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ORIGINAL ARTICLE

Evolutionary history, taxonomy, and ecology of Neotropical *Cissus* (Vitaceae) in the biotic transition zone of Mexico and Central America

Alberto J. Coello[1,*,†], **Angélica Gallego-Narbón**[1,†], **Carter Keyworth**[1,2,†],
Angélica Quintanar-Castillo[3], **Gabriel Johnson**[1], **Marcelo R. Pace**[4] and **Jun Wen**[1,*]

[1]Department of Botany, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560, USA, [2]Department of Botany and Plant Sciences, University of California, Riverside, Riverside, CA 92521, USA, [3]Posgrado en Ciencias Biológicas. Instituto de Biología, Universidad Nacional Autónoma de México, Coyoacán, Mexico City 04510, Mexico and [4]Departamento de Botánica y Herbario Nacional de México, Instituto de Biología, Universidad Nacional Autónoma de México, Coyoacán, Mexico City 04510, Mexico
[*]For correspondence. E-mails: coellogarridoa@si.edu, wenj@si.edu
[†]Alberto J. Coello, Angélica Gallego-Narbón and Carter Keyworth contributed equally to this work.

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- **Background and Aims** Mexico constitutes a biotic transition zone between Nearctic and Neotropical floras, which alongside its complex geography and climatic variability makes it an especially diverse region. *Cissus*, the largest genus of Vitaceae, has ~70 species in the Neotropics, with 20–25 species in Mexico and Central America, occupying diverse environments from tropical forests to deserts. Despite their ecological relevance in the area, Neotropical *Cissus* species have been barely studied. Here we aim to address the phylogenetic history, taxonomic delimitation, biogeography and ecological preferences of *Cissus* in the biotic transition zone of Mexico and Central America.
- **Methods** We obtained nuclear and plastid phylogenies of Neotropical *Cissus* using target enrichment Hyb-Seq, sampling ~50 % of the Neotropical species and most Mexican species. We performed time calibration and biogeographic analyses to assess the colonization history, performed ancestral reconstructions of relevant taxonomic traits, and studied geographic and climatic differences between clades and species in Mexico and Central America using climatic principal component analyses.
- **Key Results** Four main clades were retrieved in Neotropical *Cissus* via phylogenomics, with putative early hybridization detected between clades. Morphological examination and phylogenetic data provided support for taxonomic reassessment in Mexico. Biogeographic analyses pointed to several colonization events to Mexico during the Oligocene and the Miocene, with xeric species from western Mexico clustering in a single mid-Miocene lineage. Climatic and geographic analyses supported differences between clades, and mountain ranges were suggested to have acted as geographic and climatic barriers for some lineages.
- **Conclusions** Our research recovered four main clades with significantly distinct geographic and climatic preferences in Neotropical *Cissus*. Mexico was colonized several times and geographic and climatic isolations were crucial for speciation. Our results highlighted the presence of hidden species diversity in Mexico, underscoring the need for integrative biodiversity discovery in the Mexico transition zone.

Key words: Vitaceae, phylogenomics, *Cissus*, species delimitation, Mexico, climatic niche, morphology.

INTRODUCTION

Biological diversity is unequally distributed around the world, with the highest number of taxa found in particular regions known as biodiversity hotspots (Myers *et al.*, 2000). One of the most relevant hotspots for plant species is Mesoamerica. This region harbours ~24 000 plant species and extends from the southern half of Mexico to Panama. In fact, Mexico is recognized as the fourth most species-rich country in the world for plants (Villaseñor, 2016). However, species are not homogeneously distributed, and the existence of environmental gradients creates transition zones in which diversification and speciation play an important role (Smith *et al.*, 2001).

Mesoamerica is climatically divided into southern tropical and northern subtropical areas, resulting in a climatic transition between them (Coca-de-la-Iglesia *et al.*, 2024). In addition, Mesoamerica is topographically complex with several mountain systems (Fig. 1), which can provide geographic barriers to plant species, restricting gene flow and ultimately leading to genetic drift (Badgley *et al.*, 2017). Within this intricate mountainous landscape lies the Mexican Transition Zone, which emerges as a major biogeographic interface separating the Nearctic and the Neotropical regions (Halffter, 1987; Cox, 2001; Halffter and Morrone, 2017; Villaseñor *et al.*, 2020).

This conceptual framework highlights Mexico as a particularly valuable model for studying biotic transitions as it also possesses a high diversity of habitats and ecological conditions, which range from high mountain regions to deserts (Beck *et al.*, 2023). Additionally, a large section of Mexico's southern half is dominated by humid tropical forests or seasonally dry tropical forests (Villaseñor, 2016), which normally require high temperatures and consistently or seasonally high levels of precipitation. Tropical dry forests are characterized by remarkable biodiversity, including a high number of endemic species (DRYFLOR, 2012). In the Americas, average temperature and precipitation are positively correlated with species richness (Antonelli *et al.*, 2018). This pattern is reflected in Mexico's remarkably diverse flora, comprising around 297 families, 2854 genera and 23 314 species (Villaseñor, 2016).

This species richness is reflected not only in the number of taxa but also in the diversity of growth forms, among which lianas play a prominent role. Lianas constitute a fundamental component of forest structure and dynamics, particularly in tropical regions and especially in tropical dry forests, where they reach their highest abundance (Schnitzer and Bongers, 2002; Schnitzer, 2005). Among the most diverse liana groups in the Neotropics, Gentry (1992) listed Vitaceae, a widely distributed family comprising 16 genera and ~950 species, occurring in both tropical and temperate regions (Wen *et al.*, 2018).

Mexico has a good representation of the grape family (Vitaceae), especially of *Cissus* and *Vitis* (Lombardi, 2000, 2015; Nie *et al.*, 2023). *Cissus* is the largest genus in the grape family Vitaceae, with ~300 species (Wen, 2007; Wen *et al.*, 2018). The genus includes lianas and shrubs with simple and compound leaves, 4-merous flowers with well-developed floral discs, and usually bearing single-seeded fruits, though many species exhibit high morphological variability (Wen, 2007). *Cissus* is widely distributed in tropical and subtropical regions worldwide (POWO, 2025), reaching the Americas only a single time during the Eocene–Oligocene transition (Liu *et al.*, 2013). In particular, the Neotropics display high species richness (~70 species; POWO, 2025), with the species grouped in lineages related to leaf division (Rodrigues *et al.*, 2014; Lombardi, 2015). However, most of the American species have never been included in phylogenetic studies. This is especially the case for Mexico. Nowadays, ~12 species of *Cissus* have been recognized in Mexico (Fig. 2) and 22 species in Mesoamerica (Lombardi, 2015; POWO, 2025), but taxonomic studies of *Cissus* in Mexico and its neighbouring Central American regions have remained largely at the morphological level (Standley, 1923; Lombardi, 2005, 2015; Villaseñor, 2016), and little is known about phylogenetic relationships between the species in Mexico and Central America (Rodrigues *et al.*, 2014).

This study aims to shed light on the evolutionary history, taxonomic diversity and ecological differences of *Cissus* in the biotic transition zone of Mexico and Central America. We integrate morphological, phylogenomic and ecological approaches to resolve species delimitations for *Cissus* in a way that was not possible to achieve previously based on morphological data and a limited number of loci. In particular, we attempt to achieve the following specific objectives: (1) reconstruct the phylogenetic relationships of *Cissus* in Mexico and Central America in the broader framework of the Neotropics; (2) evaluate the species delimitation and evolution of several key morphological characters of Mexican *Cissus* based on phylogenomics; and (3) assess the geographic and climatic preferences of *Cissus* in Mexico and Central America and explore how they relate to evolutionary patterns in the biotic transition zone.

MATERIALS AND METHODS

Taxon sampling and library preparation

To study the evolutionary relationships of Neotropical *Cissus*, 109 samples were newly sequenced (Supplementary Data Table S1), including 108 samples representing the Neotropical *Cissus* clade (Fig. 1; Liu *et al.*, 2013) and *Cissus quadrangularis* from the Old World as the outgroup. Our sampling covered ~50 % of the currently recognized species in the Neotropics (~70 species; POWO, 2025), with an emphasis on Mexico (10 out of 12 of the recognized species were sampled). Sequenced samples included herbarium specimens from the United States National Herbarium (US, 98 samples), the herbaria of the Missouri Botanical Garden (MO, 9 samples) and the Universidad Nacional Autónoma de México (MEXU, 2 samples). DNA extraction followed the protocol of Johnson *et al.* (2023). Library preparation and target enrichment followed Talavera *et al.* (2023). To target nuclear loci, we used a Vitaceae-specific bait set targeting 1013 nuclear loci (Nie *et al.*, 2023; Talavera *et al.*, 2023) based on 15 transcriptomes representing the main Vitaceae clades (Wen *et al.*, 2013). To allow the recovery of plastid loci, the target-enriched libraries were combined with unenriched libraries in a 6:4 ratio. A NovaSeq X plus platform was used for sequencing with 150-bp paired-end reads.

Sequence assembly and alignment

Raw reads were trimmed using Trimmomatic 0.39 (Bolger *et al.*, 2014) to remove the adapters and the low-quality sequences. The resulting trimmed paired files were assembled in HybPiper 2.1.1 (Johnson *et al.*, 2016), and the paralog_investigator tool was used to detect and remove paralogues from subsequent analyses. Nuclear loci were mapped to the targeted nuclear regions (Talavera *et al.*, 2023) and plastid loci were extracted by running the HybPiper pipeline using a *C. quadrangularis* plastome as a reference (GenBank accession MW592504). Alignments were performed for each locus separately using MAFFT 7.525 (Katoh and Standley, 2013).

