence (i.e., beta error), leading to divergent nomenclatural consequences (Padial et al., 2010). Faced with these contrasting operationalizations (i.e., accumulation vs. congruence) in the delimitation of evolutionarily independent lineages (i.e., species), two approaches have been recognized in the latest proposals for IT practice: standard and sophisticated (Conix, 2018). The standard approach stipulates that lineages should be recognized when all (or nearly all) operational methods converge to the same results without necessitating theoretical support (Alström et al., 2008; Vieites et al., 2009). In contrast, the sophisticated approach does not require multiple operational methods to converge in recognizing the same groupings, as incongruences could be buffered by theoretical assumptions (Padial et al., 2010; Schlick-Steiner et al., 2010). This serves to mitigate the problem dependent on operational methods (Conix, 2018) or researcher bias (Schlick-Steiner et al., 2010).

Based on the foregoing, taxonomists who are increasingly aware of speciation mechanisms are positioning taxonomy as an integral component of evolutionary theory (Chung, 2003); and as species are treated as hypotheses, they will be better delimited when the evolutionary processes that give rise to them are understood (Pante et al., 2015). The groups of organisms that have been investigated through integrative approaches are those situated in gray zones (De Queiroz, 2007), characterized by challenging recognition at morphological and/or genetic levels (e.g., references from the supplementary material of Schlick-Steiner et al., 2010). These zones have been designated by taxonomists as species complexes (Mutanen & Pretorius, 2007), where the utilization of multiple disciplines as sources of evidence—through independent analyses—has become necessary to provide greater support for the nomenclatural decisions taken.

In the cycads of the genus *Ceratozamia* Brongn. (Zamiaceae, Cycadales), species complexes based on morphological affinity have been proposed for over 40 years (Moretti et al., 1980), which have been reconfigured as new species were discovered (Martínez-Domínguez et al., 2022; Stevenson & Sabato, 1986; Vovides et al., 2016). One of the complexes that have received significant attention in recent years is the one encompassing the type species of the genus (Stevenson & Sabato, 1986), and it has been designated by various authors as the *Ceratozamia mexicana* species complex (Medina-Villarreal & González-Astorga, 2016; Vovides et al., 2004). The recognition of the taxa within this complex has followed two paths: (i) "taxonomic authority" through subjective visual evaluation of specimens (Martínez-Domínguez et al., 2018; Vovides et al., 2016); and (ii) "non-taxonomic authority" through multivariate analyses with statistical predictions of specimens (Medina-Villarreal & González-Astorga, 2016), leading to a prolonged taxonomic dispute and ambiguity in species delimitation (criteria a and b, respectively, sensu Schlick-Steiner et al., 2010). This fulfills both criteria to be analyzed with a sophisticated integrative approach. (Conix, 2018).

The emergence of the genus *Ceratozamia* Brongn. dates back to 1846 when the French botanist Adolphe T. Brongniart assigned the type specimen *C. mexicana* Brongn. to a specimen set collected by Auguste B. Ghiesbreght in Mexico (Stevenson & Sabato, 1986). Since its discovery more than 170 years ago, the geographical origin of this specimen has been the subject of various proposals using different methodologies to approximate its location (Martínez-Domínguez et al., 2018; Medina-Villarreal & González-Astorga, 2016; Vovides et al., 1983, 2016). Nevertheless, the proposal to address taxonomic issues within the genus has been through the delimitation of species complexes, initially defined by morphological and geographical affinities (Vovides et al., 2004). In this study, we tested the *C. mexicana* complex as defined by Medina-Villarreal and González-Astorga (2016). This definition considers the geographic limits and morphological affinities proposed by Vovides et al. (2004) and Chemnick et al. (2008) as well as the historical collection records of the population from which the holotype by A. G. Ghiesbreght was recorded (Rzedowsky et al., 2009). This complex encompasses, to our knowledge, 13 populations distributed at the eastern end of the Trans-Mexican Volcanic Belt (TMVB) in central Veracruz (Figure 1), which have been regarded as ranging from two to six nominal species (Table 1). Despite the non-monophyly of this complex being demonstrated in several one-individual-based phylogenetic studies (Gutiérrez-Ortega et al., 2024; Habib et al., 2023; Medina-Villarreal et al., 2019), we examined the geographic distribution of genetic groups at the interpopulation level using phylogeographic methods (Arroyo-Santos et al., 2014; Goldstein & DeSalle, 2011) with the aim of integrating multiple operationalizations (Conix, 2018) in the recognition of independently evolving lineages (De Queiroz, 2007). For this purpose, we employed two intergenic spacers, *nrITS* (nuclear DNA) and *trnL-trnF* (chloroplast DNA), as well as individual-level morphological data and population-level bioclimatic

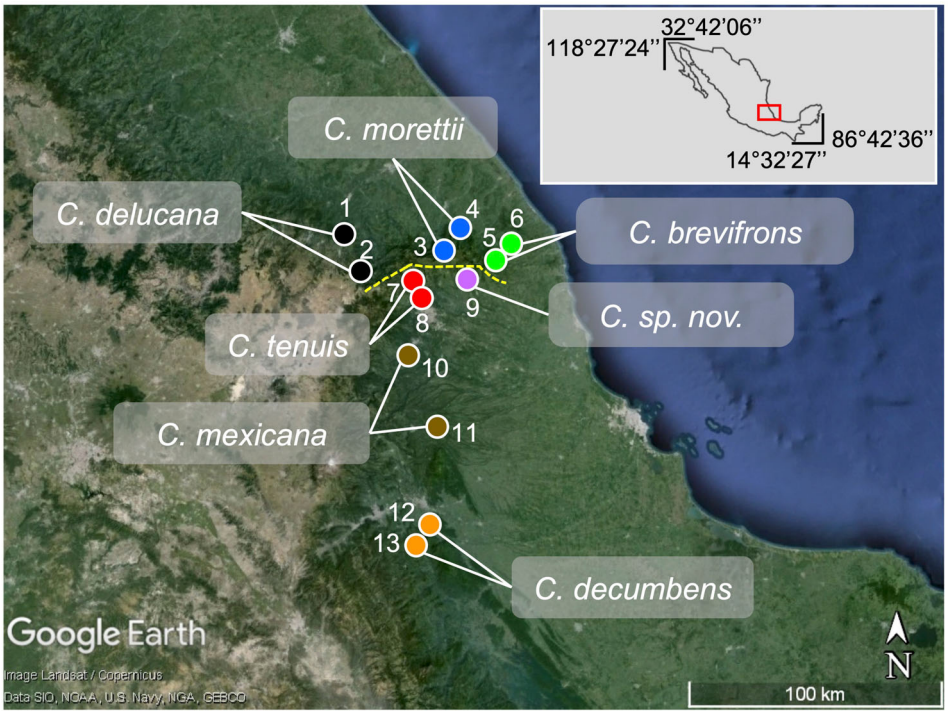


FIGURE 1 Geographic distribution of the *Ceratozamia mexicana* species complex. The population colors indicate the monophyletic groups recovered by phylogenetic analysis. The yellow dotted line represents the eastern end of the Trans Mexican Volcanic Belt (Chiconquiaco mountain range). Above this line are the species of the Northern slope of Trans-Mexican Volcanic Belt (TMVB), and below this line are the species of the Southern slope of TMVB. Population codes: 1 = ATZ, 2 = MIN, 3 = LAN, 4 = YEC, 5 = RCHO, 6 = PAL, 7 = COA, 8 = JIL, 9 = MAF, 10 = XICO, 11 = MIR, 12 = NAR, 13 = TEQ. Map data: Google Earth, Landsat/ Copernicus.

TABLE 1 Populations, locality, and taxonomic chronology of the *Ceratozamia mexicana* species complex.

Populations	Locality	1983[a]	1998[b]	2008[c]	2012[d]	2013[e]	2016[f]	2016[g]	2022[h]
Palma Sola (PAL)	Alto Lucero, Veracruz	*C. mexicana* var. *robusta*			*C. brevifrons*			*C. mexicana*	*C. brevifrons*
Rancho Nuevo (RCHO)	Alto Lucero, Veracruz	*C. mexicana* var. *robusta*						*C. mexicana*	*C. brevifrons*
Naranjal (NAR)	Naranjal, Veracruz	*C. mexicana* var. *latifolia*		*C. decumbens*				*C. mexicana*	*C. decumbens*
Tequila (TEQ)	Tequila, Veracruz	*C. mexicana* var. *latifolia*						*C. mexicana*	*C. decumbens*
Las Minas (MIN)	Las Minas, Veracruz	*C. mexicana* var. *robusta*						*C. mexicana*	*C. delucana*
Atzalan (ATZ)	Atzalan, Veracruz	Unknown record				*C. delucana*		*C. mexicana*	*C. delucana*
Landero y Coss (LAN)	Landero y Coss, Veracruz	*C. mexicana* var. *latifolia*	*C. morettii*					*C. mexicana*	*C. morettii*
Yecuatla (YEC)	Yecuatla, Veracruz	*C. mexicana* var. *robusta*						*C. mexicana*	*C. morettii*
El Mirador (MIR)	Comapa, Veracruz	*C. mexicana* var. *mexicana*					*C. mexicana*	*C. mexicana*	*C. mexicana*
Xico (XICO)	Xico, Veracruz	*C. mexicana* var. *mexicana*						*C. mexicana*	*C. mexicana*
Coacoatzintla (COA)	Coacoatzintla, Veracruz	*C. mexicana* var. *mexicana*			*C. mexicana*		*C. tenuis*	*C. sp nov*	*C. tenuis*
Jilotepec (JIL)	Jilotepec, Veracruz	*C. mexicana* var. *mexicana*			*C. mexicana*		*C. tenuis*	*C. sp nov*	*C. tenuis*
Mafafas (MAF)	Tepetlán, Veracruz	*C. mexicana* var. *mexicana*						*C. sp nov*	*C. tenuis*

[a]Vovides et al. (1983).
[b]Vázquez-Torres and Vovides (1998).
[c]Vovides et al. (2012).
[d]Vovides et al. (2008).
[e]Vázquez-Torres et al. (2013).
[f]Vovides et al. (2016).
[g]Medina-Villarreal and González-Astorga (2016).
[h]Martínez-Domínguez et al. (2018, 2022).

data. In order to answer the question: how many robustly supported species comprise the *C. mexicana* complex?

