

The evolution of paleo- and neo-endemic species of Cactaceae in the isolated Valley of Tehuacán-Cuicatlán, Mexico

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Abstract

Background and aims – Endemism may be defined according to the time of origin of taxa. Neo-endemics refer to relatively recent species that have not dispersed outside their ancestral areas. In contrast, paleo-endemics refer to species of ancient origins, which are currently geographically restricted but probably were more widespread in the past. Geographically, endemism areas may also be based on the co-occurrence of more than one species. We aimed to qualitatively identify the neo-endemism and paleo-endemism of endemic Cactaceae of the Tehuacán-Cuicatlán Valley, as well as to quantitatively assess paleo- and neo-endemics areas.

Material and methods – Using a dated molecular phylogeny of endemic Cactaceae, we defined paleo- and neo-endemics using an arbitrary boundary of 2.6 million years ago; we also assessed the significance of concentrations of these species using a categorical analysis of paleo- and neo-endemism.

Key results – Our results showed that most endemic Cactaceae in the Tehuacán-Cuicatlán Valley arose throughout the Pleistocene, while categorical analysis indicated localised mixed- and super-endemism (including both paleo- and neo-endemics) areas.

Conclusion – We suggest that paleo- and neo-endemics, as well as localised mixed-endemism areas, may have originated due to a probable high climatic stability in the Tehuacán-Cuicatlán Valley, which in addition to topographically rugged and ecologically complex zones (e.g. ecotones, isolated habitat patches) may have allowed it to function as a refuge throughout Pleistocene climatic changes, mainly promoting the speciation of neo-endemics, as well as the persistence of relatively few paleo-endemics.

Keywords

arid lands, CANAPE, endemism, North America, Pleistocene, speciation

INTRODUCTION

Endemism refers to a spatiotemporal character shown by each taxon or biotic group with a restricted geographic distribution (Anderson 1994). Although this term has

received several different meanings (see Anderson 1994; Peterson and Watson 1998; Noguera-Urbano et al. 2017), two different levels have been usually distinguished: a spatial level, in which an area is categorised as an endemism area based on the occurrence of more than

one species with rather restricted and largely congruent ranges (Haffer 1981; Anderson 1994); and a temporal level, in which endemic taxa can be classified according to their inferred evolutionary age (Stebbins 1942, 1974; Stebbins and Major 1965; Major 1988).

Spatial endemism responds to ecological, evolutionary, geographical, and climatic factors, all of which influence processes promoting the evolution of endemism areas (Stebbins and Major 1965; Anderson 1994; Linder 2008; Harrison and Noss 2017). Endemism areas have been generally attributed to historical processes, such as Pleistocene refugia (Hewitt 1996; Tzedakis et al. 2002) or major geological events (Jetz et al. 2004). Thus, endemism areas may result from either long-term climatic stability, which potentially reduces extinction events, or geographic complexity, which may promote the development of heterogeneous habitats that may enhance or limit biotic dispersal (Fine 2015; Harrison and Noss 2017). Regarding temporal endemism, two categories may be defined according to the time of origin: paleo-endemics and neo-endemics (Stebbins and Major 1965; Prentice 1976; Major 1988). Paleo-endemics refer to ancient and geographically restricted taxa for which current ranges represent remnants of formerly widespread distributions (Malik 2016). In contrast, neo-endemics refer to taxa of relatively recent origin that have not dispersed beyond their ancestral distributional range (Prentice 1976). The boundary between the recognition of paleoendemics and neo-endemics has not been clearly established. However, some authors locate the boundary between the Miocene/Pliocene transition (5–6 million years ago [Mya]; Fjelds  and Lovett 1997). Da Silva and Bates (2002) situate the boundary on the Pliocene/Pleistocene transition; thus, paleo-endemics are ancient lineages (> 2.6 Mya), while neo-endemics are recent lineages mostly originated during the Pleistocene. Areas with significant concentrations of paleo- or neo-endemic taxa are referred to as centres of paleo-endemism or neo-endemism, respectively (Mishler et al. 2014). Centres of paleo-endemism acted as places for survival ('species museums'), where taxa persisted over time. In contrast, centres of neo-endemism ('species cradles') may have played a major role in relatively recent speciation and evolutionary events (Stenseth 1984; Jablonski 1993; Gaston and Blackburn 1996).

Early studies on assemblages of paleo-endemics and neo-endemics were carried out in regions previously recognised as refugia, such as California (Stebbins and Major 1965; Kraft et al. 2010), the tropical Andes (Fjelds  1995), tropical Africa (Fjelds  and Lovett 1997), the Mediterranean Basin (Verlaque et al. 1997), the South African Cape region (Verboom et al. 2009), and southern China (L pez-Pujol et al. 2011). Recent studies have applied a phylogenetic approach and novel phylogenetic tools, including new metrics, such as relative phylogenetic diversity and relative phylogenetic endemism, and new methods, such as categorical analysis of paleo- and neo-endemism (CANAPE: Rosauer et al. 2009; Mishler et al. 2014). These analyses have located potential centres of

endemism and classified them based on branch lengths in the phylogenetic tree of inhabiting taxa, allowing for a quantitative distinction among centres of neo- and paleo-endemism (Mishler et al. 2014). These novel methods have been used in analyses including community assembly, evolutionary biogeography, bioregionalism, and conservation studies in different geographic regions, such as Australia (Mishler et al. 2014; Schmidt-Lebuhn et al. 2015), California (Thornhill et al. 2017), New Zealand (Heenan et al. 2017), Chile (Scherson et al. 2017), Mexico (Sosa et al. 2018), and North America (Mishler et al. 2020). In Mexico, endemism areas of vascular plants were recently assessed by Sosa et al. (2018) using a phylogenetic perspective on the distributional range of both paleo- and neo-endemic species. Their findings identified paleo-endemism areas (e.g. in Baja California, the Sonoran Desert, the northern Chihuahuan Desert, the Sierra Madre Oriental, the western Neovolcanic Belt, the Tehuac n-Cuicatl n Valley (TCV), and the Balsas Basin), neo-endemism areas (e.g. in the Sonoran Desert), and super-endemism areas concentrating both paleo- and neo-endemic taxa (e.g. in the northern Mexican Plateau and Sierra Madre de Chiapas).