Phylogenomic analyses

Concatenation-based and coalescent-based analyses were performed. A plastid matrix of concatenated loci

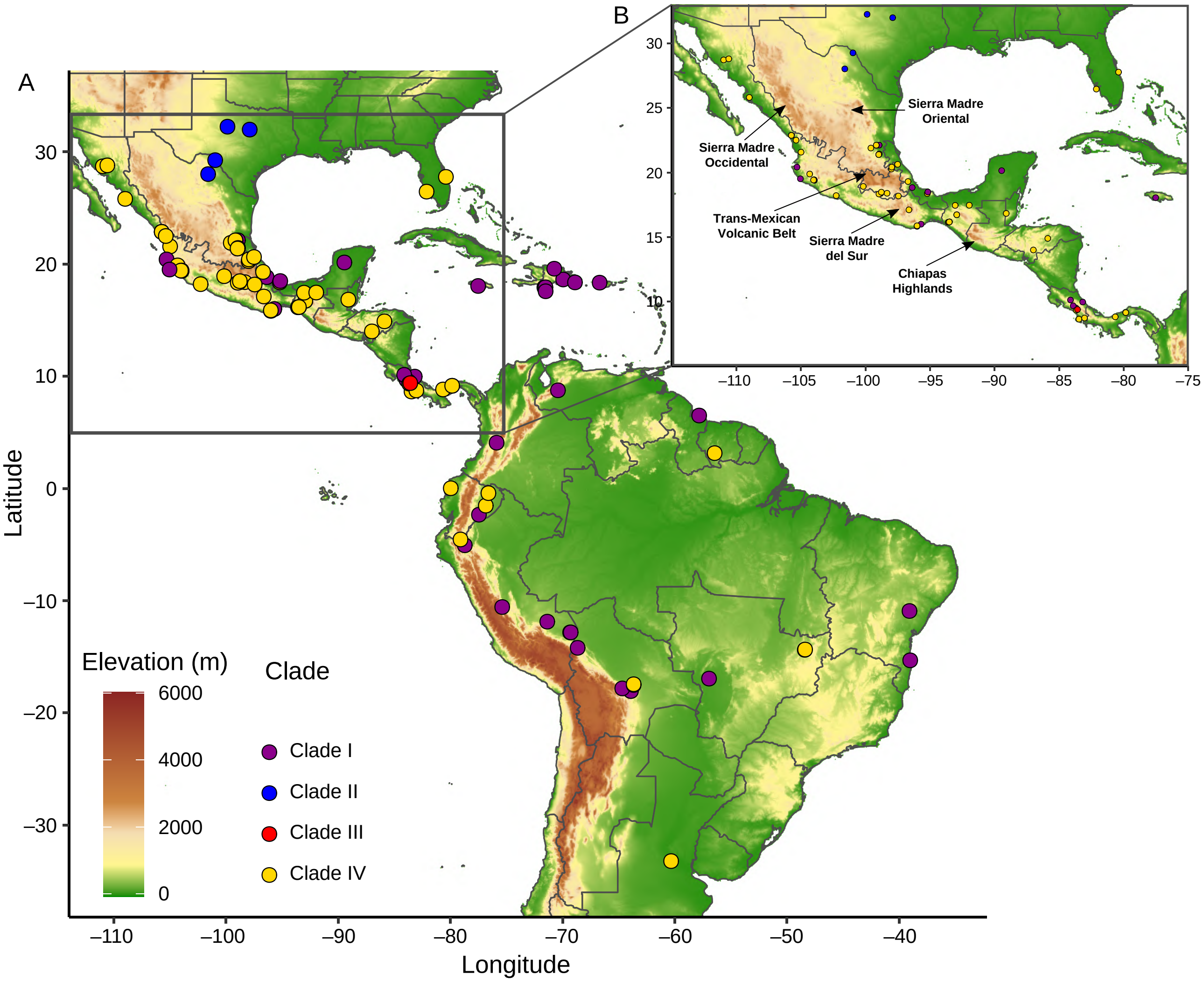

Fig. 1. (A) Distribution of all the Neotropical *Cissus* specimens included in our phylogenetic reconstructions coloured by their clade in the phylogeny (note that *C. trifoliata* in Clade II extends further north into the USA). (B) Distribution of *Cissus* specimens included in our phylogenetic reconstructions in Mexico and Central America, showing the most important mountain ranges. Elevations based on the WorldClim 2.0 5-minute resolution layer (Fick and Hijmans, 2017) and botanical countries of the geographical standard for plant distributions (Brummitt, 2001) are shown.

was built using AMAS 1.0 (Borowiec, 2016) and used as the input for a concatenation-based maximum likelihood analysis, which was performed in RAxML-HPC 8.2.13 (Stamatakis, 2014) under a GTRGAMMA evolutionary model with 1000 fast bootstrap replicates. For nuclear data, we performed a coalescent-based analysis. We obtained gene trees for each of the nuclear loci, excluding the paralogues, using the same RAxML parameters used for the plastid analysis. These trees were used as the input to obtain a species tree in ASTRAL 5.7.8 (Zhang *et al.*, 2018). The resulting coalescent-based tree was fully annotated using the –t 2 ASTRAL parameter to include both local posterior probabilities as a measure of branch support (PP_{local}), and quartet scores as a measure of gene tree discordance (Q1, main topology; Q2, main alternative topology, and Q3: second alternative topology). Nuclear and plastid topologies were compared graphically, using the tanglegram function of the dendextend R package (Galili, 2015).

Divergence time estimation and biogeographic reconstruction

In order to study divergence times in Neotropical *Cissus*, we retrieved Hyb-Seq genomic data representative of the main lineages of Vitaceae and an outgroup (*Leea indica*) available in online repositories (Coello *et al.*, 2026; BioProject PRJNA1338494), given that Neotropical *Cissus* fossils are scarce and insufficient for an appropriate time calibration. These data were analysed alongside a selection of the newly sequenced specimens, including one sample per Neotropical *Cissus* taxon except for widespread species, for which we included two to four specimens. We followed the protocol previously stated for concatenation-based analyses in RAxML in order to obtain a nuclear phylogeny of 104 specimens.

A penalized likelihood approach was applied as implemented in TreePL 1.0 (Smith and O'Meara, 2012) to perform the time calibration, following the fossil calibration points used by Coello *et al.* (2026): *C. lombardii* and *C. willardii*

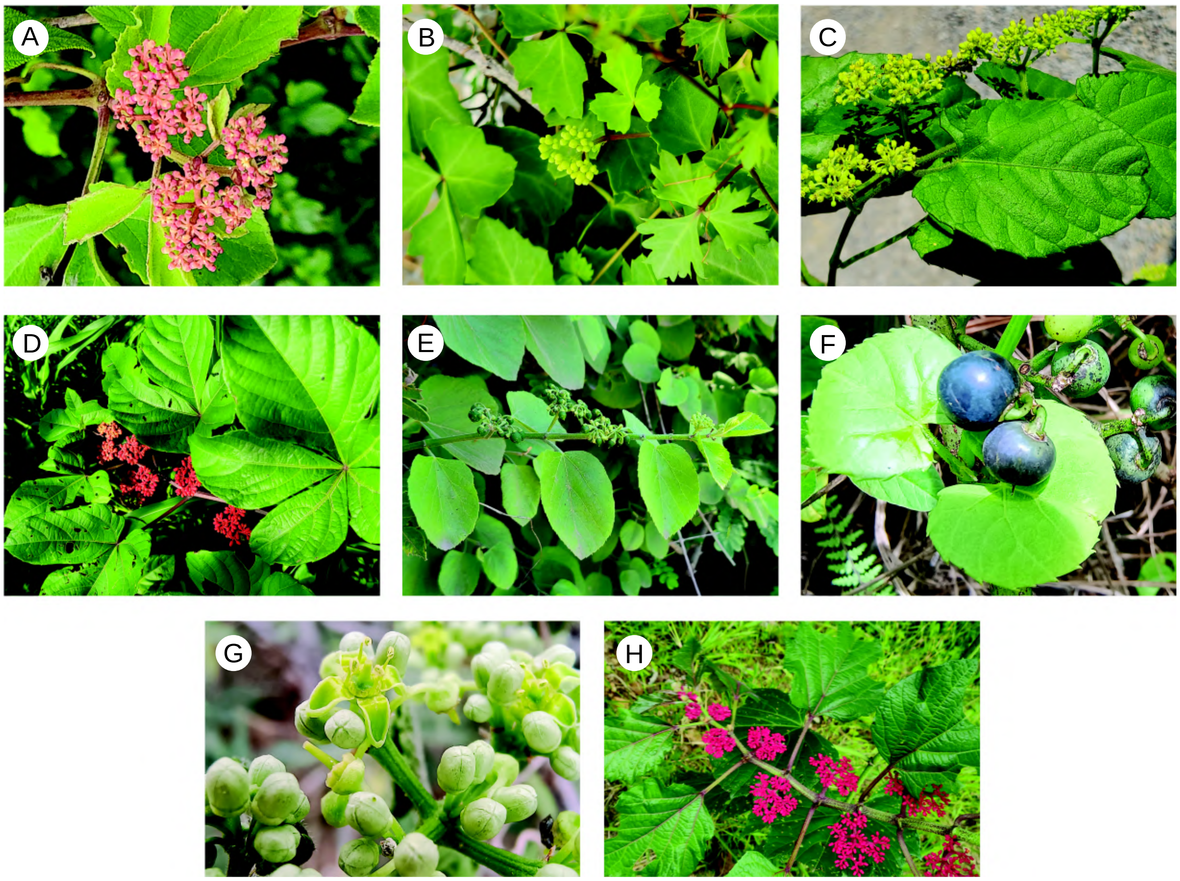

FIG. 2. Morphological representation of *Cissus* species present in Mexico. (A) *Cissus alata* (J. Wen 17892; Mexico, Chiapas); (B) *C. trifoliata* (J. Wen s.n.; Unknown); (C) *C. cacuminis* (J. Wen 18527; Mexico, San Luis Potosi); (D) *C. cucurbitina* (J. Wen 18420; Mexico, El Mixcuate); (E) *C. tiliacea* (J. Wen 17926; Mexico, Puebla); (F) *C. verticillata* (A. Quintanar-Castillo 195; Mexico, Mexico City); (G) *C. verticillata* (A. Quintanar-Castillo 195; Mexico, Mexico City); (H) *C.* sp. nov. 3 (J. Wen 17888; Mexico, Chiapas).