2 | METHODS

The protocol we have followed is the one proposed by Schlick-Steiner et al. (2010), which is divided into three stages: (i) primary or sequential exploration, (ii) discipline comparison, and (iii) integrated characterization.

2.1 | Choice of disciplines

Three disciplines were selected to achieve an acceptable trade-off between practical constraints and resolution power. Traditional morphometrics (based on linear measurements) were chosen for phenotypic information (data reused from Medina-Villarreal & González-Astorga, 2016). The *nrITS* and *trnL-trnF* genetic markers (Table 2) were selected based on a prior evaluation of plastid markers (Table S1); and finally, environmental information was incorporated through bioclimatic data from population localities (Fick & Hijmans, 2017).

2.2 | Choice of species concept

One of the historical disagreements in species recognition has been driven by the various proposed species concepts. However, the unified species concept (De Queiroz, 2007) is sufficiently robust to accommodate any delimitation

TABLE 2 GenBank accession numbers of analyzed operational taxonomic units (OTU).

Species or population (code)	Tissue source	*ITS*	*trnL-F*
Mafafas (MAF)	LGP 2013-01 [F. Nicolalde 1703 (CIB)]	OR437506	OR449109
Rancho Nuevo (RCHO)	LGP 2013-02 [F. Nicolalde 1728 (CIB)]	OR437507	OR449110
El Mirador (MIR)	LGP 2013-03 [A. Medina 198 (XAL)]	OR437508	OR449111
Las Minas (MIN)	LGP 2013-04 [A. Medina 217 (XAL)]	OR437507	OR449112
Atzalan (ATZ)	LGP 2013-05 [A. Medina 227 (XAL)]	OR437507	OR449112
Landero y Coss (LAN)	LGP 2013-06 [A. Medina 247 (XAL)]	OR437509	OR449113
Xico (XICO)	LGP 2013-07 [A. Medina 270 (XAL)]	OR437508	OR449111
Coacoatzintla (COA)	LGP 2013-08 [A. Medina 288 (XAL)]	OR437506	OR449112
Jilotepec (JIL)	LGP 2013-09 [A. Medina 308 (XAL)]	OR437506	OR449112
Palma Sola (PAL)	LGP 2013-10 [A. Medina 327 (XAL)]	OR437507	OR449110
Yecuatla (YEC)	LGP 2013-11 [A. Medina 353 (XAL)]	OR437509	OR449113
Naranjal (NAR)	LGP 2013-12 [A. Medina 418 (XAL)]	OR437510	OR449111
Tequila (TEQ)	LGP 2013-13 [A. Medina 430 (XAL)]	OR437510	OR449111
Ceratozamia alvarezii	JBC 1996-061 L/JBC 1996-012.01	GU807372	AF407310
C. becerrae	JBC 2001-036 A/JBC 1985-011.01	GU807373	AF407311
C. chamberlainii	JBC 1999-045.01	AF407297	AF407328
C. euryphyllidia	JBC 1986-316 B/JBC 1986-318	GU807376	AF407313
C. fuscoviridis	JBC 1999-055.01	AF407298	AF407329
C. hildae	JBC 2001-002 A/JBC 1977-197.2	GU807377	AF407315
C. kuesteriana	JBC 1983-465 H/JBC 1983-465.17	GU807379	AF407316
C. matudae	JBC 1986-098.01	AF407286	AF407317
C. latifolia	JBC 2001-004 A/JBC 1978-407	GU807380	AF407322
C. mirandae	JBC 1998-091 B/GK 10225	GU807385	GQ273636
C. norstogii	JBC 1993-133 B/JBC 1993-010.01	GU807388	AF407325
C. sabatoi	JBC 1991-043 A/JBC 1991-041	GU807390	AF407326

Note: LGP codes are batch designations for sample plants collected and stored in cryogenics, along with the population herbarium voucher in brackets. Abbreviations: CIB, Herbarium of Centro de Investigaciones Biológicas de la Universidad Veracruzana, Veracruz, México; GK, Kasetsart University Botanical Garden, Tailandia; XAL, Herbarium of INECOL A.C.; JBC, Jardín Botánico Clavijero, INECOL A.C.; LGP, Laboratorio de Genética de Poblaciones, INECOL A.C.

criteria. This concept provides a reconciliatory perspective by acknowledging that all alternative (i.e., incompatible) concepts share a common element: Species are metapopulation lineages that evolve independently, and the properties that define them are interpreted as the operational criteria for assessing the separation of these lineages. Accordingly, regarding the common element as the primary property of the species category enables us to position all alternative species concepts as secondary properties of the species. These become contingent properties that species may or may not acquire during their evolutionary trajectory, providing evidence of their divergence. Therefore, the delimitation hypotheses constructed here were made under the primary property of the unified species concept, and their secondary properties were based on the delimitation criteria of each chosen discipline.

2.3 | Choice of delimitation criteria

Delimitation criteria are the empirical methods that translate the properties of species concepts into a sequence of reproducibility, data analysis, and interpretation. Genetic delimitation was conducted using the discovery approach under the criterion of reciprocal monophyly ("tree-based methods") and genetic group discontinuity ("nontree-based methods") sensu Sites and Marshall (2004). Morphometric delimitation was carried out using the discovery and hypothesis-driven approach under the criterion of phenotypic discontinuity of statistically differentiated groups. Environmental delimitation was performed using the discovery and hypothesis-driven approach under the criterion of significant multivariate distance.

2.4 | Choice of data analysis methods

2.4.1 | Source of genetic evidence

The specimens used were the same for all disciplines, as suggested by Yeates et al. (2011) and Schlick-Steiner et al. (2010). The molecular markers used in the genetic analyses (i.e., *nrITS* and *trnL-trnF*) were chosen through a sequential filtering process. The summary of these steps is presented in Table S1. Briefly, the first filter involved testing amplification success across all populations. The second filter evaluated sequencing quality, prioritizing loci with minimal ambiguity in nucleotide reads. For the final filter, sequence variation within and between populations was assessed. Among the four loci initially sequenced, two (i.e., *atp-rbcl* and *trnS-trnG*) were excluded due to a lack of variation, as sequences were identical both within and across species. The remaining two loci (i.e., *trnL-trnF* and *nrITS*) were retained, as they exhibited sufficient variability to provide a phylogenetic signal without being highly polymorphic. These criteria ensured the loci selected were informative and appropriate for the analyses. The DNA was extracted from the foliar tissue of 10 individuals per population ($n = 130$) with the DNeasy extraction kit (QIAGEN, Valencia, CA, USA). Each PCR (20 μL) was carried out employing 4 μL of 5X buffer, 2 μL of 25 mM $MgCl_2$, 2.5 μL of an 8 mM dNTPs mix, 0.8 μL of each amplification primer at 10 μM, 0.3 μL of Taq polymerase, and 5 μL of template DNA (20 ng). Amplification conditions: an initial cycle was carried out at 94°C for 5 min, followed by marker-dependent cycles (see Table S1) of denaturation at 94°C for 1 min, marker-dependent annealing temperature for 1 min, extension at 72°C for 1 min, and a final extension period of 7 min at 72°C. Amplification products were inspected in 1% agarose gels stained with ethidium bromide and were directly purified with the QIAquick gel purification kit (QIAGEN). Bidirectional automated sequencing was carried out by Macrogen (South Korea; http://dna.macrogen.com). Electropherograms were revised with the software Sequencher v6 (Gene Codes Corp., Ann Arbor, MI, USA). The GenBank accession codes for populations and the outgroup species are summarized in Table 2.

