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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.

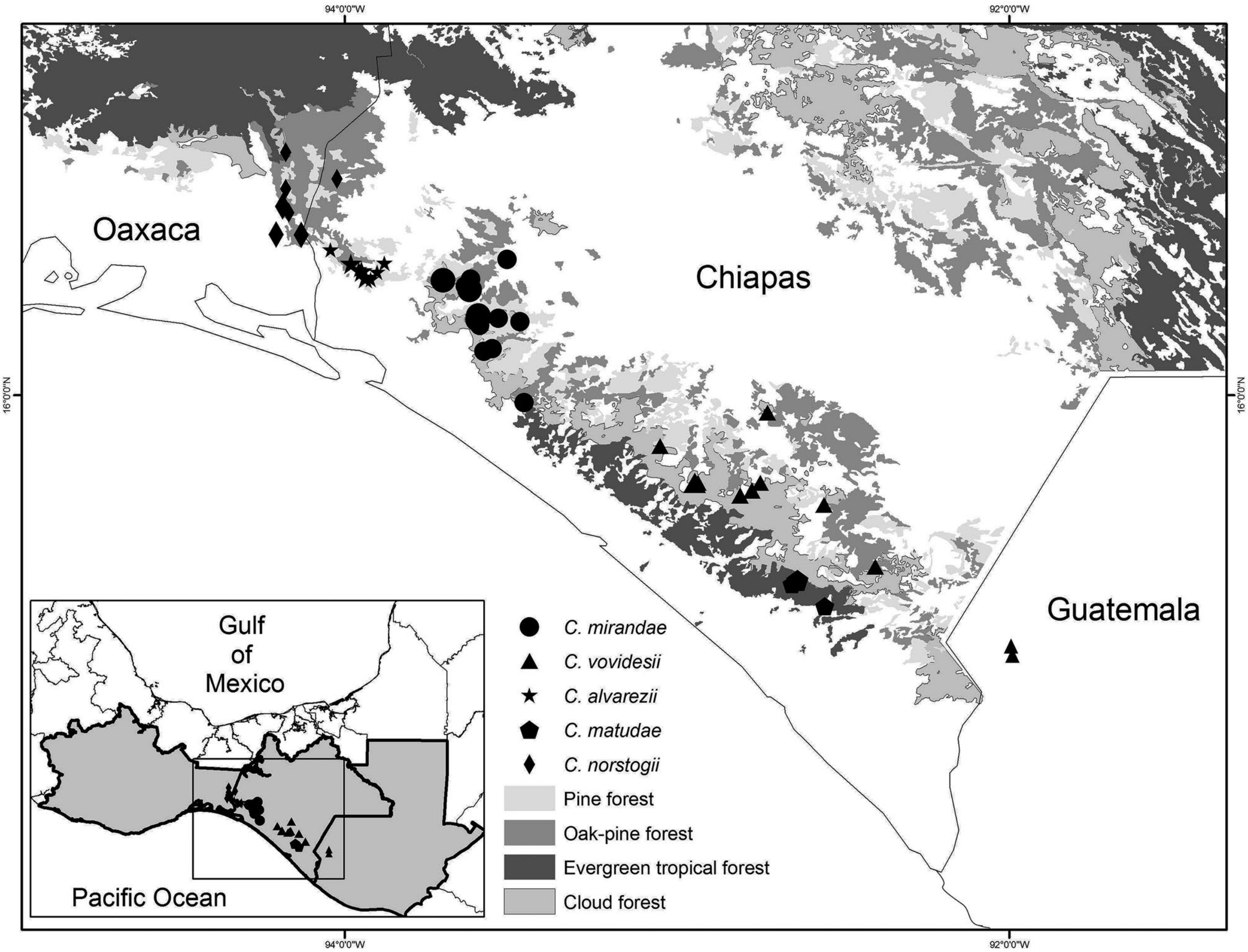


Fig. 1 Distribution of *Ceratozamia* in the Soconusco region. The small symbols indicate species records from herbaria, whereas the large symbols indicate sampled populations