The isolated TCV represents a complex physiographic mosaic of Cenozoic origin (D valos- lvarez et al. 2007) in which internal minor valleys are separated by mountain chains, therefore promoting a very heterogeneous environment. This small area bears the greatest plant diversity of the Mexican arid regions, harbouring more than 3000 species, representing approximately 13% of the estimated flora of Mexico (Casas et al. 2016; Ulloa-Ulloa et al. 2017; P rez-Valladares et al. 2019). Most of the flora in the TCV have Neotropical biogeographic affinities. However, some of Mexico's arid and semi-arid plant communities are of Nearctic origin (Rzedowski 1973; Villase or et al. 1990). Additionally, a Mexican element has been recognised and includes 13% of the total plant diversity in the valley (Villase or et al. 1990; M ndez-Larios et al. 2005). Late Pleistocene climatic changes may have largely influenced the biotic composition of the present flora of the region, suggesting that local plant communities are of recent origin (Valiente-Banuet et al. 2009). The valley is thus a complex biotic mosaic in which up to 21 plant communities have been identified (P rez-Valladares et al. 2019): xerophytic communities dominate the north-western part, while the south-eastern portion is dominated by warmer climates, favouring the development of more mesic communities (Garc a 1998; Valiente-Banuet et al. 2009; P rez-Valladares et al. 2019).

Cactaceae is nearly endemic to the Neotropics, and about 1,847 species have been recognised. The main centre of diversification for this family is located in Mexico, with a total of 670 species, 519 of which are endemic to the country (Ulloa-Ulloa et al. 2017). The highest concentrations of Cactaceae in the country occur in arid and semi-arid regions, tropical dry forests, and scrubland vegetation (Mutke et al. 2015) in the Chihuahuan and the Sonoran Deserts, and the Tehuac n-

Cuicatlán Valley (Arias-Montes et al. 2012). Significantly, the relatively small area of the TCV harbours the highest diversity of Cactaceae in Mexico (Valiente-Banuet et al. 2009), in which most plant communities are dominated by endemic species of columnar cacti, highlighting this small area as an important diversity centre for the family (Valiente-Banuet et al. 2000; Mutke et al. 2015; Pérez-Valladares et al. 2019). At least 86 Cactaceae species occur in the TCV (Arias-Montes et al. 1997, 2012), such as the “viejito” (*Cephalocereus columna-trajani*), the “tetetzos” or “teteches” (*Cephalocereus macrocephalus* and *C. tetetzo*), and the “chendes” (*Polaskia chende*) (Fig. 1).

Despite being a Cactaceae hotspot, including both the highest species richness and high levels of endemism (Méndez-Larios et al. 2004; Arias-Montes et al. 2012), no studies address the causes of this high diversity in the TCV using a phylogenetic approach. The high diversity and endemism of Cactaceae (Valiente-Banuet et al. 2009) in the isolated TCV suggests that diversification may have occurred through several pulses during the Pleistocene,

which, in addition to the complex topography, favoured both the persistence and the speciation in this group. We, therefore, expected the TCV to be an area in which both neo- and paleo-endemic species may be found. We also expected to locate mixed-endemism areas either in topographically rugged or ecologically complex zones (e.g. ecotones, isolated habitat patches). We here raised the following questions to approach the study of the endemism of Cactaceae in this region: Is the TCV a place for the persistence of ancient taxa (paleo-endemics) or promoting the speciation of new taxa (neo-endemics)? Are Pleistocene climate changes and isolation drivers of origin and diversification of endemic lineages in the TCV? To answer these questions, we first conducted a qualitative assessment of the paleo- and neo-endemism in Cactaceae in the TCV. Then, we undertook a quantitative assessment to understand if the areas in which paleo- and neo-endemic species are distributed may be considered significant endemism areas. The conjunction of both qualitative analyses on species and quantitative analyses

