

## Phylogenetic relationships and character evolution in *Yucca* (Agavoideae, Asparagaceae)

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### Abstract

The current classification of *Yucca* (Asparagaceae, Agavoideae) is based on morphological characters, mainly on fruit type, caulescence, leaf margin and inflorescence type. To investigate the evolution of these characters, and their potential taxonomic significance as synapomorphies for some groups within *Yucca*, a phylogenetic analysis was performed with 44 *Yucca* and eight outgroup species. Divergence times were estimated to produce a suitable phylogenetic framework for the investigation of morphological character evolution. Maximum likelihood and Bayesian inference analyses showed closer phylogenetic relationship between *Hesperoyucca* and *Hesperaloe* than either of these two genera with *Yucca*. The series previously proposed within the genus were not recovered as monophyletic, but based on fruit type we recovered two main clades which we name here clade Aloifolia and clade Rupicola. The ages of the *Yucca* stem and crown groups were estimated at 14.34 (95% HPD: 14.64–14.2) and 7.45 (95% HPD: 11.31–3.48) million years, respectively. More recent diversification events occurred in both the species with fleshy and dry fruits. *Yucca* is monophyletic with two main clades, corresponding to the species with dry fruit (clade Rupicola) and fleshy fruit (clade Aloifolia). Partial geographical concordances were observed in both clades. The dispersal type could be a key character in the diversification of the genus. Leaf margin, caulescence, and inflorescence type are not consistent with phylogenetic relationships.

**Key words:** ABK clade, ancestral-characters, classification, fruit type, *Hesperoyucca*, morphological-evolution, phylogeny, yuccas

### Introduction

*Yucca* Linnaeus (1753: 319) is an emblematic genus of mainly succulent, caulescent, acaulescent or semi-acauliscent plants with economic and cultural importance. Plants are used as ornamentals, living fences and windbreaks; their fibers are extracted from the leaves to make twines, baskets, and other utensils; and their fruits and flowers are edible (Matuda & Piña, 1980; Sheldon, 1980; Bartlett, 2019). The roots, stems, leaves, shoots, flowers, and seeds of several species of this genus contain antioxidant, antimicrobial, anti-inflammatory, antidiabetic and hypocholesterolemic

compounds (Cheeke *et al.*, 2006; Patel, 2012). Moreover, the genus is widely recognized because of its pollination mutualism with yucca moths (Pellmyr *et al.*, 2007; Smith *et al.*, 2008).

*Yucca* consists of 40 to 54 species that have been classified since the 19th century in sections based on the fruit type, and in series according to leaf and floral characteristics (Figure 1; Engelmann, 1871, 1873; Baker, 1880; Trelease, 1902, 1907, 1911; McKelvey, 1938, 1947; Clary, 1997). For example, McKelvey (1938, 1947) recognized nine series, six with dry fruits, and three with fleshy ones. However, to date, there is no consensus species number agreed upon by botanists who study the genus, nor is it clear whether the classification in series and sections based on morphology corresponds to natural groups, or if similarities in morphology are due to adaptive convergences to the environment in which these plants occur.

*Yucca* is, after *Agave* Linnaeus (1753: 323), the most diverse genus of the ABK clade (Agavoideae with bimodal karyotype, McKain *et al.* 2012), within Asparagaceae subfamily Agavoideae, where five chromosomes are long and 25 are short (Stevens, 2001; The Plant List, 2023). McKain *et al.* (2012; 2016) investigated the origin of the bimodal karyotype in the ABK clade and proposed that the duplication of the complete genome of the ABK clade may have provided genomic plasticity allowing rapid adaptation in arid habitats and new interactions with pollinators, creating niches for the radiation of new species. Diversification in the ABK clade has been studied and related with geographic convergence (geographic locations, similar environments) and pollinators. For example, Good-Avila *et al.* (2006) suggested that the colonization of arid environments and the relationships of *Agave* with nectarivorous bats drove its diversification and accelerated its speciation more actively in the last three million years, especially in *Agave* subgenus *Agave*. Moreover, Jiménez-Barron *et al.* (2020) considered that pollinators played an important role in the selection of the inflorescence type currently present in agaves. Heyduk *et al.* (2016a) studied CAM metabolism in *Yucca aloifolia* Linnaeus (1753: 319) and *Y. filamentosa* Linnaeus (1753: 319), which have hybridized to form *Y. gloriosa*, the latter being characterized by an intermediate C3-CAM metabolism.