2.4.2 | Source of morphological evidence

Methods compatible with the delimitation criteria of phenotypic discontinuity included the estimation of statistically significant differences in multivariate groupings. The data came from a study on the morphometric and geographical variation of the *C. mexicana* Brongn. species complex (Medina-Villarreal & González-Astorga, 2016). These data encompass information from 196 adult individuals across 13 populations. The standardized values for eight variables per individual can be found in the "Morphology_data.csv" file, and the data were analyzed using the "Morphology.R" script within the R statistical environment (R Core Team, 2025; data and code files in Supporting Information 2). The analyzed morphological variables (Figure 2) correspond to leaf number, leaflet pairs, petiole length, rachis length, middle leaflet width, middle leaflet length, the distance between middle leaflets, and the width of the middle leaflet articulation. The multivariate analysis was conducted using the "lda" function from the MASS library (Venables & Ripley, 2002). From this analysis, we obtained the axes with the highest explained variance to draw scatterplots for each individual population and for the variables with the highest

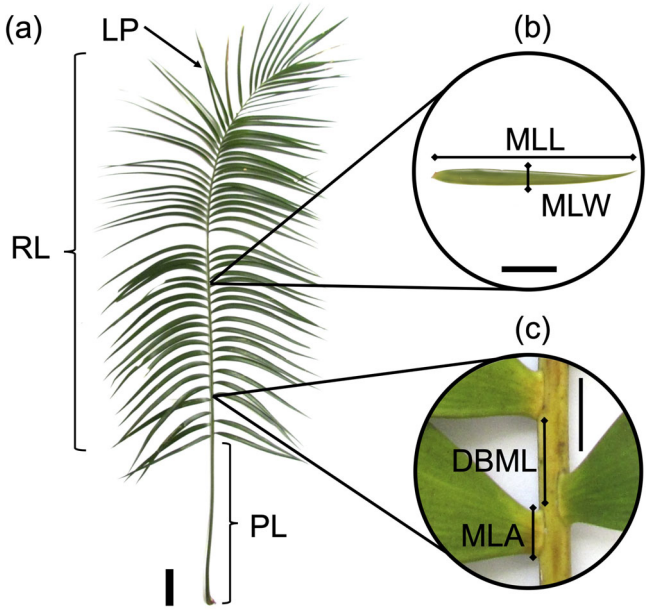


FIGURE 2 Morphological variables analyzed in the *Ceratozamia mexicana* species complex. The codes correspond as follows: leaflet pairs (LP), petiole length (PL), rachis length (RL), middle leaflet width (MLW), middle leaflet length (MLL), the distance between middle leaflets (DBML), and the width of the middle leaflet articulation (MLA). Scale bars: 10 cm in (a) and (b), 1 cm in (c).

loadings. To estimate multivariate morphological differentiation among population groups (i.e., clades /lineages), the following steps were taken: (1) centroids for each population were obtained, (2) pairwise Euclidean distances (D_{obs} = observed distances) were calculated, and (3) then these observed distances were compared to a null model (distances expected by random via 1000 bootstrap replicates) to assess their significance.

2.4.3 | Source of environmental evidence

The climatic data for each population (single occurrence points) were extracted from the 19 bioclimatic layers WorldClim 2 (Fick & Hijmans, 2017), with a spatial resolution of 30 s (approximately 1 km^2 per pixel). The standardized dataset, encompassing bioclimatic and altitude variables, is accessible in the "Environmental_data.csv" file. Analysis was performed using the "Environmental. R" script within the R statistical environment (R Core Team, 2025; data and code files in Supporting Information 2). A correlation analysis was performed to rule out autocorrelated variables. The six variables selected for further analyses correspond to isothermality (bio3), temperature seasonality (bio4), temperature annual range (bio7), annual precipitation (bio12), precipitation of driest month (bio14), and precipitation of driest quarter (bio17). Dimensional reduction was carried out using multivariate analysis with the "lda" function from the MASS library. From this analysis, we obtained the axes with the highest explained variance to plot the climatic niche position of each lineage, along with the variable loadings. Due to insufficient geographic presence data to construct the potential niche, we opted for an ecological interchangeability analysis considering the multivariate position of each population. To estimate niche differentiation among population groups (i.e., clades/lineages), we followed the multivariate method of niche equivalence testing described by Warren et al. (2008), which involves: (1) centroids for each population were obtained, (2) pairwise Euclidean distances were calculated, and (3) then these observed distances were compared to a null model (distances expected by random via 1000 bootstrap replicates) as a proxy to assess their climatic niche divergence (R Core Team, 2025).

2.5 | Data generation

Tree inference and node dating were performed with the aid of the software BEAST v1.10.4 (Suchard et al., 2018). For rooting the tree, 12 species were chosen as outgroups (i.e., *Ceratozamia alvarezii* Pérez-Farr., Vovides & Iglesias, *C. becerrae* Pérez-Farr., Vovides & Schutzman, *C. chamberlainii* Mart.-Domínguez, Nic.-Mor. & D. W. Stev., *C. euryphillidia* Vázq. Torres, Sabato & D. W. Stev., *C. fuscoviridis* W. Bull, *C. hildae* G.P.Landry & M. C. Wilson, *C. kuesteriana* Regel, *C. latifolia* Miq., *C. matudae* Lundell, *C. mirandae* Vovides, Pérez-Farr. & Iglesias, *C. norstogii* D. W. Stev., and *C. sabatoi* Vovides, Vázq. Torres, Schutzman & Iglesias). These taxa were chosen, instead of a single outgroup, because currently, there is no consensus about phylogenetic interrelationships among *Ceratozamia* species. These 12 species encompass the full geographic distribution range of the genus. Nonetheless, *C. matudae* was designated as the functional outgroup based on the phylogeny presented by González and Vovides (2002). We used Bayes factors to determine whether applying partition-specific models significantly improved the explanation of the data (Nylander et al., 2004), as molecular partitions from different origins (e.g., nuclear, chloroplast, mitochondrial) may contain incongruent phylogenetic signals due to differences in inheritance lines (e.g., effective population size). To avoid combining incongruent molecular markers, a Bayes Factor test (Brandley et al., 2005; Kass & Raftery, 1995) was performed. This test concluded that, albeit with slight differences, the phylogenetic signal encompassed in the

ITS and *trnL-trnF* sequences tend to be congruent (2LnBF = 1.28; the phylogenies recovered from the individual markers are presented in Figures S1 and S2). Therefore, both markers were used simultaneously for phylogenetic analyses but declared as unlinked partitions. To relate historic events (e.g., vulcanism; Ferrari et al., 2012) to times for speciation events within the *Ceratozamia*, the phylogeny was dated. Calibrating the tree is difficult and involves making a number of assumptions (Burleigh et al., 2012). With these data, we previously (Medina-Villarreal et al., 2019) estimated that the divergence event between the major clades of *Ceratozamia* occurred approximately 12.1 million years ago (Medina-Villarreal et al., 2019). To keep consistency with that study, this calibration point was employed for setting the root age and allowing the software to estimate nucleotide evolutionary rates for each molecular marker. The "uncorrelated relaxed clock" model was used with a lognormal distribution. The tree prior was set as "speciation: Yule process," and a random starting tree was used. All other priors were left as default (uninformative). The Markov chain Monte Carlo was run 20 times for 10 million generations, and parameters were sampled every 1000 generations. The program TRACER v 1.7.1 (Rambaut et al., 2018) was used for assessing the stationarity of the MCMC, effective sample sizes (ESSs) > 200, and posterior intervals spanning the 95% highest posterior density. The single runs were combined with LogCombiner implemented in the BEAST package. Trees parameters were summarized in median values using Tree Annotator v1.10, and displayed in FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/). Besides, a spatial analysis of molecular variance (SAMOVA) was carried out using SAMOVA 1.0 (Dupanloup et al., 2002) to determine F_{CT}, which represents the proportion of total genetic variance explained by differences between population groups (Excoffier et al., 1992). This analysis requires the geographic coordinates of populations and their corresponding individual haplotypes with the aim of identifying genetic clusters.

2.6 | Hypothesis elaboration

Based on the chosen delimitation criteria and data analysis methods, the delimitation hypotheses were constructed ("discovery approach") and then tested ("hypothesis-driven"). Our sequential analysis pathway started with the generation of the phylogenetic hypothesis, from which monophyletic groups were obtained and subsequently tested in morphometric and environmental analyses.

2.7 | Assessment of conclusiveness of disciplines

The criterion for determining the conclusiveness of a discipline lies in its power to recognize groups of specimens (Sites & Marshall, 2004). A conclusive discipline is one that recognizes significant groups of specimens that exceed the threshold of the chosen delimitation criterion and data analysis method. On the contrary, a nonconclusive discipline is one in which it is not possible to distinguish between groups of specimens (e.g., Barrett et al., 2021).

2.8 | Comparison of conclusive disciplines

This step involves contrasting the results obtained in each discipline (Schlick-Steiner et al., 2010).

2.9 | Integrated characterization

At this stage, the information from the conclusive or nonconclusive disciplines of specimen groups is presented to describe the attributes that define the species.

2.10 | Nomenclatural consequences

This is the final stage where plausible delimitation hypotheses are proposed based on the conducted analyses. Nomenclatural changes can lead to cases, such as synonyms, the resurrection of nominal species, or the assignment of a new name if the delimitation warrants it. However, non-delimitation can arise where the nature of the evolutionary process is poorly understood and could delineate the boundaries of the current praxis of IT.

3 | RESULTS

3.1 | Source of genetic evidence

3.1.1 | Phylogenetic relationships and divergence times

Based on the relationships recovered from the *ITS* and the *trnL-F* markers for these clades (Figure 3), it becomes evident that the *C. mexicana* complex is described as a polyphyletic assemblage. The overall topology of the OTUs (i.e., the populations studied here) suggests a geographic concordance. Specifically, in relation to the