at the stem of the Neotropical *Cissus* clade (28.1–33.9 mya; Manchester *et al.*, 2012*b*), *Ampelocissus parvisemina* at the crown of the clade formed by *Vitis* and *Ampelocissus robinsonii* (56.8–62 mya; Liu *et al.*, 2016), *Parthenocissus monasteriensis* at the crown of the clade formed by *Yua* and *Parthenocissus* (47.8–56 mya; You *et al.*, 2024) and *Saxuva draculoidea* at the stem of *Pseudocayratia* (33.9–37.8 mya; Herrera *et al.*, 2012). Additionally, we also constrained the crown of Vitaceae to 67.61–90.5 mya according to the plastome phylogenetic reconstruction of You *et al.* (2024). Best optimization parameters were obtained after a preliminary calibration (gradient-based optimizer opt = 1, autodifferentiation based optimizer optad =1, cross-validation based optimizer optcvad = 2), and we performed a random cross-validation with 200 000 iterations for penalized likelihood and 5000 for cross-validation to obtain the best smoothing value (1×10^{-20}). Finally, the inferred parameters and the best smoothing value were used as the input for the definitive time calibration analysis.

The resulting time-calibrated tree was pruned to include a single tip per taxon in the Neotropical *Cissus* clade (43 taxa) and all the Vitaceae specimens outside of this clade were removed. The biogeographic analyses were performed using BioGeoBEARS (Matzke, 2013). Six biogeographic areas were defined considering the distribution of *Cissus* species in the Neotropics (POWO, 2025) and the areas of endemicity observed based on our phylogenetic reconstruction: Southern USA, Western Mexico, Eastern Mexico, Central America, Caribbean and South America. Two types of models were run (DEC, dispersal event cladogenesis; and DIVALIKE, dispersal–vicariance) with and without incorporating the +J parameter, which considers the possibility of founder-event speciation (Matzke, 2014, 2022). We time-stratified the models in two periods: (1) from the crown of Neotropical *Cissus* to the closure of the Panama isthmus (32–3.5 mya; Jaramillo, 2018), and (2) from the closure of the Panama isthmus (3.5–0 mya). For each period, we built a dispersal probabilities matrix (Supplementary Data Table S2). AICc values were used to select the best model for each model type (DEC compared with DEC + J, and DIVALIKE compared with DIVALIKE + J). After that, we retained the most resolved model.

Morphological character evolution

Three macromorphological characters of taxonomic importance in *Cissus* (Lombardi, 2000; Wen, 2007) were examined based on specimen observations, specimen label annotation and species descriptions: leaf complexity (simple, trifoliate, 3- to 5-foliate or 7-foliate); flower colour (greenish or reddish flowers); and the degree of tendril ramification (branched or unbranched tendrils). Given that tendrils and inflorescences are not always present in herbarium specimens, the number of specimens considered for each trait dataset was different. The evolution of the three characters was studied by performing ancestral character reconstructions using the function ace of the R package ape (Paradis and Schliep, 2019) under different models (equal rates, ER; symmetrical rates, SYM; and all rates different, ARD). Our nuclear phylogeny for Neotropical *Cissus* was pruned to include exclusively those specimens for which we could obtain data for a certain trait (109 specimens for leaf type, 87 for tendril ramification and 73 for flower colour) and the three resulting pruned phylogenetic trees were used as the input for ancestral reconstruction models for each of the examined traits. Models were compared based on their AICc values, and the best model for each trait was retained.

In addition, seed morphology of the main Neotropical *Cissus* clades retrieved in our phylogenetic analyses was evaluated, as seed morphology is taxonomically significant for Vitaceae (Chen and Manchester, 2011). Fruits from eight representative species of different lineages of Neotropical *Cissus* were retrieved from the US National Herbarium specimens, giving priority to those species whose seed morphology has never been explored and to those specimens that were sequenced in this study. Mature fruits were boiled in water for 2 min to facilitate seed isolation from the fruit, following Chen and Manchester (2007). Ventral, dorsal and lateral images from seeds were recorded using a Leica DMC 5400 camera mounted on a Leica M205 C stereomicroscope. However, as the number of available seeds in our study and previous research (~13 Neotropical species) remains scarce and the morphological variability of *Cissus* seeds is high, showing similarity between lineages (see Results section; Chen and Manchester, 2011; Manchester *et al.*, 2012*a*), we decided not to perform an ancestral trait reconstruction for seed characters in this study.

Climatic and geographic analyses

A publicly available taxonomically curated database of *Cissus* species from Mexico and Central America was used for climatic and geographic analyses (A. Gallego-Narbón *et al.* unpubl. res.). The database includes 2767 occurrences of herbarium specimens representing 23 *Cissus* species. We filtered the database to include exclusively the species that we sequenced and combined *C. alata* and *C. microcarpa* occurrences under the name *C. alata* considering the evidence provided by our phylogenetic analyses (see Discussion section).

In order to assess the differences in the geographic and climatic conditions occupied by the species, we downloaded the 19 global bioclimatic layers and a layer of elevations from WorldClim 2.0 (Fick and Hijmans, 2017) with a resolution of 30 s and a datum of WGS84. The bioclimatic layers summarize the climatic conditions from 1970 to 2000. Climatic data were extracted from those layers for each of the localities in the database using the stack and extract functions of the package raster (Hijmans, 2022). In order to avoid spatial biases associated with sampling effort, we thinned the database using the spThin package (Aiello-Lammens *et al.*, 2015), including only localities occupied by a certain species that are separated ≥1 km from each other. The final database included 1978 occurrences. The correlation between variables for the resulting database was assessed by calculating Pearson's pairwise correlations and a hierarchical cluster analysis (functions cor and hclust implemented in R; R Core Team, 2025). For each cluster of variables with a correlation $r \geq 0.80$ we retained the most biologically relevant variable (Supplementary Data Fig. S1). A total of six bioclimatic variables were selected: bio01 (mean annual temperature), bio04 (temperature seasonality), bio10 (mean temperature of the warmest quarter), bio12 (mean annual precipitation), bio15 (precipitation seasonality) and bio18 (precipitation of the warmest quarter).

The selected variables were used as the input for a climatic principal component analysis (PCA), which allowed the assessment of the climatic space occupied by each of the taxa studied. The PCA analysis was performed using the prcomp function in R (R Core Team, 2025), and the values for each of the occurrences in the first two axes were extracted (PC1 and PC2). The results were plotted with the autoplot function (R Core Team, 2025), which scales the axes for easier visualization, considering all the occurrences together and for each of the main phylogenetic clades separately.

In order to study in detail the climatic and geographic space occupied by the studied taxa, we plotted the distribution of the six analysed climatic variables and the three geographic variables (latitude, longitude and altitude) for each clade and for each species using box plots and violin plots generated with the ggplot2 package (Wickham, 2016). We assessed if there were statistically significant differences between all the groups for a certain variable and for pairs of groups using Kruskal–Wallis and Wilcoxon tests, respectively, as implemented in the stat_compare_means function of the ggpubr package (Kassambara, 2022).

RESULTS

Sequence capture and phylogenetic relationships

Sequences were obtained for 99.7 % of the targeted nuclear regions (1010 regions) and 92.6 % of the targeted plastid regions (227 regions). After removal of low-quality samples, a total of 109 samples were considered for nuclear analyses and 108 for plastid analyses. After paralogue removal 684 loci were retained, with 1 245 859 bp in total, and the concatenated plastid alignment was 152 500 bp long.

Nuclear and plastid phylogenetic analyses (Fig. 3, Supplementary Data Figs S2 and S3) recovered high support for most of the relationships. Both nuclear and plastid analyses supported the existence of four main clades in Neotropical *Cissus* (Clades I– IV) with complete support (bootstrap support (BS) = 100 and $\mathrm{PP}_{\mathrm{local}} = 1.0$; Supplementary Data Figs S2 and S3). Clade I constituted the most diverse clade, including *C. alata* and allies, with a distribution across Mesoamerica, South America and the Caribbean region. Clade II included the widely distributed species *C. trifoliata* and the Caribbean *C. intermedia*. Clade III only included the Costa Rican endemic C. *patellicalyx*. Lastly, Clade IV was composed of *C. verticillata* and closely related species widely distributed across