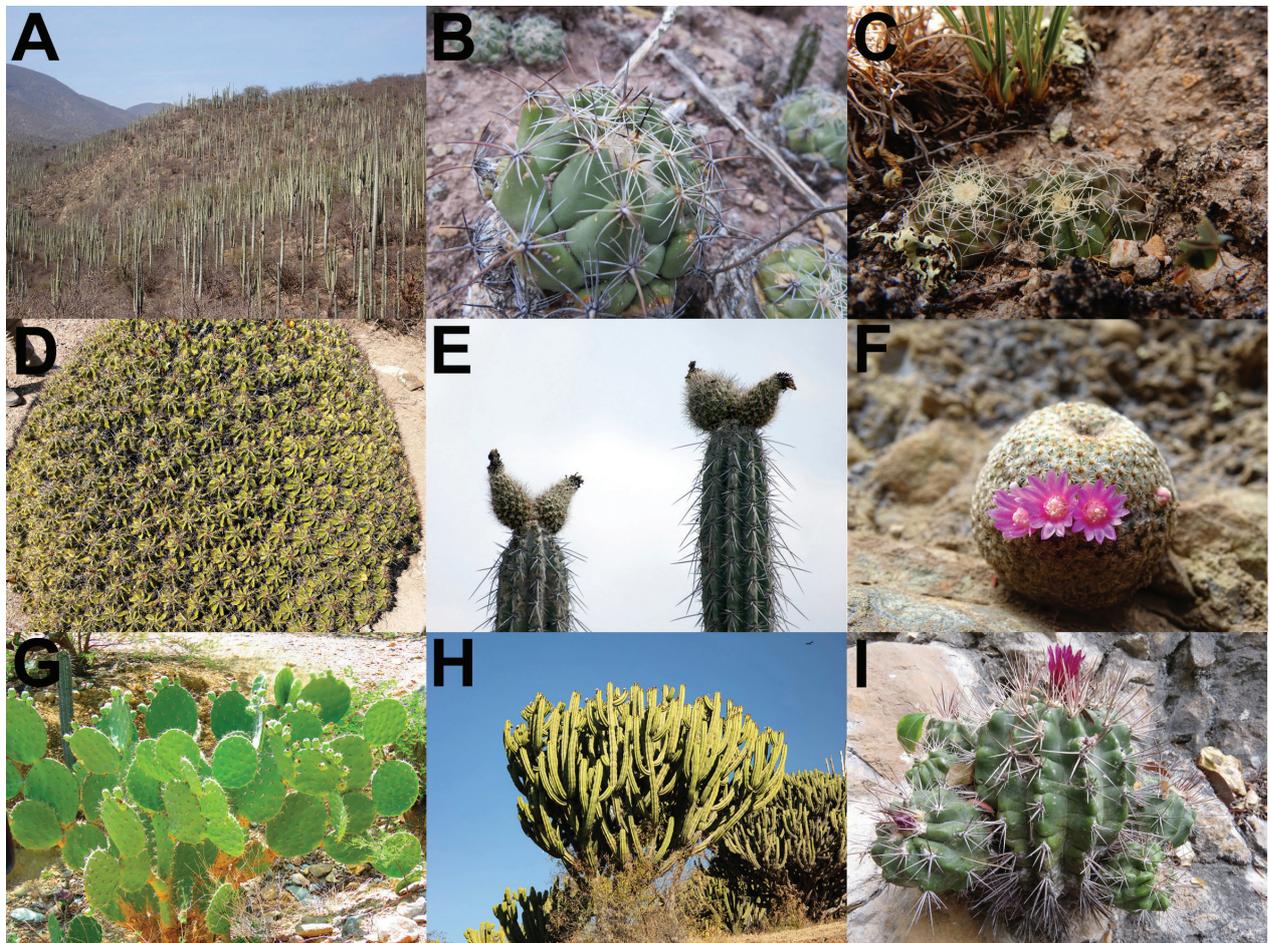


Figure 1. Some species of Cactaceae endemic to the Tehuacán-Cuicatlán Valley. A. *Cephalocereus columna-trajani*. B. *Coryphantha pallida*. C. *Echinocereus acanthosetus*. D. *Ferocactus robustus*. E. *Lemaireocereus hollianus*. F. *Mammillaria huitzilopochtli*. G. *Opuntia tehuacana*. H. *Polaskia chende*. I. *Thelocactus tepelmensis*. The photos are used under a CC BY license from Naturalista (<https://www.naturalista.mx/>). Photo credits go to Chris Fluit (A, 301214920), Leticia Soriano Flores (B, 3927206), Carlos Martorell (C, 9640218), Iván Hernández (D, 56592615), Alicia Mastretta Yanes (E, 192470225), Socorro García Méndez (F, 132245025), Leticia Soriano Flores (G, 88218728), Joseph Scheer (H, 174475303), Leticia Soriano Flores (I, 26464348). The letter inside the parenthesis indicates the figure, and the number is the photo identifier from Naturalista.

on areas may improve the understanding of the evolution of the endemism in the isolated TCV.

MATERIAL AND METHODS

Study area

The TCV covers nearly 10,000 km² in southeastern Puebla and north-western Oaxaca, in southern Mexico (Fig. 2). Most of the valley is currently protected in a biosphere reserve (Dávila et al. 2002). This small area is characterised by a high environmental heterogeneity, with warm, semi-warm, and temperate climates. The climate is predominantly dry, with annual precipitation ranging from 400 to 500 mm and temperatures averaging 22–24°C (García 1998). The TCV is formed by several minor valleys and mountain chains with elevations from 70 to 3300 m a.s.l. (Pérez-Valladares et al. 2019).

List of the endemic species and spatial data

In order to address the study of paleo- and neo-endemism in the TCV, we first generated a list of the Cactaceae species endemic to the TCV by reviewing the specialised literature, including the Flora of the Tehuacán-Cuicatlán Valley of the Instituto de Biología, UNAM (Arias-Montes

et al. 2012), as well as other floristic, taxonomic, and phytogeographic studies (e.g. Dávila et al. 1995, 2002; Méndez-Larios et al. 2004; Villaseñor 2016).

All records for Cactaceae species endemic to TCV were downloaded from the Portal de Datos Abiertos of the UNAM (<https://datosabiertos.unam.mx/>) and the Global Biodiversity Information Facility (GBIF.org 2023a, 2023b). Only collected specimens preserved in scientific herbaria were used. To clean the data, we followed the recommendations of Chapman (2005) and Castillo et al. (2014); all of the records were screened to exclude those with obvious errors in georeferencing data (i.e. data quality issues, data outside the study area or with coordinates occurring in the ocean). Records without coordinates were georeferenced following Chapman and Wieczorek (2020), taken as reference the description of the collection locality registered in the online databases. For specimens without such description, we consulted Arias-Montes et al. (2012). The geographic distribution of the endemic species was visualised using QGIS v.3.22.3 (QGIS Development Team 2022). We verified all species' names by using Plants of the World Online (POWO 2023), which allowed us to remove both synonyms and non-accepted names.