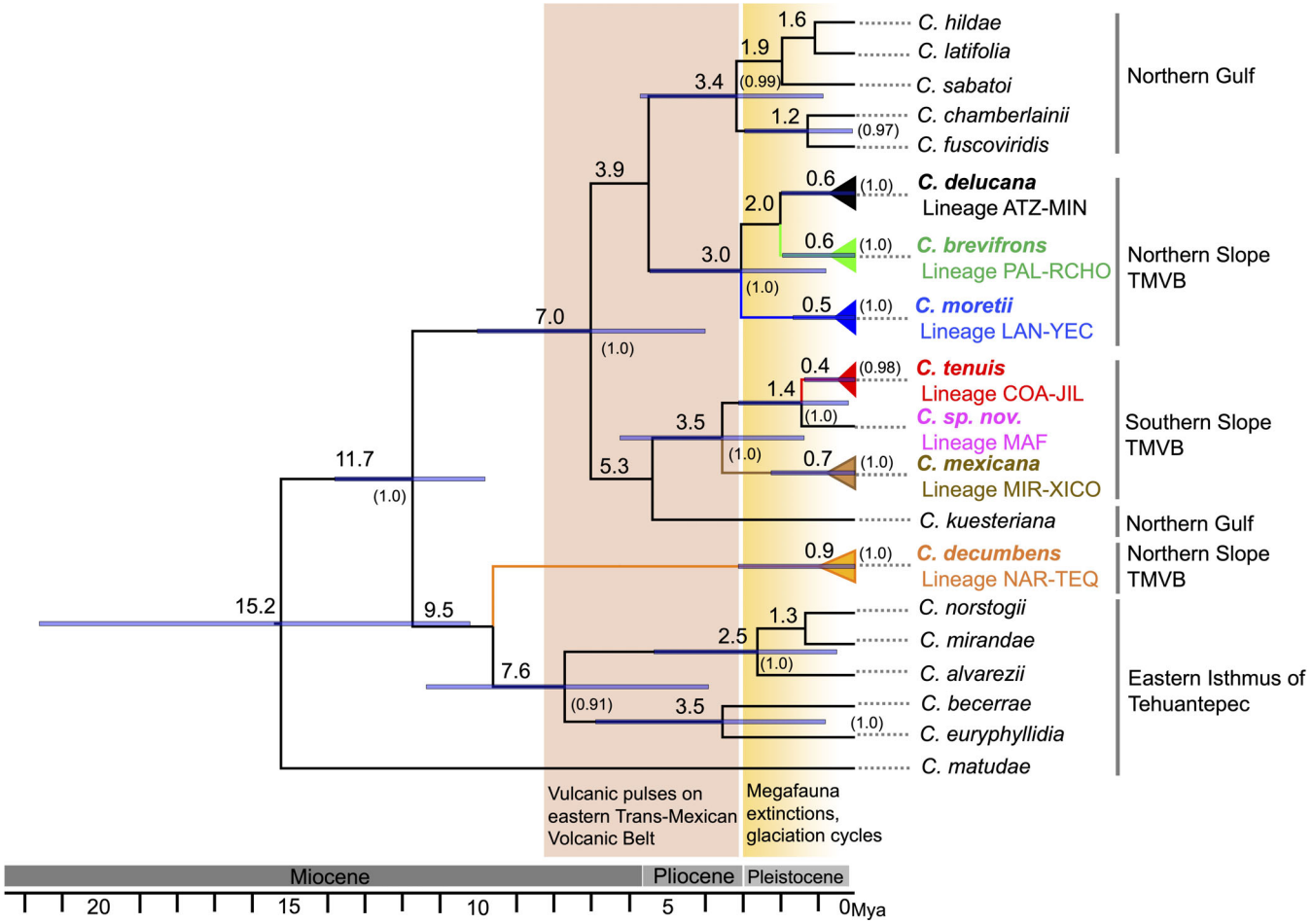


FIGURE 3 Chronogram of the *Ceratozamia mexicana* species complex. The species tree represents the timeline inferred from the Total Evidence from *nrITS* and *trnL-trnF* sequences. Colors identify the monophyletic groups within *C. mexicana* complex: orange for *C. decumbens*, brown for *C. mexicana*, magenta for *C.* sp. *nov.*, red for *C. tenuis*, blue for *C. morettii*, green for *C. brevifrons*, and black for *C. delucana*. Only nodes with a Bayesian posterior probability exceeding 0.9 are evaluated. Numbers are node ages, whereas numbers in parenthesis are Bayesian posterior probabilities. The blue bars depict 95% confidence intervals for divergence times.

TMVB, the units cluster into lineages located on the southern slope of the TMVB, as well as lineages on the northern slope of the TMVB.

The chronogram of divergence events indicates that the ancestor of *C. decumbens* Vovides, Avendaño, Pérez-Farr. & Gonz.-Astorga lineage (populations NAR-TEQ; 1.0 posterior probability (pp)) was the first to diverge within the clade of species in the Isthmus ca. 9.6 million years ago. This species is more related to the species from the Eastern Isthmus of Tehuantepec (i.e., *C. mirandae* Vovides, Pérez-Farr. & Iglesias, *C. norstogii* D.W.Stev., *C. alvarezii* Pérez-Farr., Vovides & Iglesias, *C. euryphyllidia* Vázq. Torres, Sabato & D. W. Stev. and *C. becerrae* Pérez-Farr., Vovides & Schutzman) than to those on the TMVB and the Northern Gulf (i.e., *C. latifolia* Miq., *C. sabatoi* Vovides & Vázq. Torres, *C. hildae* G. P. Landry & M. C. Wilson, *C. chamberlainii* Mart.-Domínguez, Nic.-Mor. & D. W. Stev. and *C. fuscoviridis* W. Bull.). Around seven million years ago, the species from the Northern Gulf and species located on the northern slope of TMVB split from the ancestor of species located on the southern slope of TMVB. Subsequently, this ancestor gave rise to the lineages of three species; one corresponds to *C. mexicana* Brongn. (populations MIR-XICO; 1.0 pp), another to *C. tenuis* (Dyer) D. W. Stev. & Vovides (populations COA-JIL; 0.98 pp) and the last one to an undescribed species *C.* sp. *nov* (MAF). Similarly, on the northern slope of the TMVB, a clade that originated 3.9 million years ago gave rise to the lineages of another three species. One of these corresponds to the *C. morettii* Vázq. Torres & Vovides (populations LAN-YEC; 1.0 pp), another one to *C. brevifrons* Miq. (populations PAL-RCHO; 1.0 pp); and *C. delucana* Vázq. Torres, A. Moretti & Carv.-Hern. (MIN-ATZ lineage; 1.0 pp).

3.1.2 | Genetic diversity

The maximum length of the aligned sequence matrix for *nrITS* and *trnL-trnF* was 858 and 517 bp, with 14 and

5 informative sites, respectively. The 13 populations were monomorphic, with a total of 10 detected haplotypes. Only one haplotype per population was detected for both markers; thus, there was no intrapopulation variation. Most of the haplotypes from both markers were recorded in pairs of geographically neighboring populations, with the exception of the MAF population with a unique haplotype (H1) for the *trnL-trnF* marker. Haplotype diversity was similar in both *nrITS* and *trnL-trnF* markers, whereas nucleotide diversity displayed a twofold increase in magnitude (Hd = 0.7870, $\pi = 0.0056$; Hd = 0.7630, $\pi = 0.0027$, respectively).

3.1.3 | Genetic structure

The tests for geographic clustering of cytotypes are summarized in Table 3. The minimum fixation index values ($F_{CT} \sim 0.61$) were obtained with $K = 2$; populations from the northern and southern slopes of the TMVB were grouped, respectively, that is, (MAF-MIR-XICO-COA-JIL-NAR-TEQ) (RCHO-PAL-MIN-ATZ-LAN-YEC). Meanwhile, the highest fixation index values ($F_{CT} \sim 1.0$) were achieved at $K = 7$. Figure 4 displays the geographical distribution of cytotypes (*nITS:trnL-trnF*) along the populations, with a unique combination of each species.

3.2 | Source of morphological evidence

The position of each group in the morphospace is depicted in Figure 5. The plot was constructed using LD1 and LD2, containing 53.9% and 30.9% of the explained variation, respectively. The variables that mostly describe the variation between the groups are the width of the middle leaflet, the rachis length, and leaflet pairs on axis

TABLE 3 Fixation index (F_{CT}) for different levels of K in the SAMOVA.

Loci	Genetic clusters	*K*	F_{CT}	*p*
nrITS + *trnL-trnF*	(MAF, MIR, XICO, COA, JIL) (NAR, TEQ, RCHO, MIN, ATZ, LAN, PAL, YEC)	2	0.618	<0.001
	(MAF, MIR, XICO, COA, JIL) (NAR, TEQ) (RCHO, MIN, ATZ, LAN, PAL, YEC)	3	0.788	<0.001
	(MAF, COA, JIL) (MIR, XICO) (NAR, TEQ) (RCHO, MIN, ATZ, LAN, PAL, YEC)	4	0.842	<0.001
	(MAF, COA, JIL) (MIR, XICO) (NAR, TEQ) (RCHO, MIN, ATZ, PAL) (LAN, YEC)	5	0.895	<0.001
	(MAF) (COA, JIL) (MIR, XICO) (NAR, TEQ) (RCHO, MIN, ATZ, PAL) (LAN, YEC)	6	0.954	<0.001
	(MAF) (COA, JIL) (MIR, XICO) (NAR, TEQ) (RCHO, PAL) (MIN, ATZ) (LAN, YEC)	7	1.000	<0.001

Note: See Figure 1 for populations.