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

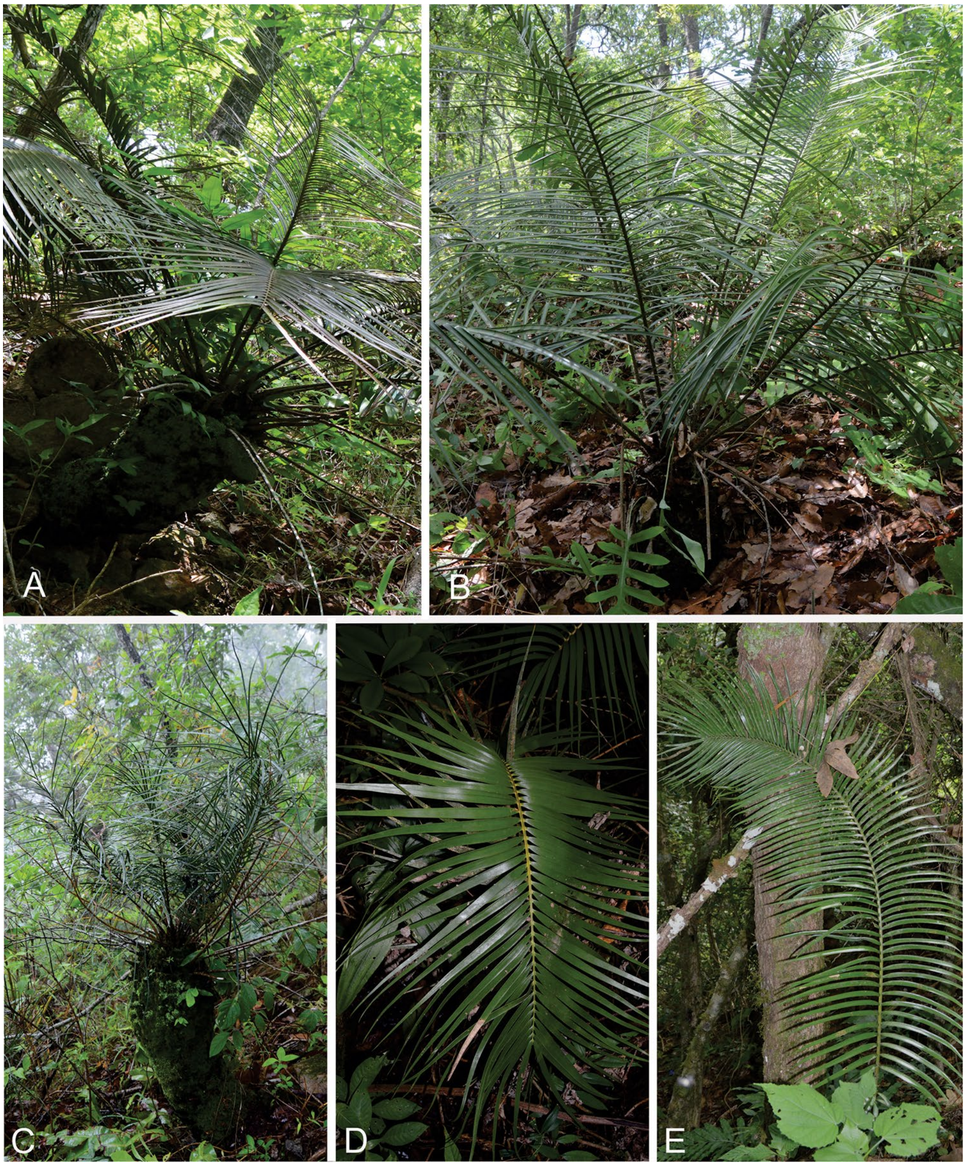


Fig. 2 Vegetative characteristics of *Ceratozamia* species in habitat. **A** *C. alvarezii* (population 1 from Cintalapa), **B** *C. mirandae* (population 4 from Villaflores), **C** *C. norstogii* (population 1 from Cintalapa), **D** *C. matudae* (population 1 from Acacoyagua), **E** *C. vovidesii* (population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