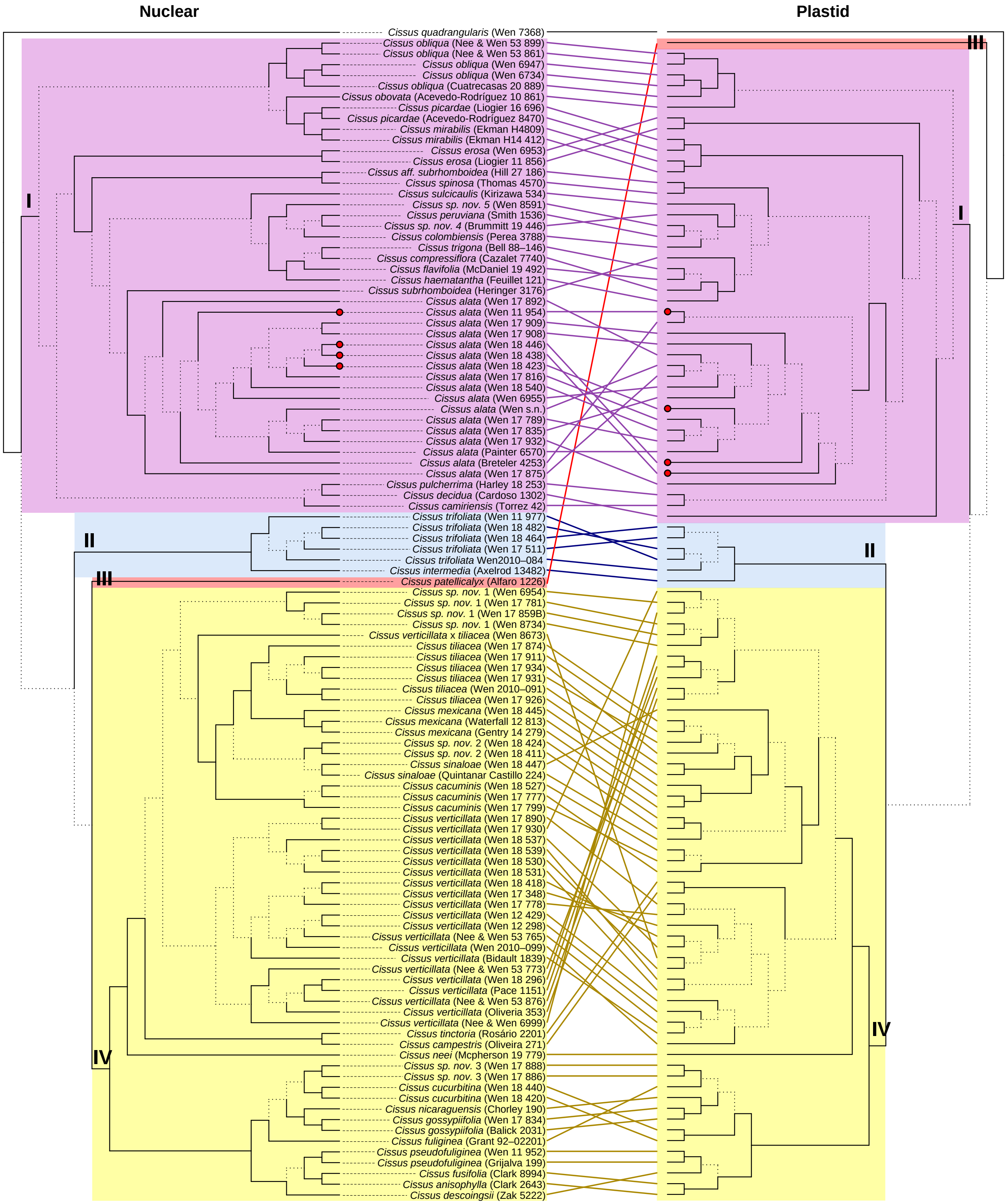

FIG. 3. Topological comparison between nuclear (left; Supplementary Data Fig. S2) and plastid (right; Supplementary Data Fig. S3) phylogenies. The four main clades of Neotropical *Cissus* retrieved in our phylogenetic reconstructions are highlighted in different colours: Clade I (purple), Clade II (blue), Clade III (red) and Clade IV (yellow). Dashed lines in the nuclear phylogenetic reconstruction represent branches not recovered in the plastid reconstruction, and vice versa. Red circles indicate samples originally identified as *C. microcarpa* (Supplementary Data Table S1).

Mexico, Central America and South America. The relationships between these four clades remained uncertain. The nuclear reconstruction (Fig. 3A, Supplementary Data Fig. S2) showed a polytomy of Clades I and II and a clade including Clades III and IV as sisters. In contrast, the plastid analysis (Fig. 3B and Supplementary Data Fig. S3) placed Clade III as the earliest-diverged clade, followed by Clade I, and Clade II and IV as sister lineages. Topological incongruence between nuclear and plastid reconstructions was also found within Clade I and Clade IV (Fig. 3).

Morphological character evolution

Simple leaves, greenish flowers and unbranched tendrils were inferred as the ancestral character states of Neotropical *Cissus*, with compound leaves, reddish flowers and branched tendrils originating several times during the evolution of the group (Fig. 4). Regarding leaf type, Clade I (*Cissus alata* clade) had mostly trifoliate species, with two independent shifts to simple leaves in *C. haematantha* and *C. picardae*, and one independent shift to more than three leaflets in the clade of the species *C. decidua* and *C. pulcherrima*. In contrast, Clade IV (*Cissus verticillata* clade) included mostly simple-leaved species, with the exceptions of two species of independent origin with three to five leaflets (*C. cucurbitina* and *C. mexicana*). Greenish flowers were ancestral for Clade I and Clade IV, and represented the most frequent state in Clade IV, with a single origin of reddish flowers in the clade including *C. cucurbitina*, *C. gossypiifolia*, and a new undescribed species (*Cissus* sp. nov. 3). Reddish flowers were the most common character state in current species of Clade I, and several shifts between both reddish and green flower colours occurred during the evolution of this clade. Unbranched tendrils were the ancestral state for Clade IV, with several independent origins of branched tendrils during the evolution of the clade, followed in some cases by reversals to the ancestral state of this character. Branched tendrils were the ancestral state for Clade I, with three independent shifts to unbranched tendrils. Seeds in Clades II (Fig. 5E, F) and IV (Fig. 5G, H) were

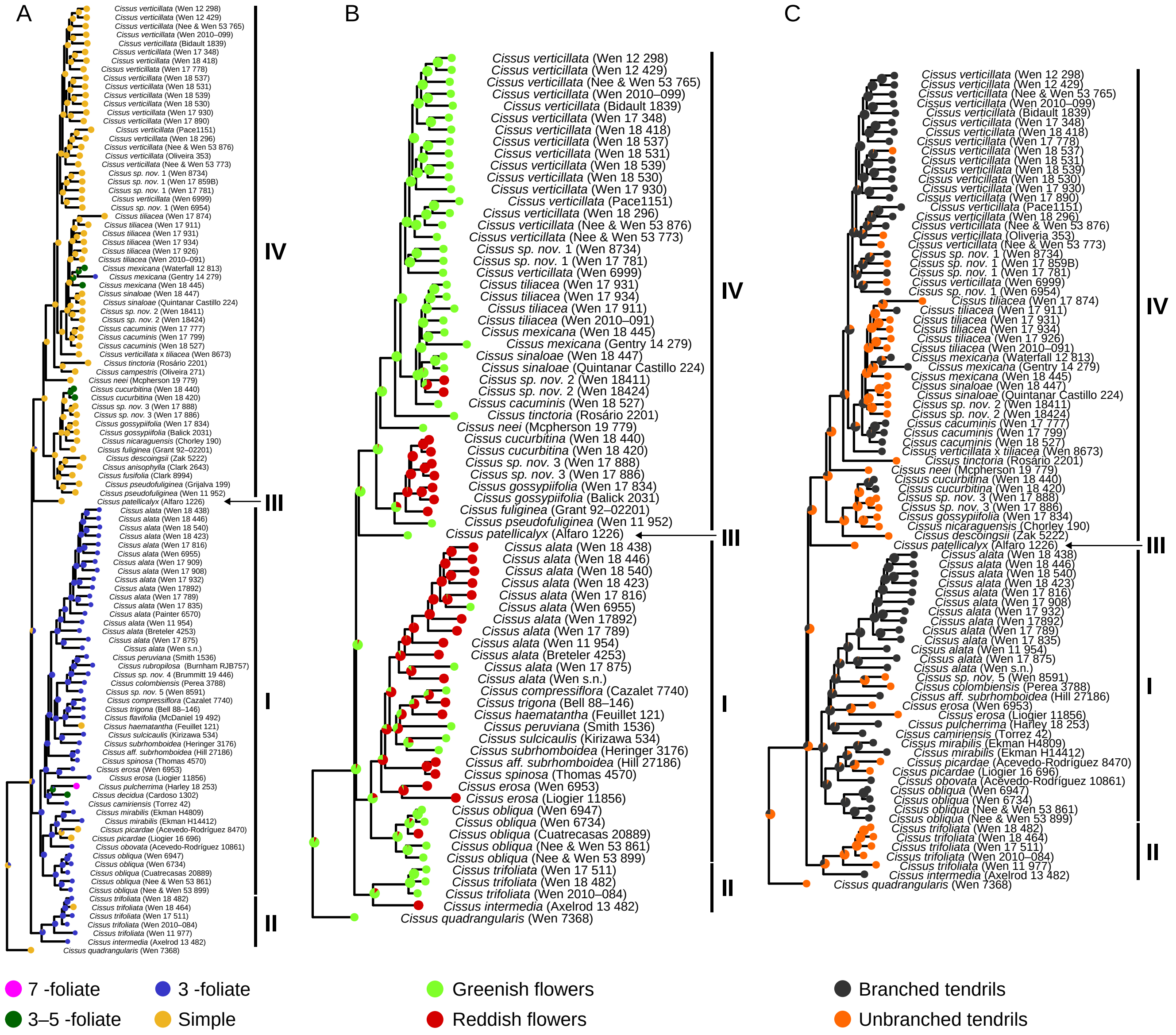

FIG. 4. Ancestral trait reconstructions for Neotropical *Cissus*. The main phylogenetic clades inferred in our phylogenetic analyses are highlighted and numbered. (A) Leaf complexity (as indicated in the key). (B) Flower colour (as indicated in the key). (C) Tendril type (as indicated in the key). Phylogenies were pruned to include exclusively those specimens with trait information.