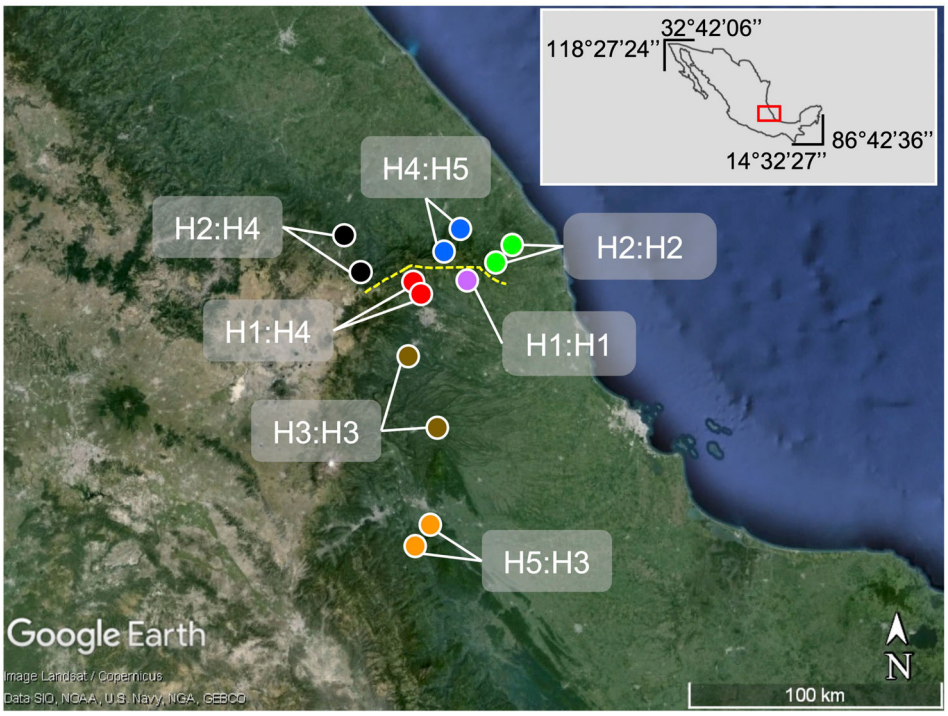


FIGURE 4 Geographical distribution of *trnL-trnF* and *nrITS* cytotypes within the *Ceratozamia mexicana* species complex. The population colors correspond to the monophyletic groups recovered by phylogenetic analysis, and the label codes are the cytotypes of the *nrITS:trnL-trnF* markers. Map data: Google Earth, Landsat/Copernicus.

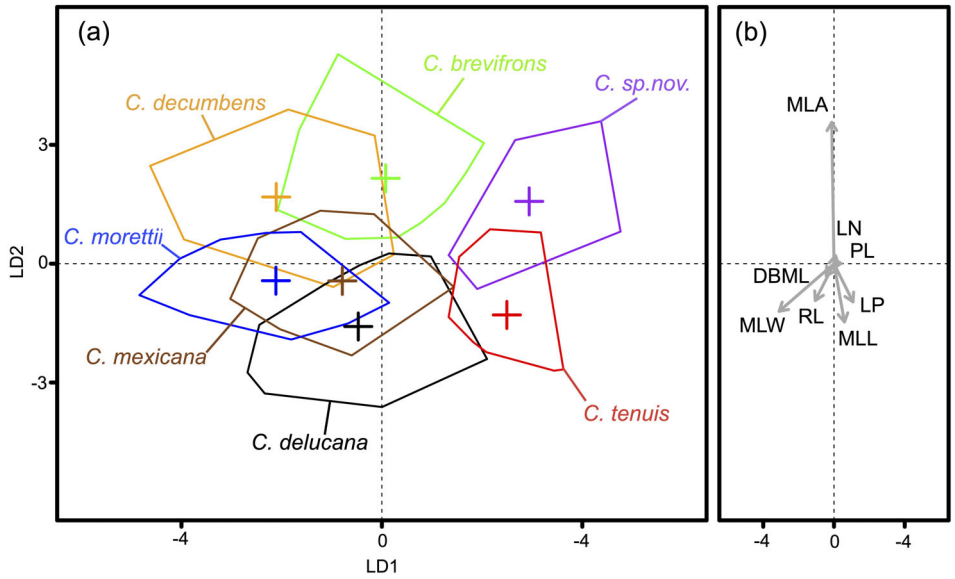


FIGURE 5 Morphospace of the *Ceratozamia mexicana* species complex. The axes correspond to the first and second linear coefficients of the discriminant analysis. In panel (a) the polygons represent the area occupied by individuals from each monophyletic group (colors corresponding to those indicated in the phylogenetic tree). In panel (b) the length and direction of the arrows represent the influence of the morphometric variables on both axes (see Figure 2 for the eight morphometric variables). Y-axis tick marks in this panel have been omitted, as they are identical to those of panel (a). The codes correspond as follows: leaflet pairs (LP), petiole length (PL), rachis length (RL), middle leaflet width (MLW), middle leaflet length (MLL), the distance between middle leaflets (DBML), and the width of the middle leaflet articulation (MLA).

TABLE 4 Morphological differentiation among monophyletic groups.

Clade	Lineages/clades paired	D_{obs}	*p*
Ceratozamia decumbens	*C. decumbens*—North TMVB	2.107	<0.001
	C. decumbens—South TMVB	4.273	<0.001
	North TMVB—South TMVB	2.422	<0.001
North TMVB	*C. brevifrons*—*C. delucana*	3.780	<0.001
	C. brevifrons—*C. morettii*	3.388	<0.001
	C. delucana—*C. morettii*	2.010	<0.001
South TMVB	*C.* sp. *nov.*—*C. mexicana*	4.234	<0.001
	C. sp. *nov.*—*C. tenuis*	2.900	<0.001
	C. mexicana—*C. tenuis*	3.393	<0.001

Note: D_{obs} corresponds to the distance measured between paired centroids in the multivariate analysis. TMVB corresponds to the Trans-Mexican Volcanic Belt.

1, while the length of the middle leaflet and the width of the articulation of the middle leaflet are on axis 2 (Table S2). This plot (Figure 5) indicates that the morphological groups vary along axis 1 from plants with few wide leaflets on large rachis on the negative side to plants with many narrow leaflets on short rachides on the positive side. On axis 2, the shapes range from plants with long leaflets and narrow bases toward the negative side to plants with short leaflets and wide bases toward the positive side. The observed distances pairwise analysis between groups revealed that the *C. decumbens* (lineage NAR-TEQ) occupies a distinct morphospace compared to the clades from the southern and northern slopes of the TMVB, and in turn, also between these clades. Within the clades of the southern and northern slopes of the TMVB, statistically significant distances were observed among all lineages, indicating that each monophyletic group occupies a distinct morphospace (Table 4).

3.3 | Source of environmental evidence

The environmental space (i.e., climatic niche) occupied by each of the groups is depicted in Figure 6. This was plotted using LD1 and LD2, which account for 74.3% and 0.2% of the variation, respectively. The variables that mainly

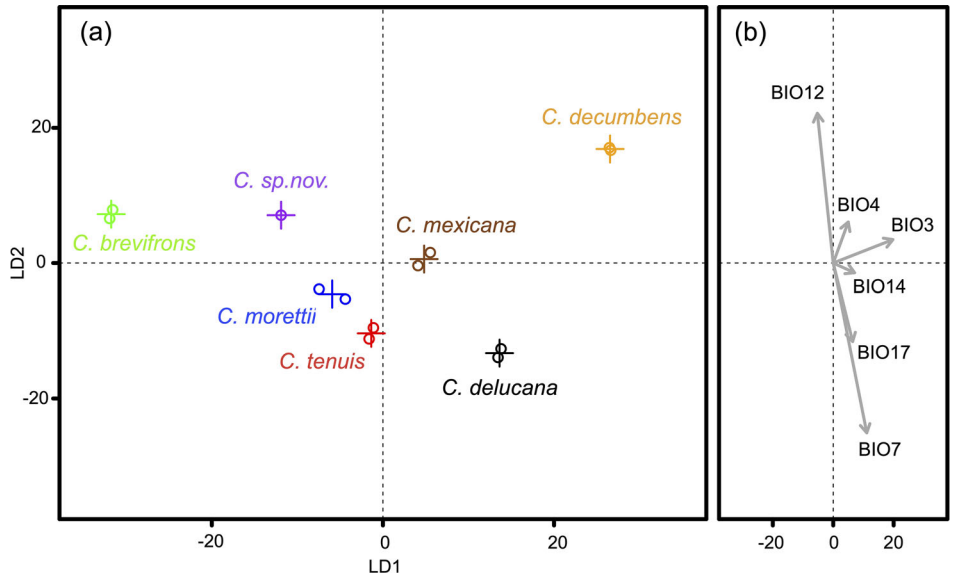


FIGURE 6 Niche space of the *Ceratozamia mexicana* species complex. The axes correspond to the first and second linear coefficients of the discriminant analysis. In panel (a) the cross-shaped dots represent the multivariate centroids of climatic variables (colors corresponding to those indicated in the phylogenetic tree). In panel (b) the length and direction of the arrows represent the influence of the six climatic variables on both axes. *Y*-axis tick marks in this panel have been omitted, as they are identical to those of panel (a). The codes correspond as follows: isothermality (BIO3), temperature seasonality (BIO4), temperature annual range (BIO7), annual precipitation (BIO12), precipitation of driest month (BIO14), and precipitation of driest quarter (BIO17).

TABLE 5 Climate niche differentiation among monophyletic groups.

Clade	Lineages/clades paired	D_{obs}	*p*
Ceratozamia decumbens	*C. decumbens*—North TMVB	40.146	<0.001
	C. decumbens—South TMVB	33.674	<0.001
	North TMVB—South TMVB	7.098	<0.001
North TMVB	*C. brevifrons*—*C. delucana*	49.811	0.001
	C. brevifrons—*C. morettii*	28.401	0.001
	C. delucana—*C. morettii*	21.411	<0.001
South TMVB	*C.* sp. *nov.*—*C. mexicana*	17.936	<0.001
	C. sp. *nov.*—*C. tenuis*	20.372	<0.001
	C. mexicana—*C. tenuis*	12.564	<0.001

Note: D_{obs} corresponds to the distance measured between paired centroids in the multivariate analysis. TMVB corresponds to the Trans-Mexican Volcanic Belt.

describe the variation between the groups in axis 1 are isothermality (Bio3) to the positive side and Annual Precipitation (Bio12) to the negative side (Table S3). Statistically significant differences in the pairwise distances between the centroids of all lineages were observed, indicating that each lineage occupies a distinct niche space (Table 5).