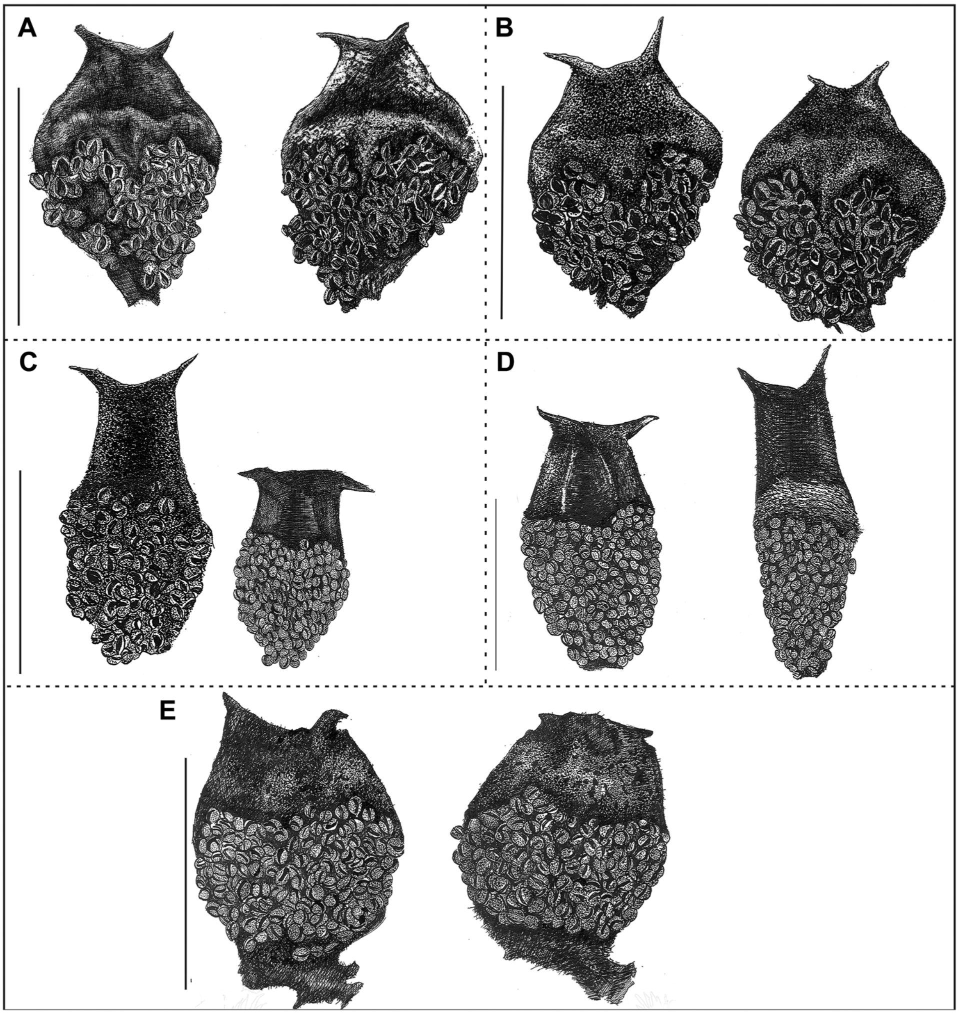


Fig. 3 Line drawing of microsporophylls. **A** *C. vovidesii*, **B** *C. matudae*, **C** *C. alvarezii*, **D** *C. mirandae*, **E** *C. norstogii*. Scale bar = 1 cm