To assess paleo- and neo-endemism patterns in the TCV, we analysed the endemism at two levels: 1) a temporal level, using a time-calibrated phylogeny, and

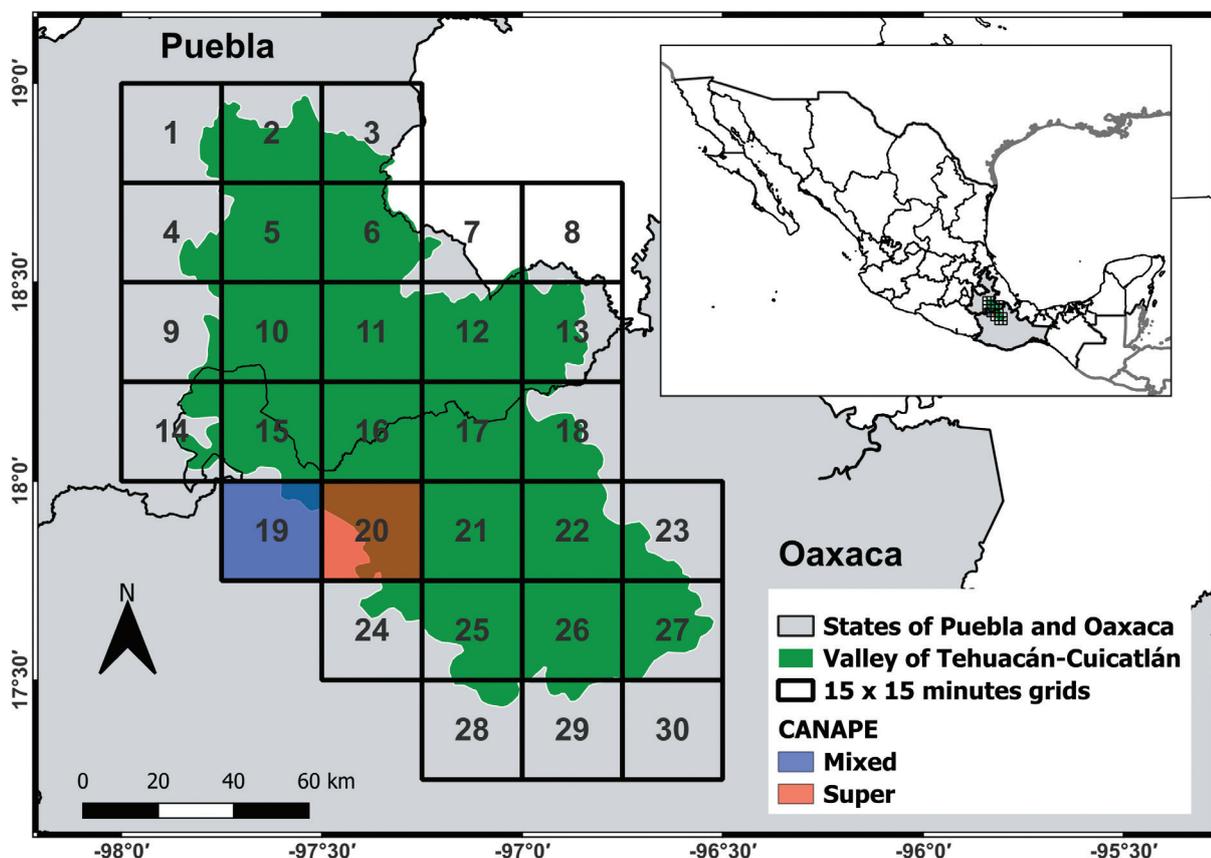


Figure 2. Map of the Tehuacán-Cuicatlán Valley. The colour-filled cells show the centres of endemism identified by CANAPE.

2) a spatial level, using a spatial phylogenetic analysis (CANAPE).

Paleo- and neo-endemism at the temporal level

We considered paleo-endemics as ancient lineages (> 2.6 Mya) and neo-endemics as recent lineages (≤ 2.6 Mya), following Da Silva and Bates 2002. This criterion was used because most geomorphological processes and climatic events in the TCV probably occurred during the Pleistocene (Cornejo-Romero et al. 2017), suggesting that this period was significant for the evolution and composition of the valley flora (Brunet 1967; Dávalos-Álvarez et al. 2007). We downloaded 166 DNA sequences from GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>; Supplementary material 1) corresponding to the chloroplast region *trnK-matK*. We included sequences from 21 of the 27 Cactaceae species recognised as endemics to the TCV and sequences of 145 non-endemic species from different genera as outgroups (e.g. *Acanthocalycium*, *Browningia*, *Cereus*, *Epiphyllum*, *Lophophora*, *Melocactus*, *Pachycereus*, *Pereskia*, and *Stenocactus*) (Supplementary material 1), including as many outgroup species as possible is desirable for estimating more accurate branch lengths, as required in branch length-based methods. Sequences were edited using BioEdit v.7.1.5.0 (Hall 1999), and alignments were conducted in Muscle v.3.6 (Edgar 2004) using default parameters or manual adjustment when necessary. Then, we estimated and selected the best nucleotide substitution model for each locus via the Akaike information criterion using MEGA v.7.0.26 (Kumar et al. 2016).

Phylogeny and divergence time estimates for Cactaceae species were inferred on the *trnK-matK* matrix using Bayesian inference methods in BEAST v.2.1.2 (Bouckaert et al. 2014). We relied on calibrations derived from a comprehensive molecular time-calibrated tree of Cactaceae, which allowed us to constrain the stem node age at 32.11 Mya (Hernández-Hernández et al. 2014). Additionally, the node age for Cactoideae was constrained at 17.15 Mya, the Opuntieae node at 9.04 Mya, and the node Core Mammilloid at 8.62 Mya. An uncorrelated relaxed lognormal clock was implemented, and the birth-death model was selected for the species tree prior (Bouckaert et al. 2014). We ran two independent runs, each consisting of four Markov chain Monte Carlo and 30 million generations, with parameters sampled every 1000 generations. Then, we used Tracer v.1.5 (Rambaut et al. 2018) to confirm chain convergence and to estimate effective sample sizes (> 200) for all model parameters. The two independent runs were combined in LogCombiner v.2.1.2 (Bouckaert et al. 2014), with 25% of the initial trees discarded as burn-in. Finally, TreeAnnotator v.2.1.2 (Bouckaert et al. 2014) was used to summarise the information on the trees and to derive a maximum clade credibility (MCC) tree, which was visualised and edited using FigTree v.1.4.2 (Rambaut 2014).