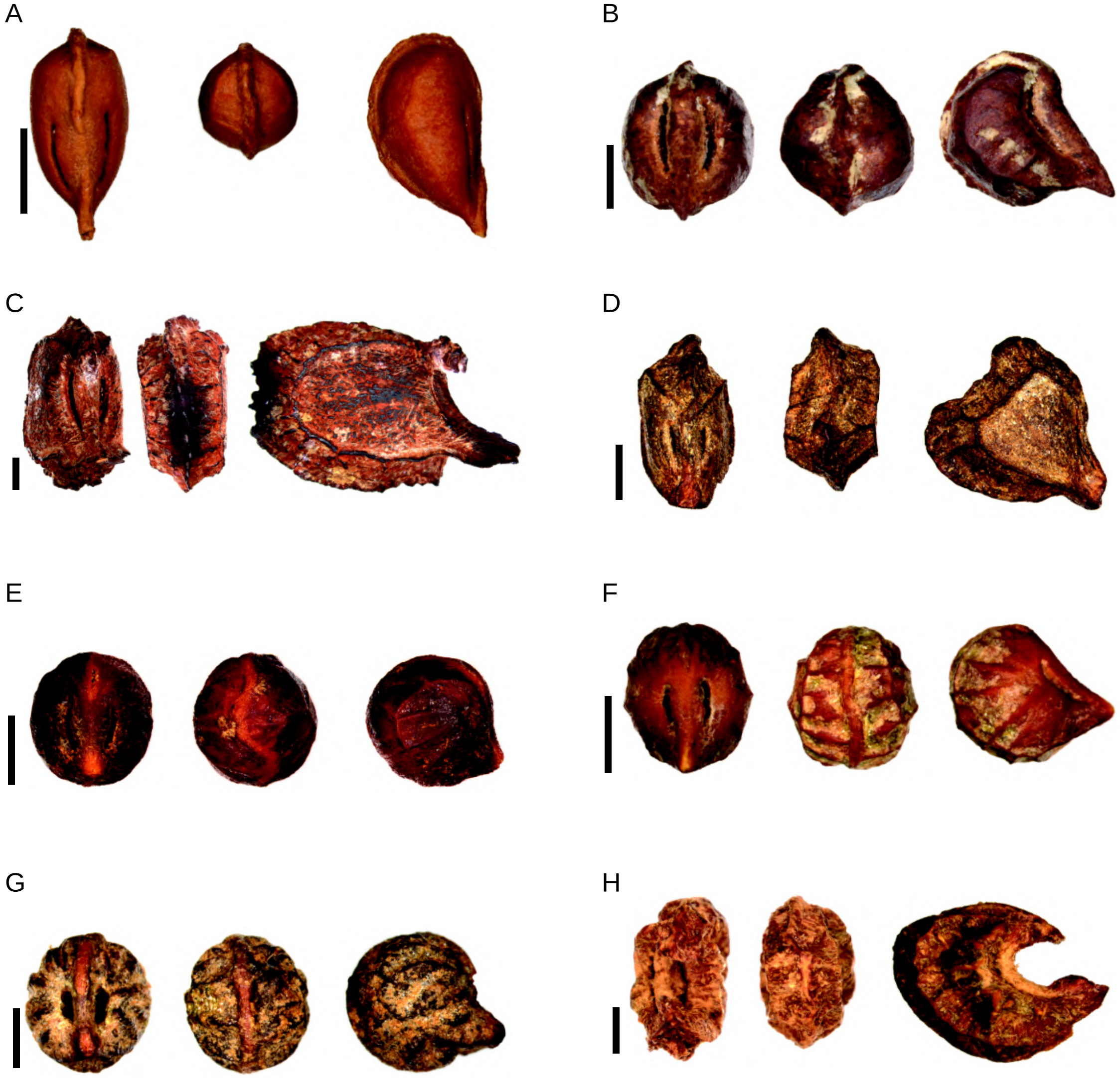

FIG. 5. Seed morphology of Neotropical *Cissus*. (A) *Cissus picardae* (P. Acevedo-Rodríguez 8470; Dominican Republic, La Altagracia); (B) *C. mirabilis* (E. L. Ekman H4809; Haiti, Port-Margot); (C) *C. flavifolia* (S. McDaniel 19492; Peru, Maynas); (D) *C. alata* (S. Mori 1786; Panama, Panamá); (E) *C. trifoliata* (D. S. Correll 14864; USA, Tex); (Fas) *C. intermedia* (F. Axelrod 13482; Dominican Republic, Pedernales); (G) *C. mexicana* (H. S. Gentry 14279; Mexico, Sonora); (H) *C. fusifolia* (J. L. Clark 8994; Ecuador, Zamora-Chinchipe). Ventral, dorsal and lateral views are shown from left to right. Scale bar = 2 mm.

mainly globose in shape, while those in Clade I (Fig. 5A–D) displayed diverse shapes from globose to elongated and laterally flattened. Seeds from *C. patellicalyx* (Clade III) could not be examined due to the lack of available material.

Time divergence and biogeographic analyses

The origin of the Neotropical *Cissus* clade was inferred during the early Oligocene (Supplementary Data Fig. S4), with an uncertain ancestral distribution according to the best biogeographic model (DIVALIKE, LnL = −95.96, AICc = 196.2; Fig. 6, Supplementary Data Table S3). However, one of the models with lower resolution (DEC; Supplementary Data Fig. S5A) inferred a widespread origin in Central America, South America and the Caribbean. Clade II (*Cissus trifoliata* clade) was the earliest-diverged lineage during the early Oligocene, and its crown distribution area was also uncertain. Its sister clade, including the rest of the New World *Cissus* lineages, exhibited a distribution across Central America and South America and diverged into two main lineages with crown ages during the middle Oligocene: (1) Clade I, with an ancestral South American distribution, and (2) Clade IV and Clade III, with an ancestral distribution in Central America.

The Caribbean was colonized twice from South America in Clade I, with one ancestral colonization during the Oligocene that led to a Caribbean clade (*C. obovata*, *C. picardae* and *C. mirabilis*), and a colonization by *C. erosa*. Central America was colonized four times from South America in Clade I by *C. alata*, *C. erosa*, *C. flavifolia* and *C. obliqua*, with *C. erosa* also spreading across Eastern Mexico and *C. alata* spreading across both Eastern and Western Mexico. During the evolutionary history of Clade IV, South America was colonized from Central America six independent times, with two ancestral colonizations during the early Miocene and four colonizations by *C. fuliginea*, *C. gossypiifolia*, *C. neei* and *C. verticillata*.

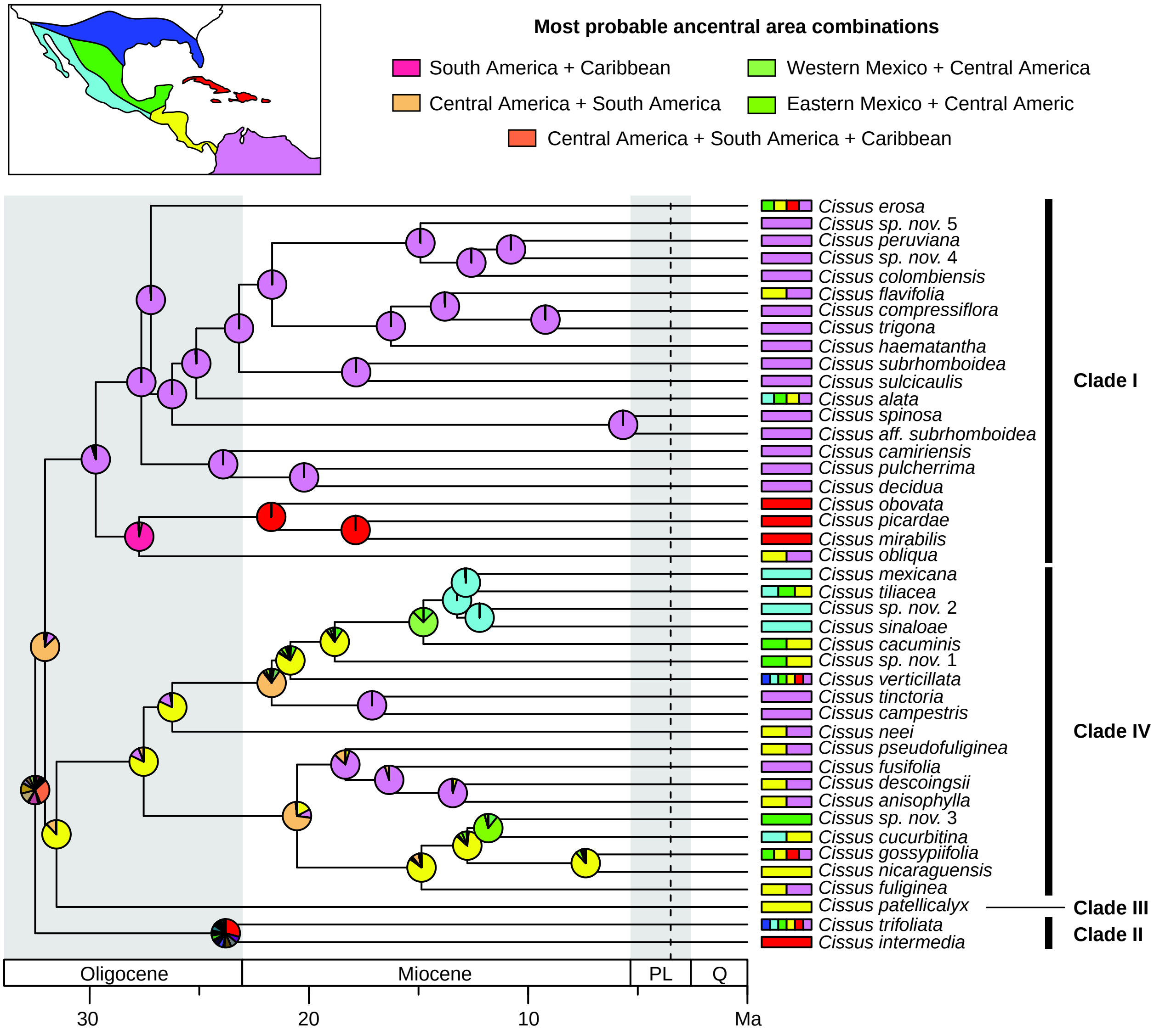

FIG. 6. Biogeographic reconstruction obtained with BioGeoBEARS using the DIVALIKE model, based on a pruned nuclear phylogeny including one sample for each of the Neotropical *Cissus* taxa. The outgroup was pruned. Six geographic areas were considered (as indicated in the key). Pie charts show ancestral range probabilities (see the key for the most probable area combinations). The dashed line represents the formation of the Isthmus of Panama (3.5 mya; Jaramillo, 2018). Time-calibrated phylogeny is based on Supplementary Data Fig. S4.