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

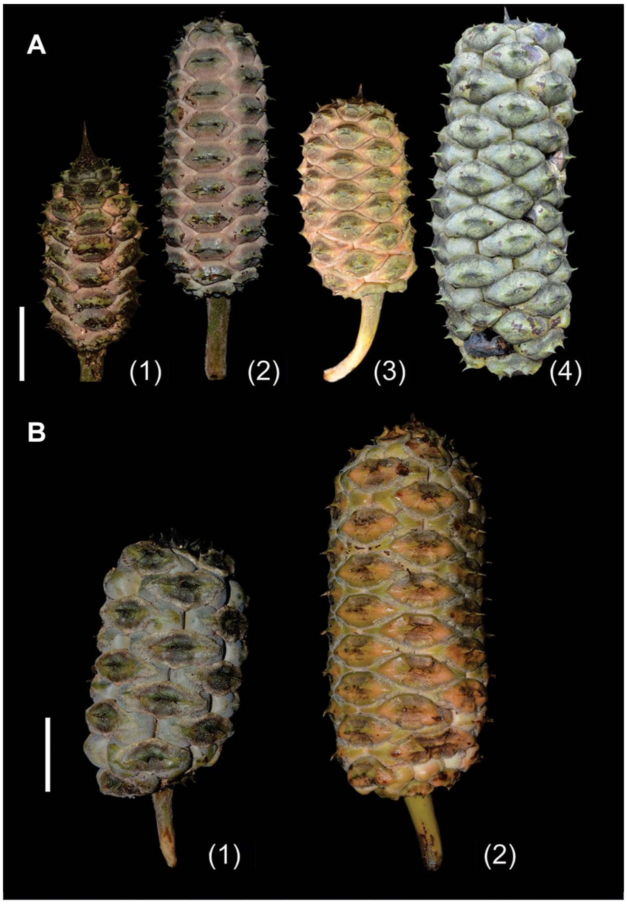


Fig. 4 Phenotypic variation of ovulate strobili. **A** *Ceratozamia mirandae* from Villaflores (1), (2) population 4; (3) population 1; (4) population 3. **B** *C. alvarezii* from Cintalapa (1), (2) populations 1 and 2. Scale bar = 5 cm

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.