The geographical distribution of *Yucca* ranges from Alberta in southern Canada to Guatemala (Engelmann, 1871, 1873; Speirs, 1979; Hurlburt, 2004). However, its greatest diversity and endemism occur in the arid and semi-arid zones of the southern United States of America and northern Mexico (Matuda & Piña, 1980; Clary, 1997). More than 50% of its species are microendemic and some species, such as *Yucca capensis* L.W. Lenz (1998: 289), *Y. coahuilensis* Matuda & Piña (1980: 120), *Y. linearifolia* Clary (1995: 394), *Y. necopina* Shinnars (1958: 408) and *Y. queretaroensis* Piña (1989: 52), have populations with few individuals (Lenz, 1998; Matuda & Piña, 1980; Clary, 1995; Locklear, 2017; Magallán-Hernández *et al.* 2014). The IUCN Red List (2021) includes 54 *Yucca* species: seven have been listed as Endangered (EN), one is Near Threatened (NT), 34 are Least Concern (LC), seven are Vulnerable (VU), and five are Data Deficient (DD). It should be mentioned that a few species on the IUCN Red List are considered as synonyms of other species according to The Plant List (2023). Caballero (2022) assessed eight species according to the Mexican conservation status assessment tool MER “Método de Evaluación de Riesgo de Extinción de Especies Silvestres en México” in Spanish (SEMARNAT, 2010), and proposed *Y. lacandonica* Gómez-Pompa & J. Valdés (1962: 43) as a species threatened with extinction. Some species are associated with a very particular habitat; for example, *Y. necopina* grows in deep coarse-textured soils eroded from sandstone strata by colluvial or alluvial processes (Locklear, 2017). Knowledge of the ecology, biogeography, and anatomy of many *Yucca* species is limited. Furthermore, the relationships with its most closely related genera remain controversial, and there is uncertainty regarding *Yucca*’s sister genus.

Previous studies have pointed out that *Hesperaloe* Engelmann (1871: 497) and *Hesperoyucca* (Engelmann) Baker (1892: 8) have a closer relationship with each other and that they are sister to *Yucca* (Bogler & Simpson, 1995; 1996; Clary and Simpson, 1995; Clary, 1997; Rocha *et al.* 2006; Smith *et al.* 2008; Halpin & Fishbein, 2013; Flores-Abreu *et al.* 2019). In turn, *Yucca* is the sister group of *Agave* sensu stricto, *Beschorneria* Kunth (1850: 844), *Polianthes* Linnaeus (1753: 216) and *Manfreda* Salisbury (1866: 78), according to Smith *et al.* (2008) and Halpin & Fishbein (2013). However, according to other studies, *Agave* sensu lato is more closely related to *Furcraea* Ventenat (1793: 65) and *Beschorneria* than to *Yucca* (Bogler *et al.* 2006; Jiménez-Barron *et al.* 2020).

With respect to the species relationships within the genus *Yucca*, Clary (1997) studied phylogenetic relationships using morphological characters and molecular data (ITS1 and ITS2), including 45 taxa of *Yucca* and four outgroup species. Her analysis showed only section *Yucca* to be monophyletic, whereas section *Clistocarpa* Engelmann (1871: 496) and series *Rupicolae* McKelvey (1947: 5) were more closely related to each other. Pellmyr *et al.* (2007), based on Amplified Fragment Length Polymorphism (AFLP) data of 38 *Yucca* taxa, recovered the three recognized sections *Chaenocarpa* Engelmann (1871: 496), *Clistocarpa* Engelmann (1871: 496) and *Sarcocarpa* Engelmann (1871: 496), and series *Rupicolae*, as monophyletic. Moreover, *Y. queretaroensis* was resolved as sister to the remainder of the species, some of which were paraphyletic in this study. Smith *et al.* (2008), studying the relationships of 34 *Yucca* species, based on six chloroplast regions and the AFLP markers of Pellmyr *et al.* (2007), recovered *Yucca* as