Paleo- and neo-endemism at the spatial level

Paleo- and neo-endemism was spatially assessed using CANAPE (Mishler et al. 2014), as implemented in the R package *canaper* v.1.0 (Nitta et al. 2023). This approach uses inferred branch lengths from a phylogenetic tree. Therefore, paleo- and neo-endemism areas are interpreted as those containing a significantly high concentration of range-restricted species showing either long or short branches. Thus, paleo-endemic areas are characterised by non-random concentrations of species with long branch lengths, while neo-endemic areas include non-random concentrations of species with short branch lengths (Mishler et al. 2014; Thornhill et al. 2016). These areas act as “cradles” and “museums” of biodiversity, respectively. We applied CANAPE to our MCC tree; the species in our dataset that were absent from the tree were added using the R package *phytools* v. 2.0 (Revell 2012). We overlapped a grid of 15 minutes of longitude and latitude on the TCV, and each quadrant was used as a geographic unit from which we calculated both the phylogenetic endemism (PE, Rosauer et al. 2009) and the relative phylogenetic endemism (RPE, Mishler et al. 2014). To assess the statistical significance of PE and RPE, we compared the observed PE and RPE values of each grid cell to 999 values generated from a null distribution that randomises the terminals in the phylogeny while holding constant the total taxa per cell and the total cells per taxon. P-values were estimated from a two-tailed distribution value, which allowed us to identify areas with higher (> 0.9) or lower (< 0.1) PE or RPE than the null distribution. Higher or lower PE/RPE values were compared to the null distribution in grid cells, indicating paleo- or neo-endemism areas, respectively (Mishler et al. 2014). In addition to identifying endemic centres and classifying them into different categories, CANAPE is also used to support suggestions for biodiversity conservation (Wang et al. 2022; Cai et al. 2023).

RESULTS

List of the endemic species and spatial data

Our revision based on specialised literature generated a list of 27 Cactaceae species endemic to the TCV (Table 1), which were grouped into the following clades based on Hernández-Hernández et al. (2014): the Cactaceae clade, including only *Thelocactus* (1 sp.); the Core Mammilloid clade grouping *Mammillaria* (13 spp.) and *Coryphantha* (1 sp.); the Core Pachycereeae clade, which groups *Cephalocereus* (4 sp.), *Echinocereus* (1 sp.), *Lemaireocereus* (1 sp.), and *Polaskia* (1 sp.); the Ferocactus clade, including only *Ferocactus* (3 spp.); and the Opuntioideae clade, including only *Opuntia* (2 spp.). *Mammillaria* is the largest genus in the TCV, with 13 endemic species comprising 48% of the total endemic Cactaceae species. Additional genera with high numbers of endemics are

Table 1. Estimated divergence times for the Cactaceae species endemic to the Tehuacán-Cuicatlán Valley. Species are classified as paleo- or neo-endemics based on the criterion of Da Silva and Bates (2002).

Species	Divergence time (mya)	Paleo- or neo-endemic
<i>Cephalocereus columna-trajani</i>	0.86 (0.16–1.92)	Neo-endemic
<i>Cephalocereus fulviceps</i>	0.61 (0.00–1.99)	Neo-endemic
<i>Cephalocereus macrocephalus</i>	0.61 (0.00–1.99)	Neo-endemic
<i>Cephalocereus tetetzo</i>	0.86 (0.28–2.37)	Neo-endemic
<i>Coryphantha pallida</i> subsp. <i>calipensis</i>	0.72 (0.00–2.19)	Neo-endemic
<i>Echinocereus acanthosetus</i>	–	–
<i>Ferocactus flavovirens</i>	0.98 (0.12–2.56)	Neo-endemic
<i>Ferocactus latispinus</i> subsp. <i>spiralis</i>	1.80 (0.08–4.41)	Neo-endemic
<i>Ferocactus robustus</i>	1.27 (0.10–3.10)	Neo-endemic
<i>Lemaireocereus hollianus</i>	4.43 (2.61–6.81)	Paleo-endemic
<i>Mammillaria crucigera</i>	0.83 (0.13–2.00)	Neo-endemic
<i>Mammillaria dixanthocentron</i>	0.83 (0.13–2.00)	Neo-endemic
<i>Mammillaria haageana</i> subsp. <i>vaupelii</i>	0.20 (0.00–1.02)	Neo-endemic
<i>Mammillaria hernandezii</i>	3.01 (1.03–4.37)	Paleo-endemic
<i>Mammillaria huitzilopochtli</i>	3.11 (0.83–5.21)	Paleo-endemic
<i>Mammillaria kraehenbuehlii</i>	–	–
<i>Mammillaria napina</i>	5.18 (3.37–7.15)	Paleo-endemic
<i>Mammillaria oteroi</i>	–	–
<i>Mammillaria pectinifera</i>	1.06 (0.07–2.73)	Neo-endemic
<i>Mammillaria sphaclata</i>	4.32 (2.65–6.84)	Paleo-endemic
<i>Mammillaria supertexta</i>	1.14 (0.26–2.27)	Neo-endemic
<i>Mammillaria tepexicensis</i>	–	–
<i>Mammillaria varieaculeata</i>	1.06 (0.00–2.73)	Neo-endemic
<i>Opuntia parviclada</i>	–	–
<i>Opuntia tehuacana</i>	1.79 (0.75–3.04)	Neo-endemic
<i>Polaskia chende</i>	1.70 (0.36–3.60)	Neo-endemic
<i>Thelocactus tepelmensis</i>	–	–

Cephalocereus and *Ferocactus*, with four and three species, respectively.