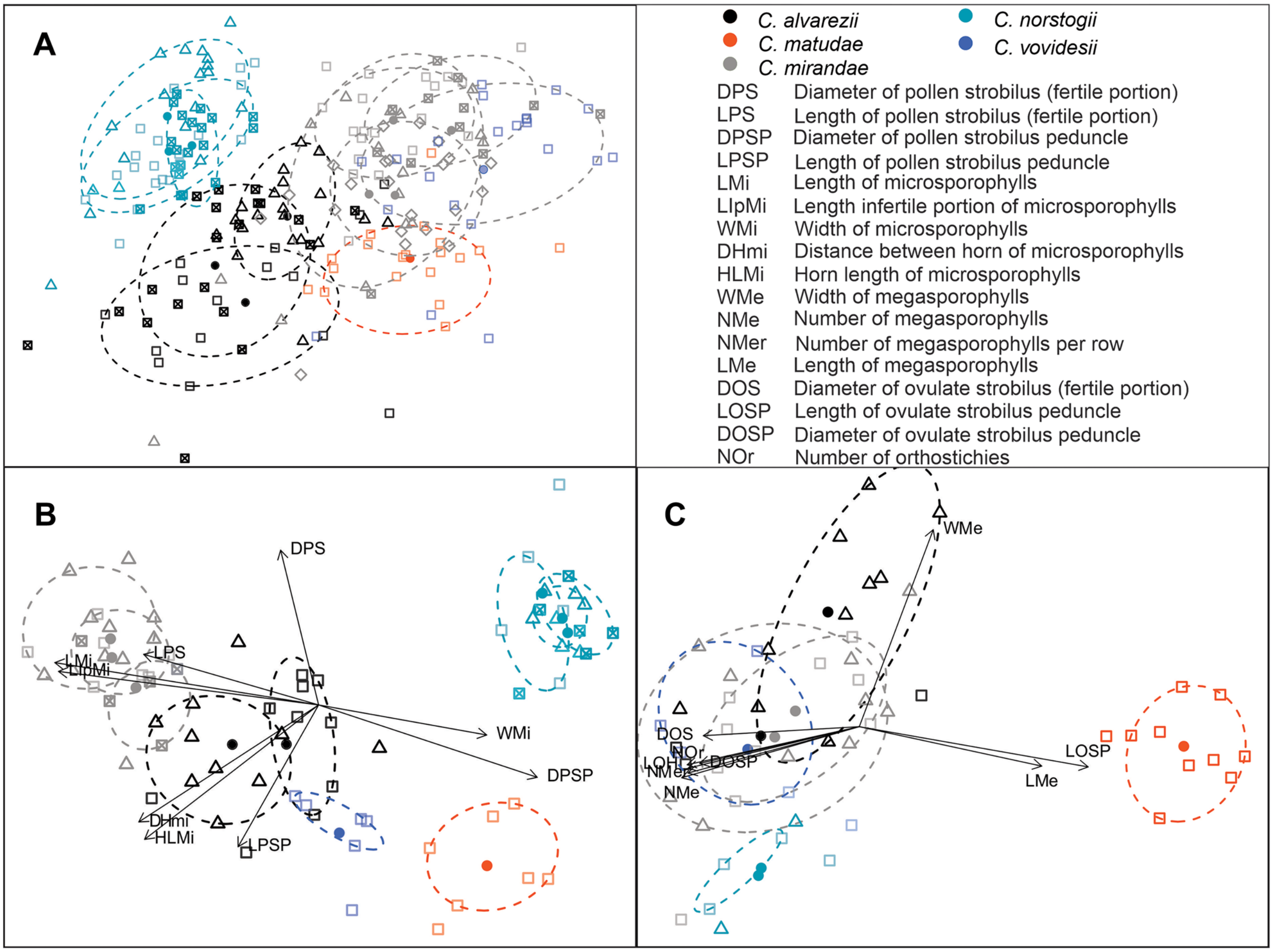


Fig. 5 Phenotypic variation in quantitative characters. **A** Vegetative characters. **B** Reproductive characters for pollen plants. **C** Reproductive characters for ovulate plants