Western Mexico was colonized at least once from Central America, with one ancestral colonization during the middle Miocene forming a clade that includes most Western Mexico species (*C. mexicana*, *C. sinaloae*, *C.* sp. nov. 3 and *C. tiliacea*), and a putative colonization by *C. cucurbitina*. Eastern Mexico was colonized both from Central America (*C. cacuminis*, *C.* sp. nov. 1, *C.* sp. nov. 3) and from western Mexico (*C. tiliacea*).

Climatic space of Mexican and Central American Cissus

The first two components of the climatic PCA accounted for 67.79 % of the climatic variability in the study area (Fig. 7A). The first axis (PC1) was mostly determined by the variables bio12 (mean annual precipitation, 28 % of PC1), bio04 (temperature seasonality, 28 %) and bio18 (precipitation of the warmest quarter, 21 %). This component goes from localities with high precipitations and very stable temperatures throughout the year (negative PC1) to localities with low precipitations and high temperature variability throughout the year (positive PC1). The second axis (PC2) was mostly determined by bio01 (mean annual temperature, 25 % of PC2), bio10 (mean temperature of the warmest quarter, 25 %) and bio15 (precipitation seasonality, 17 %). This component goes from localities with warm temperatures and high precipitation seasonality (negative PC2), to localities with mild temperatures and stable precipitations throughout the year (positive PC2).

Three of the four major clades exhibited broad climatic niches (Clade I, Clade II and Clade IV; Fig. 7B, C, E), On the other hand, Clade III, which included exclusively *C. patellicalyx*, comprised a narrow area of the climatic space in the upper left quadrant (low PC1 and high PC2) characterized by cold temperatures and high precipitation stable

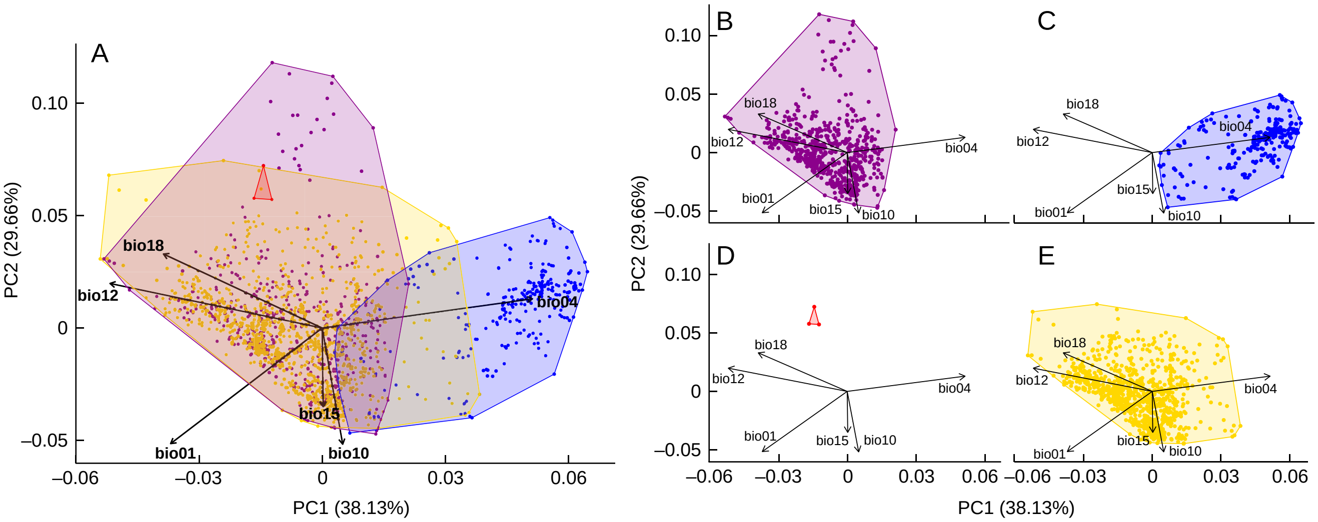

Fig. 7. Climatic PCA showing the climatic space occupied by each of the occurrences (A) and showing the occurrences for each phylogenetic clade separately (B–E). Polygons delimit the climatic space occupied by each clade. The colours represent the phylogenetic clades (Clade I, purple; Clade II, blue; Clade III, red; Clade IV, yellow). The contributions of the climatic variables are included: bio01 (mean annual temperature), bio04 (temperature seasonality), bio10 (mean temperature of the warmest quarter), bio12 (mean annual precipitation), bio15 (precipitation seasonality), bio18 (precipitation of the warmest quarter). The axes were scaled for easier visualization.

throughout the year. Clades I and IV exhibited the broadest climatic niches (Fig. 7B, E), which overlapped significantly. Most of the localities occupied by both clades were distributed in the lower left quadrant (low PC1 and low PC2), under warm temperatures and high precipitation, stable throughout the year, characteristic of typical tropical climates. However, Clade I occupied the coldest areas (highest PC2) not endured by Clade IV, while Clade IV endured drier areas (medium to high PC1) that cannot be occupied by Clade I. Clade II, encompassing *C. trifoliata*, occupied mostly the lower right quadrant (high PC1 and low PC2), occupying dry localities with high temperature and precipitation variability that cannot be endured by the other clades, characteristic of temperate climates. Violin plots showed significant differences in PC1 for the four clades ($P < 0.001$; Supplementary Data Fig. S6D), with lowest mean values in Clade III, followed by Clade I, Clade IV and Clade II. Clade III exhibited the highest PC2, followed by Clade II ($P < 0.001$; Supplementary Data Fig. S6E), with Clades I and IV similar in this axis.

Geographic and climatic differences in Cissus

Clade comparisons showed strong latitudinal, longitudinal and altitudinal differences between clades ($P < 0.001$; Supplementary Data Fig. S6A–C). Clade II occupied northwestern areas not occupied by the rest of the clades. Clades I and IV exhibited wide geographic distributions that significantly overlapped and encompassed also the narrow geographic space occupied by Clade III. However, pairwise comparisons showed that Clade IV expanded to the northwest while Clade I expanded to the southeastern areas ($P < 0.0001$; Supplementary Data Fig. S6A, B). Most localities occupied by Clades I, II, and IV were at lower elevations, <1000 m, while the highest elevations were occupied by Clade III ($P < 0.01$; Supplementary Data Fig. S6C).

Clade II differed strongly from Clade I and Clade IV for all the studied climatic variables ($P < 0.0001$; Supplementary Data Fig. S6F–K), generally occupying colder and drier areas with higher temperature and precipitation seasonality than clades I and IV. Clades I and IV exhibited differences in temperature seasonality (bio04, $P < 0.0001$), temperature of the warmest quarter (bio10, $P < 0.001$), and mean annual precipitation (bio12, $P < 0.0001$), with higher temperatures in Clade IV and higher precipitations in Clade I. Differences in geographic and climatic variables between the species can be observed in Supplementary Data Fig. S7.

DISCUSSION

Phylogenetic relationships and cytological incongruence

We confirmed the monophyly of Neotropical *Cissus* (Supplementary Data Fig. S4), in agreement with previous studies (Liu *et al.*, 2013; You *et al.*, 2024). We observed that the Neotropical clade is sister to a clade that includes mostly African species (Supplementary Data Fig. S4), but extensive analyses with more species outside the Neotropics are needed to test this relationship. Based on the nuclear and plastome trees from our phylogenomic data, four clades can be recognized for the Neotropical species (Fig. 3). Rodrigues *et al.* (2014) provided a phylogenetic analysis of *Cissus* species, emphasizing the Neotropical species but sampling only a small proportion of the species from Mexico and Central America. However, the main *Cissus* lineages inferred by Rodrigues *et al.* (2014) are congruent with our phylogenetic reconstructions (Fig. 3).

Clade I includes *C. alata* and its closely related species, including mostly trifoliate species with the exceptions of *C. picardae* and *C. haematantha* (with simple leaves), *C. decidua* (3- to 5-foliate) and *C. pulcherrima* (7-foliate; Figs 3 and 4).

According to our sampling, Clade I seeds are characterized by a prominent beak (Fig. 5A–D), while displaying a highly variable morphology. Two Caribbean species (*C. picardae* and *C. mirabilis*; Fig. 5A, B) possess globose to elongated seeds with smooth surfaces. On the other hand, elongated and laterally flattened seeds with rugose surfaces were found in samples in Central America and South America (*C. flavifolia* and *C. alata*; Fig. 5C, D). This seed morphology is similar to that of the seed fossil *C. lombardii* from Peru (Manchester *et al.*, 2012*a*), and we hypothesize that Clade I is evolutionarily most closely related to this fossil. However, several African species in early-diverged clades of *Cissus* exhibit a similar seed morphology (Adams *et al.*, 2016). Additional research with increased seed sampling is needed to test this hypothesis.