Paleo- and neo-endemism at the temporal level

The aligned *trnK-matK* sequences were 2703 base pairs (bp) in length, and the best nucleotide substitution model to analyse this alignment was the GTR+G+I. Our phylogenetic tree included 21 of the 27 Cactaceae species recognised here as endemic to the TCV. The dated phylogeny showed that most of the endemic species diverged throughout the Pleistocene-Holocene (Table 1; Fig. 3 shows a synthesized phylogenetic tree; see Supplementary material 2 for a detailed tree). Divergence time estimates are moderately variable, ranging from 0.20 Mya in *Mammillaria haageana* subsp. *vaupelii* to 5.18 Mya in *M. napina* (Table 1). Following the criterion of Da Silva and Bates (2002), divergence time estimates showed that of the 21 species included in our phylogenetic tree, 5 (24%) were classified as paleo-endemics and 16 (76%) as

neo-endemics (Table 1). *Mammillaria napina* is the paleo-endemic with the oldest divergence time at 5.18 Mya (highest posterior density (HPD) 3.37–7.15 Mya). This globose cactus occurs mostly in a restricted geographic range in the north-western TCV.

Paleo- and neo-endemism at the spatial level

The CANAPE analysis identified two cells (19 and 20) of high phylogenetic endemism (one with mixed-endemism and one with super-endemism) in the TCV (Fig. 2). The two cells included 11 (41%) of the 27 endemic species. Species such as *Cephalocereus fulviceps*, *Ferocactus latispinus* subsp. *spiralis*, *Mammillaria dixanthocentron*, *M. haageana* subsp. *vaupelii*, and *Opuntia tehuacana* are categorized as neo-endemics. Other endemic species, such as *Echinocereus acanthosetus*, *M. kraehenbuehlii*, *M. oteroi*, *M. tepexicensis*, and *Thelocactus tepelmensis* are not included in our phylogeny, so they were not classified. Both cells are dominated by temperate forests

(oak, mixed, and pine forest), with scattered patches of xerophytic scrub and seasonally dry forest (Valiente-Banuet et al. 2000).

DISCUSSION

Recent work supports that centres of endemism occur in regions with long-term climatic stability, which likely buffered surrounding unfavourable climatic conditions (Wiens and Donoghue 2004; Jablonski et al. 2006;

Fine 2015; Harrison and Noss 2017). In addition, the topographic complexity and the isolation of some of these regions enhance speciation rates, producing high concentrations of endemic species (Cai et al. 2023). Climatic fluctuations during the Pleistocene strongly impacted the diversification of taxa by limiting the distribution of many species to isolated regions of long-term spatiotemporal climate stability, which contributed to the development of refugia (Haffer 1969; Fjeldså 1995; Davis and Shaw 2001; Jetz et al. 2004; Harrison and Noss 2017). These refugia harbour high biodiversity and