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,

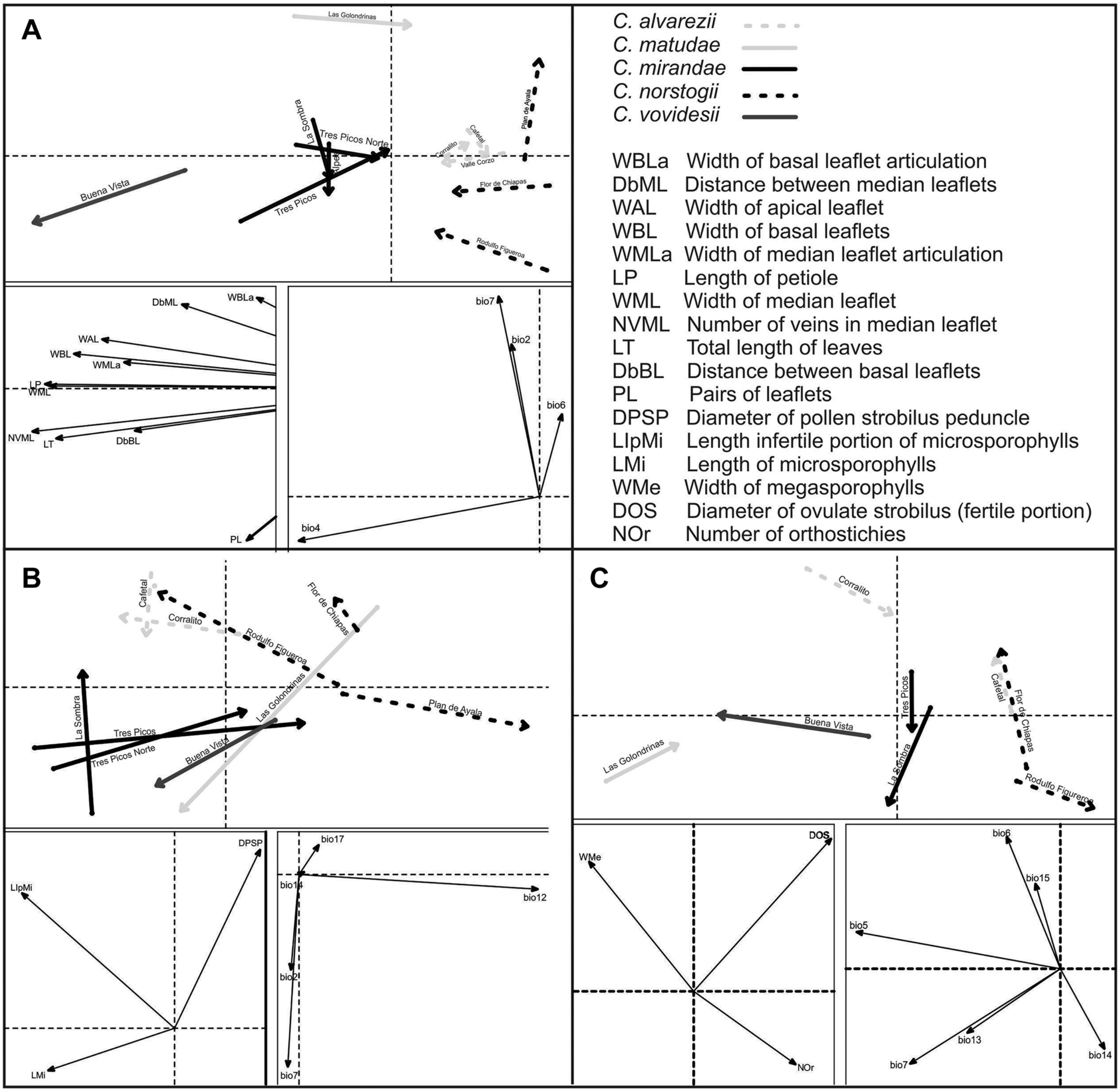


Fig. 6 Co-inertia analysis of five species of *Ceratozamia* in Soconusco. **A** Vegetative characters. **B** Reproductive characters for pollen plants. **C** Reproductive characters for ovulate plants. Circles indicate populations based on morphological characters, and arrowheads represent the environments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

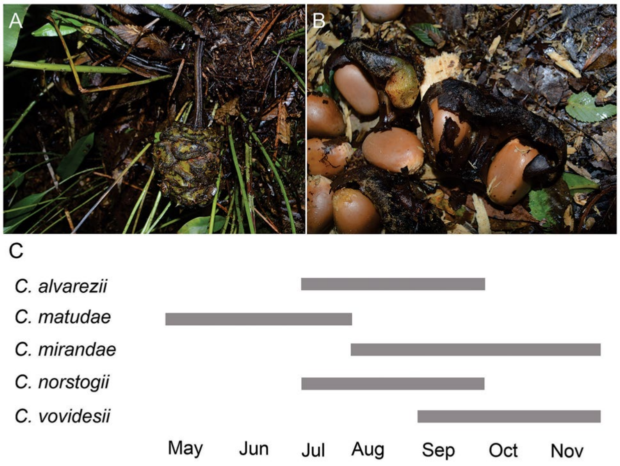


Fig. 7 Disintegration phenophase of *Ceratozamia* in Soconusco. **A** Beginning of disintegration phenophase of *C. matudae*. **B** Seed release of *C. matudae*. **C** Timing of disintegration of *Ceratozamia*

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.

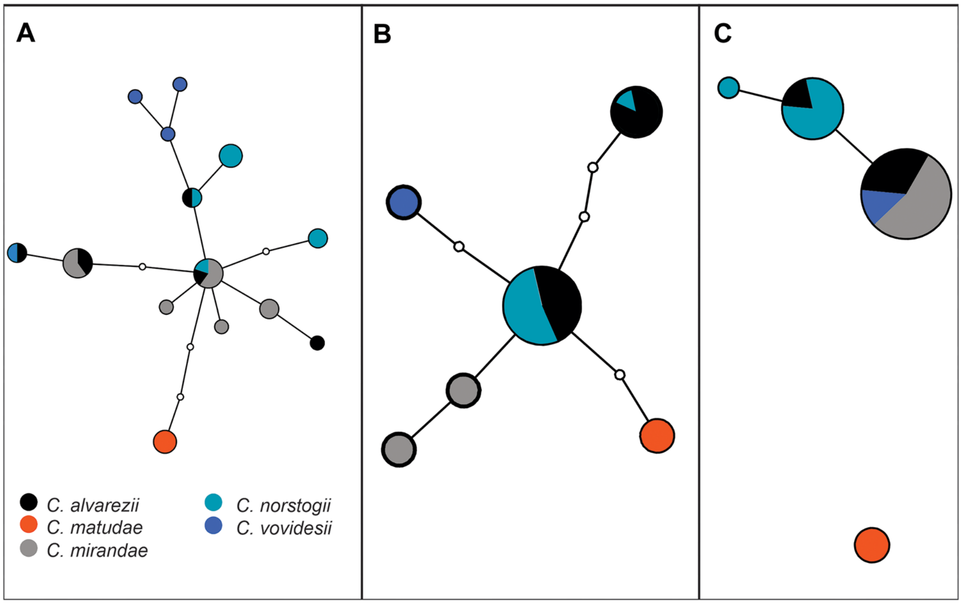
The main characters used to distinguish species in *Ceratozamia* have been quantitative vegetative such as WML and LML (c.f. Pérez-Farrera et al., 2014; Whitelock, 2004). In this group from Soconusco, we found that these characters are highly variable within and among populations (Figs. 2 and 5A). The 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