4 | DISCUSSION

4.1 | Comparison of conclusive disciplines

In terms of genetic evidence, it is noteworthy that the highest F_{CT} value of the cytotypes corresponds to the lineages identified through the phylogenetic analysis. This geographic pattern of restricted gene flow may be related to the foraging home range of seed consumers/dispersers (Hall & Walter, 2013) such as small rodents (Pérez-Farrera et al., 2000), corvid birds (e.g., *Psilorhinus morio* Wagler 1829), or marsupials (e.g., *Didelphis virginiana* Kerr 1792; watch the video recorded by camera-traps in Supporting Information 3), and erotylid pollinators of the genus *Pharaxonota* Reitter 1875 (Santiago-Jiménez et al., 2019). As in previous studies (González & Vovides, 2002; Gutiérrez-Ortega et al., 2024; Habib et al., 2023), *C. decumbens* is solely recovered in a long branch that separates it from all other species. Of the species of the northern slope of the TMVB clade (i.e., *C. morettii, C. brevifrons*, and *C. delucana*) most have

posterior probability values greater than 0.95. This is congruent with the conclusion of Habib et al. (2023) that placed *C. brevifrons* and *C. delucana* as sister species and near to *C. morettii* within the latifolia subclade. Their results were based on transcriptomic data comprising 3954 single-copy nuclear genes. As for the species in the southern slope of the TMVB clade, all have support values greater than 0.95 pp. The ancestors of the *C. mexicana* species complex seem to have arisen during and after periods of great volcanic activity (Ferrari et al., 2012), particularly toward the end of the Pliocene with the uplift of the eastern end of the TMVB (i.e., the Chiconquiaco mountain range), which separated the ancestors of the species at the north slope of the TMVB and the Northern Gulf of Mexico from the species at the south slope of the TMVB. The vicariant origin of the lineages within the northern and southern TMVB clades may be related to the extinction of large mammals during the Pleistocene. This is because these species display characteristics of megafaunal dispersal syndrome, like discontinuous distribution of dense populations with large fruits and seeds (Hall & Walter, 2013). However, we are aware that the timing of speciation events estimated in this study may be consistent with some (Nagalingum et al., 2011; Palacios-Chávez & Rzedowski, 1993) or differ from others due to the markers employed, calibration points, and substitution rate assumptions (Gutiérrez-Ortega et al., 2024; Habib et al., 2023).

Regarding the morphological evidence, recent studies involving the same populations within the genus *Ceratozamia* have yielded mixed results, with some not finding differential attributes (Martínez-Domínguez et al., 2017), while others have identified such distinctions (Vovides et al., 2020). In our case, there is a clear observation of phenotypic overlap in a general sense. However, our multivariate tests show a statistically significant differentiation when considering their phylogenetic relationships. Neglecting the phylogenetic component could potentially obscure the presence of two distinct lineages with similar morphology within what might be perceived as a single species (Barrett et al., 2021; Bickford et al., 2007). This phenomenon has been demonstrated previously in the *Ceratozamia* genus (Medina-Villarreal et al., 2019; Vovides et al., 2020), underscoring the importance of accounting for evolutionary convergence.

Regarding the environmental evidence, conflicting conclusions have been reported for the same species studied (McCormack et al., 2009; Rice et al., 2003) in determining whether niche divergence accompanies speciation (Rissler & Apodaca, 2007). However, the multivariate approach used here, following Warren et al. (2008), provides more detailed information about niche divergence, as it aligns better with the Hutchinsonian concept of a niche as a multidimensional hypervolume, where certain axes remain conserved while others diverge. Taking this into account, our results are consistent with the identification of niche divergence at all levels compared (i.e., clades and lineages) by finding that the position they occupy within the hypervolume is sufficiently distant to determine that they are different. Nevertheless, we are aware of the paucity of climatic data to compare potential niches, so the differences found here are only for the realized niche of the species analyzed. However, Gómez-Díaz et al. (2024) when generating the potential distribution model of some cycad species (i.e., *C. brevifrons*, *C. morettii*, and *C. tenuis*), found that each one occupies a unique ecoregion and climatic niche, despite living less than ~10 km from each other and having an exceptionally small distribution area in central Veracruz. This may be an explanation for the detection of significant differences between species in our analysis, even with only a limited amount of data. Furthermore, something important to highlight in the study by Gómez-Díaz et al. (2024) is the fact that the predicted potential area of *C. tenuis* does not include the point of presence of the new species (Mafafas population), considering that it was included in the training of geographic distribution modeling. This allows us to support that the climatic

TABLE 6 Contrasts in recognized specimen groups (populations) across disciplines.

Specimen groups	Genetic evidence	Morphological evidence	Environmental evidence	Taxon
PAL-RCHO	✓	✓	✓	*Ceratozamia brevifrons*
NAR-TEQ	✓	✓	✓	*C. decumbens*
MIN-ATZ	✓	✓	✓	*C. delucana*
LAN-YEC	✓	✓	✓	*C. morettii*
MIR-XICO	✓	✓	✓	*C. mexicana*
COA-JIL	✓	✓	✓	*C. tenuis*
MAF	✓	✓	✓	*C.* sp. *nov.*

Note: See Figure 1 for populations.

niche of *C.* sp. *nov.* is different from its sister species *C. tenuis*.

In accordance with the IT framework proposed by Schlick-Steiner et al. (2010), our study has achieved "whole congruence" among the three disciplines (Table 6). In other words, the monophyletic groups identified by genetic evidence were supported by both the multivariate morphometric analysis and the climatic niche analysis. Based on the foregoing and under the unified species concept adopted here (De Queiroz, 2007), our results provide distinguishable attributes for the delimitation of evolutionarily independent lineages (Padial et al., 2010).

4.2 | Integrated characterization

The seven recognized lineages (Pante et al., 2015) differ significantly from the morphological species hypotheses proposed by Medina-Villarreal and González-Astorga (2016). These authors identified two lineages based on the leaflet shape, one consisting of three populations with long and narrow leaflets, and another group of 10 populations with short and wide leaflets. Although none of their groups correspond to our terminal monophyletic groups, one of these coincides with the clade of southern slope TMVB species, namely Mafafas, Coacoatzintla, and Jilotepec populations, which have long and narrow leaflets. This serves as an example of the complexity involved in identifying groups based on a single character (e.g., Martínez-Domínguez et al., 2017), especially when recent research has demonstrated convergent evolution in the morphology of the *Ceratozamia* genus (Medina-Villarreal et al., 2019).

In a monograph on the genus *Ceratozamia*, Martínez-Domínguez et al. (2022) included 36 species; however, the current consensus, as accepted by the World List of Cycadas (Calonje et al., 2025), recognizes 45 taxa. Nevertheless, most of the groups identified in this study are associated with some of the accepted (i.e., characterized) species. With this in mind, and based on the species described in Martínez-Domínguez et al. (2022) plus the non-shared cytotypes (*nrITS:trnL-trnF*), the species would be: the Palma Sola and Rancho Nuevo populations belong to the taxon *Ceratozamia brevifrons* Miq. (H2:H2); the Naranjal and Tequila populations to *Ceratozamia decumbens* Vovides, Avendaño, Pérez-Farr. and Gonz.-Astorga (H5:H3); the Minas and Atzalan populations to *Ceratozamia delucana* Vázq. Torres, A. Moretti & Carv.-Hern. (H2:H4); the Landero and Coss, and Yecuatla populations to *Ceratozamia morettii* Vázq. Torres & Vovides (H4:H5); and the Mirador and Xico populations to *C. mexicana* Brongn. (H3:H3). Concerning the Coacoatzintla, Jilotepec, and Mafafas populations, the monograph includes them within *Ceratozamia tenuis* (Dyer) D. W. Stev. & Vovides (H1:H4), but our evidence indicates the presence of two evolutionarily independent lineages within this taxon. The phylogenetic analysis positions the Mafafas population as a sister lineage to the populations of Coacoatzintla and Jilotepec, with a support value of 1.0 pp., and with the presence of a non-shared cytotype (H1:H1) that is fixed in all individuals of the population.

4.3 | Nomenclatural consequences

The delimitation hypotheses of the recognized lineages presented here agree with previously described nominal species, except for *Ceratozamia tenuis* (Dyer) D. W. Stev. & Vovides. Therefore, we consider that the Coacoatzintla and Jilotepec populations, which are geographically closer to each other than to the Mafafas population, should retain the name *Ceratozamia tenuis* (Dyer) D. W. Stev. & Vovides. Consequently, the Mafafas population should be considered a newly discovered species (description is in progress) in a cryptic speciation scenario (Bickford et al., 2007; DeSalle et al., 2005).

Finally, this study has facilitated the delimitation, recognition, and discovery of genetic lineages (Yeates et al., 2011) within the *C. mexicana* Brongn. complex. The secondary properties (sensu De Queiroz, 2007) of the recognized species here included: phylogenetic, based on reciprocal monophyly and non-shared cytotypes; morphological via phenotypic differentiation; and ecological via realized-niche divergence. Furthermore, this genealogical characterization will serve as the basis for future efforts aimed at unraveling the genetic identity of the holotype (Stevenson & Sabato, 1986) of *C. mexicana* Brongn., which in turn represents the type species for the entire genus.