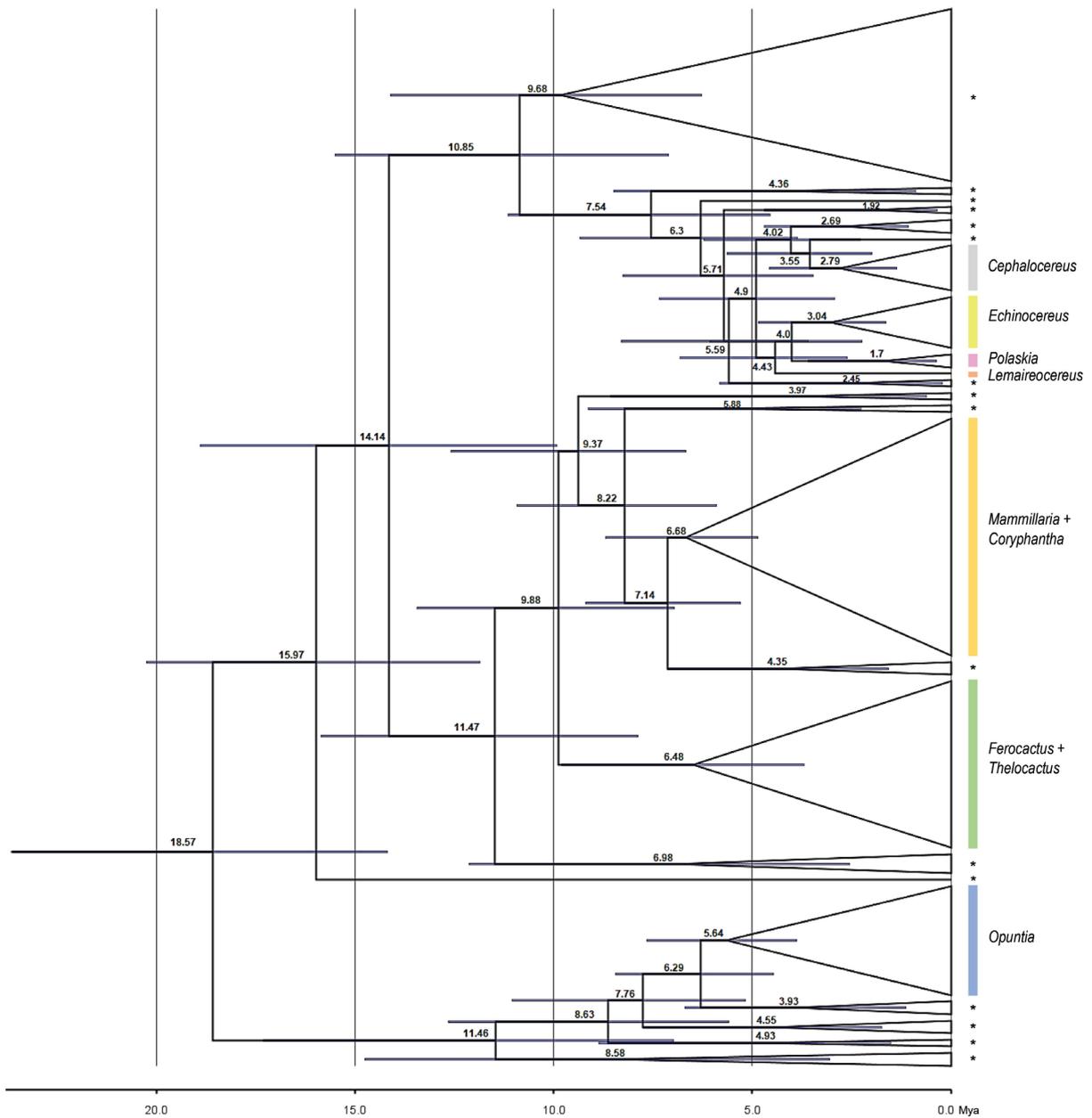


Figure 3. Synthesized phylogenetic tree of Cactaceae estimated from *trnK-matK* sequences using BEAST (see Supplementary material 2 for a detailed tree). Node bars represent the 95% HPD for the age of that node. Numbers at the nodes indicate mean ages. The asterisk (*) indicates groups with no endemic species in the Tehuacán-Cuicatlán Valley.

endemism, enabling the persistence of paleo-endemic taxa but also promoting the speciation of novel taxa (neo-endemics) (Harrison and Noss 2017; Cai et al. 2023).

In Mexico, several regions, including mountain chains and desert areas, have been widely recognised as hotspots for plant and animal richness and endemism and have frequently been suggested as Pleistocene refugia (e.g. Becerra 2005; Weeks et al. 2005; Delgado-Salinas et al. 2006; Flores-Villela and Martínez-Salazar 2009). These refugia likely show a mixture of potential paleo- and neo-endemic taxa, pointing to multiple historical processes involved in the origin and maintenance of biodiversity. Particularly, the isolated TCV is a small area surrounded by mountains likely acting as barriers that promoted the isolation of mainly arid and semi-arid climate-adapted taxa, leading to the evolution of an area with high biodiversity and a high proportion of endemic species (Dávila et al. 2002; Méndez-Larios et al. 2004). The family Cactaceae has 27 endemic species in the TCV. This number does not seem very large compared to the 519 species of Cactaceae endemic to the country, which could be interpreted as a limitation to this analysis, but this pattern of narrow endemism is common to other areas of the country, for example, the Chihuahuan Desert has 229 endemic species. However, this is 50 times more extensive than the TCV (Hernández et al. 2004). Still, if regional floras are analysed, local endemism also records few species, 23 species in Cuatro Ciénegas, Coahuila (Pinkava 1984), six in Mapimí, Durango (García-Arévalo 2002), or ten in El Huizache, San Luis Potosí (Hernández et al. 2001). In TCV, most endemic species are geographically restricted, such as *Thelocactus tepelmensis*, recently described by Davis et al. (2018) and whose only known population has been found growing on limestone rock faces in a narrow canyon in northern Oaxaca. Some species, however, are widely distributed, such as *Cephalocereus tetetzo*, a branched columnar cactus widely distributed in xerophytic shrublands and tropical dry forests across the valley (Arias-Montes et al. 1997).

The distinction between paleo-endemics and neo-endemics has relied on various criteria, including geography, taxonomy, cytology, geology, climate, and phylogeography (Favarger and Contandriopoulos 1961; Bramwell 1972; Major 1988; Cronk 1992); however, these criteria may be ambiguous. A bounded timescale allowed us to discriminate between palaeo- and neo-endemics, suggesting that molecular phylogenies may be one of the most accurate methods to estimate temporal endemism in plant lineages. We, therefore, considered the criterion of Da Silva and Bates (2002) as accurate for our study because it is based on a historical framework from a molecular phylogenetic analysis, which seems to fit well with the local geological history of the region (Dávalos-Álvarez et al. 2007). Thus, according to our dated phylogeny, 76% (16) of the 21 endemic species were classified as neo-endemics, suggesting that the TCV may have played a major role in recent speciation and diversification in Cactaceae. We found that only 24%

(5) of the endemic Cactaceae species were classified as paleo-endemic species (> 2.6 Mya), suggesting a relatively minor role of the TCV region in the persistence of ancient taxa. *Mammillaria napina* and *Lemaireocereus hollianus* are paleo-endemic species with a divergence estimated at 5.18 and 4.43 Mya, respectively. Divergence times of other probable paleo-endemics, such as *Mammillaria huitzilopochtli* (3.11 Mya) and *M. hernandezii* (3.01 Mya), are not clear because these values are near the boundary of 2.6 Mya. Furthermore, the HPD values show that some neo-endemic taxa could also be classified as paleo-endemic, such as *Ferocactus latispinus* subsp. *spiralis* with a divergence time estimated at 1.8 Mya (HPD 0.08–4.41 Mya), *Opuntia tehuacana* 1.79 Mya (HPD 0.75–3.04 Mya), and *Polaskia chende* 1.7 Mya (HPD 0.36–3.6 Mya).