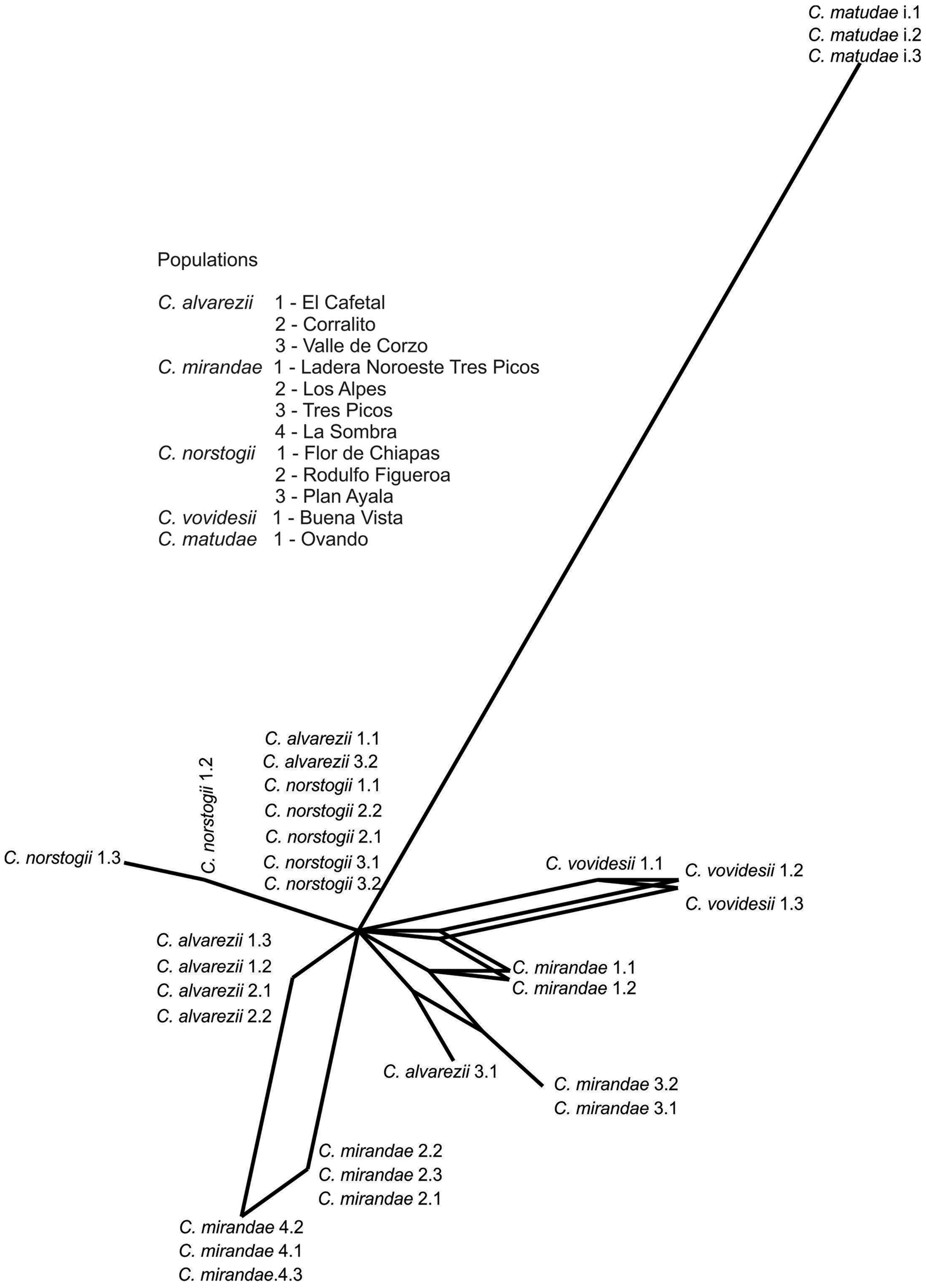


Fig. 9 Split graph for *Ceratozamia* species based on the concatenated matrix

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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