Clade II consists of only two species: *C. trifoliata* and *C. intermedia*, with globose seeds and curved chalazas (Fig. 5E, F). Clade III has only one species from Central America, *C. patellicalyx*, which shows highly discordant placement between the nuclear and plastid trees (Fig. 3). This species has simple leaves, greenish flowers and unbranched tendrils (Fig. 4). Due to the rarity of this species (a narrow endemic of Costa Rica; Lombardi, 2005), its seed morphology could not be assessed in this study. Clade IV is dominated by simple-leaved, green-flowered species, including the widespread *C. verticillata* and *C. tiliacea*. Clade IV also contains a 3- to 5-foliate, red-flowered subclade including *C. cucurbitina*, *C. gossypiifolia* and *C.* sp. nov. 3, as well as the green-flowered and 3- to 5-foliate species *C. mexicana* (Fig. 4). Seeds from Clade IV display two main morphology types, which correlate with two different subclades. Globose seeds, similar in shape and size to those in Clade II, were found in the subclade that includes mostly Western Mexican species (*C. mexicana*, *C. tiliacea*, *C. verticillata*, *C. campestris*; Fig. 5G; Chen and Manchester, 2011; Manchester *et al.*, 2012*a*). Seeds with irregular shapes and a prominent beak were found in the subclade that includes *C. fusifolia*, *C. descoingsii* and *C. fuliginea* (Fig. 5H; Chen and Manchester, 2011). In general, several current Neotropical species show seed morphologies similar to those of fossils of *Cissus* seeds from Peru (*C. willardii*; Manchester *et al.*, 2012*a*) and Panama (*C. correae*; Herrera *et al.*, 2024). However, the current diversity of seed morphology in the Neotropical species (Fig. 5; Chen and Manchester, 2011; Manchester *et al.*, 2012*a*) and the lack of data for several crucial lineages (e.g. *C. patellicalyx*) require additional studies to fully understand seed morphology evolution in Neotropical *Cissus*, as seed fossils and their accurate placement are extremely relevant for accurate time calibrations of *Cissus* and Vitaceae.

Previous studies on Neotropical *Cissus* have relied heavily on morphological characters, as treated by Lombardi (2007), although he acknowledged the need to study the relationships between species through DNA analyses. Prior molecular analyses of Neotropical *Cissus* are scarce, based on a few loci, and/or mainly focused on South American species (Liu *et al.*, 2013; Rodrigues *et al.*, 2014). Therefore, this study constitutes the most complete molecular study of Mexican and Central American *Cissus*, and the first Neotropical *Cissus* research to incorporate hundreds of nuclear loci. We observed that most of the Mexican species of *Cissus* are grouped into Clade IV (Fig. 3). Rodrigues *et al.* (2014) inferred that *C. verticillata* and *C. tiliacea* were part of the same group, as we detected in our phylogenetic reconstructions (Fig. 3). Regarding the remaining Mexican species, Liu *et al.* (2013) suggested a close relationship between *C. alata* and *C. erosa*. This pattern is partially supported by our phylogenomic reconstructions, as both species are included in Clade I, comprising mostly South American species (Fig. 3). However, our additional sampling showed that these species are not closely related in Clade I and colonized Mexico and Central America independently (Figs 3 and 6). Finally, the widespread species *C. trifoliata* is part of an independent clade alongside the Caribbean *C. intermedia* (Fig. 3), in agreement with previous phylogenetic studies that show that *C. trifoliata* is isolated from the main Neotropical *Cissus* lineages (Liu *et al.*, 2013; Rodrigues *et al.*, 2014). In addition, the close relationship of these two species is also supported by their similar seed morphology (Fig. 5E, F).

Our phylogenetic reconstructions (Fig. 3) do not show a strong correlation with the morphological groups described by Lombardi (2007), who recognized high morphological variability of *Cissus* in the Neotropics. He divided Neotropical *Cissus* into 13 informal groups based on morphology, of which three were endemic to South America. Although some of these informal groups matched related species on our nuclear coalescent tree (Fig. 3A), discrepancies prevailed. For instance, Lombardi (2007) speculated that *C. mexicana* was related to *C. trifoliata* because of their similar morphological characteristics. However, we observed that the two species belonged to different lineages, with *C. trifoliata* forming a distinct clade with *C. intermedia*, and *C. mexicana* grouping with *C. tiliacea* in Clade IV (Fig. 3). In addition, *C. alata* and *C. microcarpa* are intermixed in Mexico and Central America (Fig. 3), so the division of these two taxa in two different morphological groups according to Lombardi (2000) is not supported in our phylogenetic analyses.

It is also remarkable that we observed signals of cytonuclear discordance in our reconstructions (Fig. 3). In this regard, *C. patellicalyx* (Clade III) exhibited incongruent phylogenetic placement between nuclear and plastid reconstructions, being sister to Clade IV according to our nuclear analysis (Fig. 3A) and showing the earliest divergence in the Neotropical *Cissus* clade in our plastid analysis (Fig. 3B). *Cissus patellicalyx* reflects ancestral morphological characters according to our morphological reconstruction (Fig. 4), which could provide support for an early divergence of the species. In future analyses, seed morphology can help shed light on the evolution of this unique species. On the other hand, Lombardi (2005) reported similarities between this species and the species of Clade IV *C. verticillata* and *C. tiliacea*. Therefore, both phylogenetic relationships and morphological characters seem to suggest a hybrid origin of *C. patellicalyx*. In addition, we found an uncertain placement of Clade II, forming a polytomy with clades I and III–IV according to the nuclear reconstruction (Fig. 3A) and being sister to Clade IV according to the plastid reconstruction (Fig. 3B). The low resolution for the placement of this clade alongside its morphological characters intermediate between Clade I and Clade IV (trifoliate leaves in *C. trifoliata* typical of Clade I and green flowers typical of Clade IV; Fig. 4) suggest a hybrid origin of Clade II. Lastly, while *C.*

verticillata and the Western Mexican species clade were different entities according to our nuclear reconstruction (Fig. 3A), they were intertwined according to the plastid reconstruction (Fig. 3B). Lombardi (2000) differentiated *C. verticillata* from the most widespread species of this clade (*C. tiliacea*) by pedicel morphology and bud corolla shape. Lombardi (2000) described both species as capable of developing branched and unbranched tendrils, which is in agreement with our morphological analysis (Fig. 4). However, *C. verticillata* has predominantly branched tendrils, and *C. tiliacea* usually bears unbranched tendrils. Our study thus suggests an influence of hybridization in the evolution of the Neotropical *Cissus* clade.

Phylogenetic assessment on taxonomy of several Mexican Cissus *species*

The Cissus alata *complex.* Various researchers have relied on different characters to differentiate *Cissus alata* (= *C. rhombifolia*) and *C. microcarpa*. For example, Standley (1923) used leaflet pubescence, with *C. microcarpa* having a glabrous abaxial leaflet surface, while *C. rhombifolia* (= *C. alata*) was considered to have pubescent leaflets beneath, at least along the veins. Krings (1997) regarded *C. rhombifolia* (i.e. *C. alata*) as having more or less rhombic terminal leaflets, and petioles and stems frequently winged, whereas *C. microcarpa* would have ovate to obovate, elliptic or lanceolate terminal leaflets, and usually lacking winged stems. Lombardi (2000, 2015) described the leaflets in the reproductive portion of the branches as notably reduced in relation to the leaflets in the lower vegetative portion of the stem in *C. alata*, while *C. microcarpa* does not have the leaflets conspicuously reduced in size on reproductive branches. He also considered that the inflorescences of *C. microcarpa* may or may not contain malpighiaceous (i.e. armed) trichomes, while the inflorescences of *C. alata* lack this type of armed trichome, suggesting that the morphology of *C. microcarpa* is rather plastic. Through our herbarium studies, we found that the leaflet pubescence, terminal leaflet shape and presence/absence of wings on stems did not correspond to the phylogenetic subclades of the *C. alata* complex clade in Mexico. The lack of discernible differences between *C. alata* and *C. microcarpa* through phylogenetic (Fig. 3) and morphological (Fig. 4) analyses resulted in the tentative hypothesis that the two taxa should be considered as one broadly defined species or species complex, at least in Mexico and the northern part of Central America. The *C. alata* complex is not only morphologically variable; geographically it also ranges from Mexico to the Caribbean and southward to Bolivia. Phylogeographic sampling across its geographic range is required to untangle the taxonomy in the complex. Yet it seems taxonomically sound to recognize the complex as *C. alata*, which has the nomenclatural priority, as the working hypothesis.

Cissus mexicana. *Cissus mexicana* has been a poorly known species from Western Mexico. It is a perennial succulent climber with palmate leaves, each consisting of three to six narrow leaflets, green flowers and unbranched tendrils. Standley (1923) regarded it as a doubtful species, possibly a synonym of *Parthenocissus quinquefolia* (L.) Planch. Lombardi (2007) suggested that it is probably related to *C. trifoliata*. Our phylogenetic analyses place it in Clade IV (Fig. 3), sister to the morphologically highly distinct *C. tiliacea*, which also grows in the xeric habitats in Western Mexico but bears simple leaves (Fig. 4). *Cissus mexicana* has globose and rugose seeds with a prominent beak, similar to those of the same lineage of Clade IV (Fig. 5G). *Cissus mexicana* clearly needs to be recognized and deserves conservation efforts as a narrow endemic species in the xeric habitats of western Mexico.