Neo-endemic species are found in the genera *Cephalocereus*, *Echinocereus*, *Ferocactus*, *Mammillaria*, *Opuntia*, and *Polaskia*. *Mammillaria* is the most speciose genus in Cactaceae with approximately 180 species, from which more than 90% of the species are distributed in Mexico, and about 85% are endemic to the country (Butterworth and Wallace 2004; Crozier 2005; Hunt 2006; Hernández and Gómez-Hinostrosa 2015). Although the origin and high diversification rates of the genus might be associated with geographic expansion during the aridification of North America in the Miocene 8.62 Mya (HPD 5.83–12.56 Mya; Hernández-Hernández et al. 2014), a high percentage of *Mammillaria* species could have evolved due to Pleistocene climatic changes (Cervantes et al. 2021). Our results showed the divergence time estimates for TCV *Mammillaria* endemic species ranged from 0.20 Mya (HPD 0.00–1.02 Mya) for *Mammillaria haageana* subsp. *vaupelii* to 5.18 Mya (HPD 3.37–7.15 Mya) for *M. napina*. Thus, six out of ten species included in our phylogenetic analysis are neo-endemics. Remarkably, most of these taxa are geographically restricted; for example, the neo-endemics *M. crucigera* and *M. supertexta* are restricted to the south-eastern TCV region, which is geologically recent and mainly comprised of alluvial fans dating back to the Pleistocene-Holocene (Brunet 1967; Dávalos-Álvarez et al. 2007). This region is exposed to humidity from the Gulf slope, which has promoted the development of more mesic vegetation, such as tropical seasonally dry forests. Speciation processes in tropical seasonally dry forests were probably favoured by Pleistocene climatic changes, which might have promoted high rates of diversification in these isolated and climatically stable environments (Pennington et al. 2004).

Other plant groups also show similar patterns to the one found for *Mammillaria*. Recent studies of ancient lineages, such as cycads, have shown that this gymnosperm group dates to the late Palaeozoic (Norstog and Nicholls 1997); however, extant species such as *Dioon* (Zamiaceae) are recently differentiated lineages (Gregory and Chemnick 2004; Dorsey et al. 2018). Dorsey et al. (2018) found that *Dioon* originated at 7.86 Mya (HPD 7.09–8.71 Mya) in the Miocene, and the diversification of extant species

occurred during the Pleistocene, suggesting that modern species are not paleo-endemics but rather recently derived neo-endemic species. Notably, *Dioon purpusii* Rose, *D. argenteum* De Luca, Sabato & Vázquez-Torres, *D. califanoi* De Luca & Sabato, and *D. caputoi* T.J.Greg., Chemnick, Salas-Mor. & Vovides are all neo-endemics restricted to the TCV. According to Dorsey et al. (2018), these species have evolved from populations periodically shifting to lower elevations in response to Pleistocene climatic fluctuations, supporting our hypothesis that these climatic changes and isolation might have driven divergence and speciation in endemic lineages of the TCV.

Regarding the spatial level, CANAPE identified two cells of high phylogenetic endemism (one of mixed-endemism and one of super-endemism) in the TCV (Fig. 2). Our results partially agree with Sosa et al. (2018), in which the whole TCV was included within a mixed-endemism centre and no super-endemism areas were detected. Similarly, Mishler et al. (2020) identified centres of mixed-endemism around the TCV, but no centres of super-endemism were reported. Furthermore, a recent study on Cactaceae also detected mixed-endemism and super-endemism areas around the TCV (Amaral et al. 2022). These differences in the categorisation assigned to the endemism areas may be scale-dependent, as has been observed in the estimation of species richness and endemism (Whittaker et al. 2001; Hartley and Kunin 2003; Laffan and Crisp 2003; Chase et al. 2019; Luebert et al. 2022), as well as in spatial phylogenetics (Daru et al. 2020). Recent studies have found that using small scales may recover significantly high values of phylogenetic diversity (Thornhill et al. 2016, 2017; Scherson et al. 2017; Allen et al. 2019; Mishler et al. 2020). We used a small grid-cell size, which allowed us to recover a centre of super-endemism not previously reported, underlying the significance of the TCV in the diversification and maintenance of species diversity in Cactaceae. Therefore, small scales may reveal emergent patterns related to phylogenetic endemism, thus changing the categorisation of endemism centres.

Both the mixed-endemism area (grid-cell 19) and the super-endemism area (grid-cell 20) are dominated by temperate forests, with scattered patches of xerophytic scrub and seasonally dry forest. In the TCV, oak, mixed, and pine forests occur as isolated patches in altitudes between 1,630 and 2,200 m (Valiente-Banuet et al. 2000). The super-endemism area included two exclusive species: *Thelocactus tepelmensis* in xerophytic scrub and *Mammillaria oteroi* in *Quercus* forests, all other species in this area are widely distributed in the TCV. Species in the mixed-endemism area include *Echinocereus acanthosetus*, *M. hernandezii*, and *M. tepexicensis* (the latter endemic to this grid cell). Collected specimens in herbariums suggest that these species are also distributed in temperate forests, such as *Pinus*, *Quercus*, and *Juniperus* forests. These results support our hypothesis that mixed- and super-endemism areas may be found in topographically or environmentally complex regions.

Our results suggest that the TCV region has played a predominant role as a cradle, promoting the recent evolution of endemic plant species, which have been enhanced by landscape heterogeneity and isolation. Isolation has promoted in situ speciation, which led to high neo-endemism. Additionally, environmental drivers such as the long-term stability of climate and habitats on a reduced spatial scale have favoured the evolution of the TCV endemic plant species.

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SUPPLEMENTARY MATERIALS

Supplementary material 1

GenBank accession numbers of the *trnK-matK* sequences included in this work for taxa in Cactaceae. The asterisk (*) indicates taxa endemic to the Tehuacán-Cuicatlán Valley.

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Supplementary material 2

Detailed phylogenetic tree of Cactaceae estimated from *trnK-matK* sequences using BEAST. Node bars represent the 95% highest posterior density for the age of that node. Numbers at the nodes indicate mean ages. A timescale is shown at the bottom, with units in millions of years. Species names written in blue are endemic to the Tehuacán-Cuicatlán Valley.

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