ACKNOWLEDGMENTS

The present study was funded by the INECOL A.C. project 2003/10776 awarded to JG-A, with additional support provided by the CONACyT doctoral scholarship (282059) granted to AM-V. The authors extend their gratitude to Dra. Janet Nolasco-Soto for her technical assistance in the laboratory work. Finally, the first author dedicates this work to the Medina-Villarreal and Medina-Guerrero families.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ORCID

Anwar Medina-Villarreal https://orcid.org/0000-0002-2982-7648

Jorge González-Astorga https://orcid.org/0000-0003-4755-8493

Alejandro Espinosa de los Monteros https://orcid.org/0000-0002-7742-5977

REFERENCES

Alström, P., Rasmussen, P. C., Olsson, U., & Sundberg, P. (2008). Species delimitation based on multiple criteria: The spotted bush warbler *Bradypterus thoracicus* complex (Aves: Megaluridae). *Zoological Journal of the Linnean Society*, *154*(2), 291–307. https://doi.org/10.1111/j.1096-3642.2008.00418.x

Arroyo-Santos, A., Olson, M. E., & Vergara-Silva, F. (2014). The phylogeography debate and the epistemology of model-based evolutionary biology. *Biology & Philosophy*, *29*(6), 833–850. https://doi.org/10.1007/s10539-013-9391-1

Barrett, C. F., Lambert, J., Santee, M. V., Sinn, B. T., Skibicki, S. V., Stephens, H. M., & Thixton, H. (2021). Genetic, morphological, and niche variation in the widely hybridizing *Rhus integrifolia* – *Rhus ovata* species complex. *Plant Species Biology*, *36*(1), 17–35. https://doi.org/10.1111/1442-1984.12293

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., Ingram, K. K., & Das, I. (2007). Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution*, *22*(3), 148–155. https://doi.org/10.1016/j.tree.2006.11.004

Brandley, M. C., Schmitz, A., & Reeder, T. W. (2005). Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. *Systematic Biology*, *54*(3), 373–390. https://doi.org/10.1080/10635150590946808

Burleigh, J. G., Barbazuk, W. B., Davis, J. M., Morse, A. M., & Soltis, P. S. (2012). Exploring diversification and genome size evolution in extant gymnosperms through phylogenetic synthesis. *Journal of Botany*, *2012*, 1–6. https://doi.org/10.1155/2012/292857

Calonje, M., Stevenson, D. W., & Stanberg, L. (2025). The World List of Cycads, online edition. http://www.cycadlist.org

Chemnick, J., Gregory, T., Haynes, J., Pérez-Farrera, M. A., & Whitelock, L. (2008). Key to species of Ceratozamia. 8th International Conference on Cycad Biology [Poster]. http://www.cycad.org/documents/Key-to-the-Species-of-Ceratozamia.pdf

Chung, C. (2003). On the origin of the typological/population distinction in Ernst Mayr's changing views of species, 1942–1959. *Studies in History and Philosophy of Science Part C: Studies in History and Philosophy of Biological and Biomedical Sciences*, *34*(2), 277–296. https://doi.org/10.1016/S1369-8486(03)00026-8

Conix, S. (2018). Integrative taxonomy and the operationalization of evolutionary independence. *European Journal for Philosophy of Science*, *8*(3), 587–603. https://doi.org/10.1007/s13194-018-0202-z

Dayrat, B. (2005). Towards integrative taxonomy. *Biological Journal of the Linnean Society*, *85*(3), 407–415. https://doi.org/10.1111/j.1095-8312.2005.00503.x

De Queiroz, K. (2007). Species concepts and species delimitation. *Systematic Biology*, *56*(6), 879–886. https://doi.org/10.1080/10635150701701083

DeSalle, R., Egan, M. G., & Siddall, M. (2005). The unholy trinity: Taxonomy, species delimitation and DNA barcoding. *Philosophical Transactions of the Royal Society B*, *360*(1462), 1905–1916. https://doi.org/10.1098/rstb.2005.1722

Dupanloup, I., Schneider, S., & Excoffier, L. (2002). A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology*, *11*(12), 2571–2581. https://doi.org/10.1046/j.1365-294X.2002.01650.x

Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics*, *131*(2), 479–491. https://doi.org/10.1093/genetics/131.2.479

Ferrari, L., Orozco-Esquivel, T., Manea, V., & Manea, M. (2012). The dynamic history of the Trans-Mexican Volcanic Belt and the Mexico subduction zone. *Tectonophysics*, *522–523*, 122–149. https://doi.org/10.1016/j.tecto.2011.09.018

Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, *37*(12), 4302–4315. https://doi.org/10.1002/joc.5086

Fisher, B. L., & Smith, M. A. (2008). A revision of Malagasy species of *Anochetus* Mayr and *Odontomachus* Latreille (Hymenoptera: Formicidae). *PLoS One*, *3*(5), e1787. https://doi.org/10.1371/journal.pone.0001787

Goldstein, P. Z., & DeSalle, R. (2011). Integrating DNA barcode data and taxonomic practice: Determination, discovery, and description. *BioEssays*, *33*(2), 135–147. https://doi.org/10.1002/bies.201000036

Gómez-Díaz, J. A., Carvajal-Hernández, C. I., & Dáttilo, W. (2024). Past, present and future in the geographical distribution of Mexican Tepezmaite cycads: Genus *Ceratozamia*. *PLoS One*, *19*(2), e0284007. https://doi.org/10.1371/journal.pone.0284007

González, D., & Vovides, A. P. (2002). Low intralineage divergence in Ceratozamia (Zamiaceae) detected with nuclear ribosomal DNA ITS and chloroplast DNA trnL-F non-coding region. *Systematic Botany*, *27*(4), 654–661. https://doi.org/10.1043/0363-6445-27.4.654

Gutiérrez-Ortega, J. S., Pérez-Farrera, M. A., Matsuo, A., Sato, M. P., Suyama, Y., Calonje, M., Vovides, A. P., Kajita, T., & Watano, Y. (2024). The phylogenetic reconstruction of the neotropical cycad genus *Ceratozamia* (Zamiaceae) reveals disparate patterns of niche evolution. *Molecular Phylogenetics and Evolution*, *190*, 107960. https://doi.org/10.1016/j.ympev.2023.107960

Habib, S., Gong, Y., Dong, S., Lindstrom, A., Stevenson, D. W., Wu, H., & Zhang, S. (2023). Phylotranscriptomics shed light on intrageneric relationships and historical biogeography of *Ceratozamia* (Cycadales). *Plants*, *12*(3), 478. https://doi.org/10.3390/plants12030478

Hall, J. A., & Walter, G. H. (2013). Seed dispersal of the Australian cycad *Macrozamia miquelii* (Zamiaceae): Are cycads megafauna-dispersed "grove forming" plants? *American Journal of Botany*, *100*(6), 1127–1136. https://doi.org/10.3732/ajb.1200115

Kass, R. E., & Raftery, A. E. (1995). Bayes factors. *Journal of the American Statistical Association*, *90*(430), 773–795. https://doi.org/10.1080/01621459.1995.10476572

Mace, G. M. (2004). The role of taxonomy in species conservation. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *359*(1444), 711–719. https://doi.org/10.1098/rstb.2003.1454

Malhotra, A., & Thorpe, R. S. (2004). Maximizing information in systematic revisions: A combined molecular and morphological analysis of a cryptic green pitviper complex (*Trimeresurus stejnegeri*). *Biological Journal of the Linnean Society*, *82*(2), 219–235. https://doi.org/10.1111/j.1095-8312.2004.00354.x

Martínez-Domínguez, L., Nicolalde-Morejón, F., Vergara-Silva, F., & Stevenson, D. W. (2018). Taxonomic review of *Ceratozamia* (Zamiaceae) in the Sierra Madre Oriental, Mexico. *PhytoKeys*, *100*, 91–124. https://doi.org/10.3897/phytokeys.100.23152

Martínez-Domínguez, L., Nicolalde-Morejón, F., Vergara-Silva, F., & Stevenson, D. W. (2022). Monograph of *Ceratozamia* (Zamiaceae, Cycadales): An endangered genus. *PhytoKeys*, *208*, 1–102. https://doi.org/10.3897/phytokeys.208.80382

Martínez-Domínguez, L., Nicolalde-Morejón, F., Vergara-Silva, F., Stevenson, D. W., & Del Callejo, E. (2017). Cryptic diversity, sympatry, and other integrative taxonomy scenarios in the Mexican *Ceratozamia miqueliana* complex (Zamiaceae). *Organisms Diversity & Evolution*, *17*(4), 727–752. https://doi.org/10.1007/s13127-017-0341-7

McCormack, J. E., Zellmer, A. J., & Knowles, L. L. (2009). Does niche divergence accompany allopatric divergence in *Aphelocoma* jays as predicted under ecological speciation?: Insights from tests with niche models. *Evolution*, *64*(5), 1231–1244. https://doi.org/10.1111/j.1558-5646.2009.00900.x

Medina-Villarreal, A., & González-Astorga, J. (2016). Morphometric and geographical variation in the *Ceratozamia mexicana* Brongn. (Zamiaceae) complex: Evolutionary and taxonomic implications. *Biological Journal of the Linnean Society*, *119*(1), 213–233. https://doi.org/10.1111/bij.12806

Medina-Villarreal, A., González-Astorga, J., & de Espinosa los Monteros, A. (2019). Evolution of *Ceratozamia* cycads: A proximate-ultimate approach. *Molecular Phylogenetics and Evolution*, *139*, 106530. https://doi.org/10.1016/j.ympev.2019.106530

Moretti, A., Sabato, S., & Vázquez-Torres, M. (1980). The distribution of *Ceratozamia* Brongn. (Zamiaceae). *Delpinoa*, *21*, 13–21. https://www.ortobotaniconapoli.it/images/Delpinoa/Delpinoa_n_s_21-22_1979-80_[1981]/Delpinoa_21-22_1979-80_[1981]_013-021.pdf