Cissus sinaloae *as a distinct species.* *Cissus sinaloae* was described by Standley (1923) as a narrow endemic from the state of Sinaloa. It is defined as glabrous with 8–12 cm long oblong–ovate leaves and 6–12 mm long petioles (Standley, 1923). The inflorescences are long, pedunculate and umbellate cymes that develop obovoid fruits. Phylogenetically, *C. sinaloae* groups with the clade of *C. mexicana* and *C. tiliacea* (Fig. 3), with the latter exhibiting high morphological variation. Treating *C. sinaloae* as part of *C. tiliacea* would require synonymizing the morphologically highly distinct *C. mexicana* under *C. tiliacea*. Considering the morphological distinction and the phylogenetic evidence, we argue for the recognition of *C. sinaloae* as a distinct species and call for future integrative analyses of the *C. tiliacea* complex based on morphology, phylogenetics, population biology and ecology, across the xeric zone of Western Mexico. The highly broad delimitation of *C. tiliacea* does not benefit the conservation of narrow endemics such as *C. mexicana* and *C. sinaloae*.

Climatic and geographic heterogeneity in the Mexican transition zone

Our geographic and climatic analyses provide evidence of spatial and climatic differentiation between the recovered phylogenetic clades of *Cissus* in Mexico and Central America and between the different Mexican species (Fig. 7, Supplementary Data Figs S6 and S7). We detected heterogeneous patterns among the different lineages, which was expected considering the wide heterogeneity provided by the transition zone. In this regard, Mexico has been considered a transitional area from a biogeographic perspective, dividing Nearctic and Neotropical biotas (Morrone, 2020). Additional research also pointed out that Mexico encompasses areas with climatic conditions typical of tropical latitudes, typical of subtropical latitudes, and also transitional areas with climates between tropical and subtropical climates (Coca-de-la-Iglesia *et al.*, 2024). Geographic heterogeneity also plays a relevant role in the transitional character of Mexico, as five main mountain ranges with different altitudes are located in the country (Fig. 1B). These mountain ranges also differ in their origin, with the Sierra Madre Oriental originated during the Eocene, the Sierra Madre Occidental and the Sierra Madre del Sur during the Oligocene, and the Trans Mexican Volcanic Belt during the Miocene (Morrone, 2010; Mastretta-Yanes *et al.*, 2015). These heterogeneous geographic and climatic conditions led to two main ecological strategies of *Cissus* in Mexico.

On the one hand, we detected several widespread species in Mexico and Central America. *Cissus alata* and *C. verticillata* exhibit wide geographic ranges generally under 1500 m of elevation (Supplementary Data Fig. S7A–C) and endure heterogeneous and very similar ecological conditions (Supplementary Data Fig. S7D, E). These two species belong to different phylogenetic clades (Fig. 3) and exhibit differences in relevant traits (Figs 2, 4 and 5). Their similar distribution range could be partly explained by their early divergence during the evolution of Neotropical *Cissus* in the Oligocene (Fig. 6), as previous research suggests that distribution range size in plant lineages tends to be similar in lineages of similar age (Alzate *et al.*, 2025). However, the long branches in the phylogeny for these two species can also be interpreted as extinction events (García-Verdugo *et al.*, 2019). In that scenario, the ecological convergence between the two species could be the result of environmental filtering.

On the other hand, we found endemic species with restricted geographic and ecological ranges in Mexico (e.g. *C. mexicana*, *C. sinaloae*; Supplementary Data Fig. S7). These species inhabit the arid environments of northwestern Mexico and are part of Clade IV in a single clade that colonized western Mexico around the middle Miocene (Fig. 6) and also show climatic differentiation relative to other *Cissus* species distributed in Mexico and Central America (Supplementary Data Fig. S7). This result is in agreement with existing literature supporting the idea that Western Mexico's xeric environments constitute relevant areas of endemicity for different animal and plant taxa (Marshall and Liebherr, 2000; Pliego-Sánchez *et al.*, 2021). In fact, xeric environments constitute the main hotspots of ancient and recent endemics (palaeo- and neo-endemism) in Mexico for different plant families (Sosa *et al.*, 2018). The period of origin of the arid clade of Neotropical *Cissus* is temporally consistent with the Miocene Climatic Optimum, which is related to a global tendency towards warming and aridification (Steinthorsdottir *et al.*, 2021). In fact, this period is considered the origin of the 'succulent biome', encompassing succulent plant species that are distributed in arid and semiarid environments, mostly in tropical latitudes (Hernández-Hernández *et al.*, 2014). In this regard, western Mexico constitutes one of the areas with the highest probability of finding succulent species according to models (Ringelberg *et al.*, 2020). Several Western Mexican *Cissus* species are succulent (Xin *et al.*, 2022), showing adaptation to the arid conditions of this area. The evolution of this western arid lineage in *Cissus* can also be explained by geographic reasons. The formation of the Trans-Mexican Volcanic Belt (Fig. 1) during the Miocene could have acted as a geographic and climatic barrier isolating this area (Morrone, 2010). This mountain system includes elevations up to 5000 m, providing conditions similar to those encountered in temperate latitudes (Ruiz-Sanchez and Specht, 2013). These conditions make the Trans-Mexican Volcanic Belt a physical and climatic barrier that has traditionally been considered as a north–south axis dividing Mexico's biotas (Marshall and Liebherr, 2000; Morrone, 2010). In fact, it has been previously identified as a barrier to gene flow between northern Mexico and southern Mexico in several plant taxa (e.g. *Hunnemannia fumariifolia*, Papaveraceae; Ruiz-Sanchez *et al.*, 2012; *Liquidambar styraciflua*, Altingiaceae; Ruiz-Sanchez and Ornelas, 2014). In addition, there is growing evidence that Mexico also is biogeographically divided in an east–west axis (Morrone, 2010). In the case of *Cissus*, the Sierra Madre Occidental could have acted as a driver of isolation. Therefore, climatic and geographic isolation from the south (the Trans-Mexican Volcanic Belt) and from the east (the Sierra Madre Oriental) could explain the origin and speciation of this arid endemic lineage of *Cissus* in Mexico.

Despite the geographic and ecological differentiation of *Cissus* in Mexico, it is important to underline that most of the species are limited to lower and middle elevations under 2000 m (Supplementary Data Figs S6 and S7), not enduring the colder conditions in high elevations. In fact, climatic analyses indicate that *Cissus* cannot endure extremely cold conditions (higher PC1 values; Fig. 7). This is congruent with the ecological character of *Cissus* as a whole, which is regarded as a pantropical genus (Wen *et al.*, 2018). Therefore, this distribution supports the idea that mountain ranges act as ecological barriers for several *Cissus* species in Mexico, which is not the case in Central America, where *Cissus* can survive at higher elevations (*C. patellicalyx* and *C. obliqua*; Supplementary Data Fig. S7C). This pattern is probably associated with the transitional character of Mexico (Morrone, 2020), which constitutes the north latitudinal limit for several *Cissus* species in the Americas (e.g. *C. alata* and *C. tiliacea*; Fig. 2). Considering that northern latitudinal limits often constitute the most stressful temperature conditions for plants (Normand *et al.*, 2009), it is reasonable that this tropical genus cannot endure the colder conditions and the higher temperature contrasts found in the mountain ranges in Mexico in contrast with those in Central America. Further research is necessary to estimate the influence of the different geographic barriers as drivers of speciation of *Cissus* in the Neotropics.

Conclusions

This work represents a significant integrative evolutionary study on *Cissus* in the Neotropics, incorporating phylogenomics, morphology and geographic and climatic data, with an emphasis on Mexico and Central America. We recovered four main clades of *Cissus* in the Neotropics and inferred several independent origins for the species currently distributed in the Mexico biogeographic transition zone. The discordance in relationships inferred from nuclear vs plastid genomes among the major clades may reflect a pattern of early hybridization/introgression. Our high genetic and species sampling allowed the detection of hidden diversity in Mexico (*C. sinaloae* and several potential undescribed new species) and suggested the conspecificity of two taxa (*C. alata* and *C. microcarpa*), at least in Mexico. Additionally, we detected significant geographic and climatic differences between the different phylogenetic clades and between the species in Mexico and Central America. While mountain ranges constituted geographic and climatic barriers that could act as drivers of speciation in northwestern Mexico, several other taxa exhibited a widespread distribution and wide climatic niches and could overcome those barriers. Further morphological and genomic research is needed to refine the taxonomy of *Cissus* in Mexico and Central America, which has been greatly underexplored. In addition, the roles of climate, colonization events and geographic barriers in the evolution and diversification of Neotropical *Cissus* must be studied in greater detail.

SUPPLEMENTARY DATA

Supplementary data are available at *Annals of Botany* online and consist of the following. Figure S1: cluster analysis for variable selection. Figure S2: nuclear coalescent-based phylogenetic reconstruction. Figure S3: plastid maximum likelihood phylogenetic reconstruction. Figure S4: time-calibrated phylogeny. Figure S5: additional biogeographic reconstructions. Figure S6: violin plots of climatic and geographic variables by clade. Figure S7: violin plots of climatic and geographic variables by species. Table S1: sample list. Table S2: dispersal probabilities for biogeographic analyses. Table S3: biogeographic model comparison.

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AUTHOR CONTRIBUTIONS

A.J.C.: conceptualization, data curation, formal analysis, funding acquisition, investigation, resources, visualization, writing – original draft, writing – review and editing; A.G.-N.: conceptualization, data curation, formal analysis, funding acquisition, investigation, resources, visualization, writing – original draft, writing – review and editing; C.K.: data curation, formal analysis, investigation, visualization, writing – original draft, writing – review and editing; A.Q.-C.: – investigation, resources, writing – original draft, writing – review and editing; G.J.: investigation, writing – review and editing; M.R.P.: investigation, resources, writing – review and editing; J.W.: conceptualization, funding acquisition, investigation, project administration, resources, writing – original draft, writing – review and editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY

Raw reads are available in the BioProject PRJNA1338494.

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