Mutanen, M., & Pretorius, E. (2007). Subjective visual evaluation vs. traditional and geometric morphometrics in species delimitation: A comparison of moth genitalia. *Systematic Entomology*, *32*(2), 371–386. https://doi.org/10.1111/j.1365-3113.2006.00372.x

Nagalingum, N. S., Marshall, C. R., Quental, T. B., Rai, H. S., Little, D. P., & Mathews, S. (2011). Recent synchronous radiation of a living fossil. *Science*, *334*(6057), 796–799. https://doi.org/10.1126/science.1209926

Nylander, J. A. A., Ronquist, F., Huelsenbeck, J. P., & Nieves-Aldrey, J. L. (2004). Bayesian phylogenetic analysis of combined data. *Systematic Biology*, *53*(1), 47–67. https://doi.org/10.1080/10635150490264699

Padial, J. M., Miralles, A., De la Riva, I., & Vences, M. (2010). The integrative future of taxonomy. *Frontiers in Zoology*, *7*(1), 16. https://doi.org/10.1186/1742-9994-7-16

Palacios-Chávez, R., & Rzedowski, J. (1993). Estudio palinológico de las floras fósiles del Mioceno Inferior y principios del Mioceno Medio de la región de Pichucalco, Chiapas, México. *Acta Botánica Mexicana*, *24*, 1–96. https://doi.org/10.21829/abm24.1993.677

Pante, E., Puillandre, N., Viricel, A., Arnaud-Haond, S., Aurelle, D., Castelin, M., Chenuil, A., Destombe, C., Forcioli, D., Valero, M., Viard, F., & Samadi, S. (2015). Species are hypotheses: Avoid connectivity assessments based on pillars of sand. *Molecular Ecology*, *24*(3), 525–544. https://doi.org/10.1111/mec.13048

Paz, O. (1972). *El Arco y la Lira* (3.a ed.). Fondo de Cultura Económica.

Pérez-Farrera, M. A., Quintana-Ascencio, P. F., Izaba, B. S., & Vovides, A. P. (2000). Population dynamics of *Ceratozamia matudai* Lundell (Zamiaceae) in El Triunfo Biosphere Reserve, Chiapas, Mexico. *Journal of the Torrey Botanical Society*, *127*(4), 291. https://doi.org/10.2307/3088647

R Core Team. (2025). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.r-project.org

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using tracer 1.7. *Systematic Biology*, *67*(5), 901–904. https://doi.org/10.1093/sysbio/syy032

Rice, N. H., Martínez-Meyer, E., & Peterson, A. T. (2003). Ecological niche differentiation in the *Aphelocoma* jays: A phylogenetic perspective. *Biological Journal of the Linnean Society*, *80*(3), 369–383. https://doi.org/10.1046/j.1095-8312.2003.00242.x

Rissler, L. J., & Apodaca, J. J. (2007). Adding more ecology into species delimitation: Ecological niche models and phylogeography help define cryptic species in the black salamander (*Aneides flavipunctatus*). *Systematic Biology*, *56*(6), 924–942. https://doi.org/10.1080/10635150701703063

Rzedowsky, J., Calderón, G., & Butanda, A. (2009). *Los principales colectores de plantas activos en México entre los años 1700 y 1930*. Instituto de Ecología, A.C. (INECOL-Bajío) y Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO). https://bioteca.biodiversidad.gob.mx/janium/Documentos/6333.pdf

Santiago-Jiménez, Q. J., Martínez-Domínguez, L., & Nicolalde-Morejón, F. (2019). Two new Mexican species of Pharaxonotha Reitter, 1875 (Coleoptera: Erotylidae) from *Ceratozamia tenuis* (Cycadales: Zamiaceae). *Dugesiana*, *26*(1), 15–25. https://doi.org/10.32870/dugesiana.v26i1.7054

Schlick-Steiner, B. C., Steiner, F. M., Moder, K., Seifert, B., Sanetra, M., Dyreson, E., Stauffer, C., & Christian, E. (2006). A multidisciplinary approach reveals cryptic diversity in Western Palearctic *Tetramorium* ants (Hymenoptera: Formicidae). *Molecular Phylogenetics and Evolution*, *40*(1), 259–273. https://doi.org/10.1016/j.ympev.2006.03.005

Schlick-Steiner, B. C., Steiner, F. M., Seifert, B., Stauffer, C., Christian, E., & Crozier, R. H. (2010). Integrative taxonomy: A multisource approach to exploring biodiversity. *Annual Review of Entomology*, *55*(1), 421–438. https://doi.org/10.1146/annurev-ento-112408-085432

Sites, J. W., & Marshall, J. C. (2004). Operational criteria for delimiting species. *Annual Review of Ecology, Evolution, and*

Systematics, *35*(1), 199–227. https://doi.org/10.1146/annurev.ecolsys.35.112202.130128

Stevenson, D. W., & Sabato, S. (1986). Typification of names in *Ceratozamia* Brongn., *Dion* Lindl., and *Microcycas* A. DC. (Zamiaceae). *Taxon*, *35*(3), 578. https://doi.org/10.2307/1221921

Suchard, M. A., Lemey, P., Baele, G., Ayres, D. L., Drummond, A. J., & Rambaut, A. (2018). Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evolution*, *4*(1), vey016. https://doi.org/10.1093/ve/vey016

Vázquez-Torres, M., & Vovides, A. P. (1998). A new species of *Ceratozamia* (Zamiaceae) from Veracruz, Mexico. *Novon*, *8*(1), 87–90. https://doi.org/10.2307/3391902

Vázquez-Torres, M., Moretti, A., & Carvajal-Hernández, C. I. (2013). A new species of Ceratozamia (Zamiaceae) from Veracruz, Mexico. *Delpinoa*, *50-51*, 129–133. https://doi.org/10.13140/RG.2.1.2036.1447

Venables, W. N., & Ripley, B. D. (2002). *Modern applied statistics with S* (Fourth ed.). Springer. https://www.stats.ox.ac.uk/pub/MASS4/

Vieites, D. R., Wollenberg, K. C., Andreone, F., Kohler, J., Glaw, F., & Vences, M. (2009). Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(20), 8267–8272. https://doi.org/10.1073/pnas.0810821106

Vovides, A. P., Avendaño, S., Pérez-Farrera, M. A., & González-Astorga, J. (2008). A new species of *Ceratozamia* (Cycadales, Zamiaceae) from Veracruz, Mexico. *Novon*, *18*(1), 109–114. https://doi.org/10.3417/2006063

Vovides, A. P., Avendaño, S., Pérez-Farrera, M. A., & Stevenson, D. W. M. (2012). What is *Ceratozamia brevifrons* (Zamiaceae)? *Brittonia*, *64*(1), 35–42. https://doi.org/10.1007/s12228-011-9199-8

Vovides, A. P., Pérez-Farrera, M. A., González, D., & Avendaño, S. (2004). Relationships and phytogeography in *Ceratozamia* (Zamiaceae). In T. Walters & R. Osborne (Eds.), *Cycad classification: Concepts and recommendations* (pp. 109–125). CABI.

Vovides, A. P., Pérez-Farrera, M. A., Gutiérrez-Ortega, J. S., Avendaño, S., Medina-Villarreal, A., González-Astorga, J., & Galicia, S. (2020). A revision of the *Ceratozamia miqueliana* (Zamiaceae) species complex based on analyses of leaflet anatomical characters. *Flora*, *270*, 151649. https://doi.org/10.1016/j.flora.2020.151649

Vovides, A. P., Rees, J. D., & Vázquez-Torres, M. (1983). Zamiaceae. *Flora de Veracruz*, *26*, 1–31. https://doi.org/10.21829/fv.462.1983.26

Vovides, A. P., Stevenson, D. W., Pérez-Farrera, M. A., López, S., & Avendaño, S. (2016). What is *Ceratozamia mexicana* (Zamiaceae)? *Botanical Sciences*, *94*(2), 419–429. https://doi.org/10.17129/botsci.449

Warren, D. L., Glor, R. E., & Turelli, M. (2008). Environmental niche equivalency versus conservatism: Quantitative approaches to niche evolution. *Evolution*, *62*(11), 2868–2883. https://doi.org/10.1111/j.1558-5646.2008.00482.x

Wilson, E. O. (2004). Taxonomy as a fundamental discipline. *Philosophical Transactions of the Royal Society B*, *359*(1444), 739. https://doi.org/10.1098/rstb.2003.1440

Wilson, E. O. (2005). Systematics and the future of biology. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(Supplement 1), 6520–6521. https://doi.org/10.1073/pnas.0501936102

Yeates, D. K., Seago, A., Nelson, L., Cameron, S. L., Joseph, L., & Trueman, J. W. H. (2011). Integrative taxonomy, or iterative taxonomy? *Systematic Entomology*, *36*(2), 209–217. https://doi.org/10.1111/j.1365-3113.2010.00558.x

Yoder, A. D., Olson, L. E., Hanley, C., Heckman, K. L., Rasoloarison, R., Russell, A. L., Ranivo, J., Soarimalala, V., Karanth, K. P., Raselimanana, A. P., & Goodman, S. M. (2005). A multidimensional approach for detecting species patterns in Malagasy vertebrates. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(Supplement 1), 6587–6594. https://doi.org/10.1073/pnas.050209210

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Medina-Villarreal, A., González-Astorga, J., & Espinosa de los Monteros, A. (2025). Integrative taxonomy of the *Ceratozamia mexicana* Brongn. (Zamiaceae) species complex from a phylogeographic perspective. *Plant Species Biology*, *40*(6), 530–546. https://doi.org/10.1111/1442-1984.70008