**FIGURE 1.** 1) *Yucca lacandonica*, 2) *Y. queretaroensis*, 3) *Y. reverchonii*, 4) *Y. brevifolia*, 5) *Y. linearifolia*, 6) *Y. gloriosa*, 7) *Y. baccata*, 8) *Y. valida*, 9) *Y. faxoniana*, 10) *Y. rostrata*, 11) *Y. sp.* Chihuahua, 12) *Y. aloifolia*, 13) *Y. madrensis*, 14) *Y. arizonica*, 15) *Y. thompsoniana*, 16) *Y. capensis*, 17–18) *Y. jaliscensis*, 19–20) *Y. rigida*, 21–22) *Y. grandiflora*, 23–24) *Y. elata*, 25–26) *Y. gigantea*, 27–28) *Y. torreyi*, 29–30) *Y. filifera*, 31–32) *Y. carnerosana*, 33–34) *Hesperoyucca whipplei*, 35–36) *Y. schidigera*, 37–38) *Y. coahuilensis*, 39–40) *Y. schottii* (Photographs by E. Solano & R. Ríos-Gómez; Figure 1 was prepared by B. Ayala-García).



**TABLE 1.** *Yucca* primers used in this study and references.

Nuclear	Region	Primer	Region	Primer	Reference
ITS	ITS-26S	TTTCTTTTCTCCGCT	<i>ITS</i> -18S	AAGTCGTAACAAGGTTTCCGTAGGTG	Bogler and Simpson 1996
ITS	ITS5	GGAAGTAAAGTCGTAACAAGG	<i>ITS</i> 4	TCCTCCGCTTATTGATATGC	White, 1990
ETS	18S-ETS	ACTTACACATGCATGGCTTAATCT	<i>Ast</i> -8	TTCCTCTCGTATCGTGCGGT	Baldwin and Markos 1998; Markos and Baldwin 2001
PPC	PPCX4F	ACTCCACAGGATGAGATGAG	PPCX5R	GCGCCATCATCTCTAGCCAA	Olson, 2002
<b>Chloroplast</b>					
<i>trnQ-rps</i> 16	<i>trnQ</i> (UUG)	GCG TGG CCA AGY GGT AAG GC	<i>rps</i> 16x1	GTT GCT TTY TAC CAC ATC GTT T	Shaw <i>et al.</i> 2007
<i>trnV-ndhC</i>	<i>trnV</i> (UAC)x2	GTC TAC GGT TCG ART CCG TA	<i>ndhC</i>	TAT TAT TAG AAA TGY CCA RAA AAT ATC ATA TTC	Shaw <i>et al.</i> 2007
<i>trnT-trnL</i>	<i>trnT</i> UGU F(TabA)	CAT TAC AAA TGC GAT GCT CT	<i>trnL</i> UAA R (TabB)	TCT ACC GAT TTC GCC ATA TC	Taberlet <i>et al.</i> 1991
<i>rps</i> 16 intron	<i>rps</i> 16-for-alt	CACCATTTTCTATAGGAATGAAAGATGC	<i>rps</i> 16-rev-alt	CGATAGATCGCTCATTTGGGATAGATGT	Smith <i>et al.</i> 2008
<i>rps</i> 16 intron	<i>rps</i> 16-ex1-for	CGTTTGAAAACGATGTGGTAG	<i>rps</i> 16-ex2-rev	CGTATCGGATCGTAAACCTAC	Downie and Katz-Downie 1999
<i>trnH-psbA</i>	<i>trnH</i> (GUG)	CGC GCA TGG TGG ATT CAC AAT CC	<i>psbA</i>	GTT ATG CAT GAA CGT AAT GCT C	Tate and Simpson, 2003; Sang <i>et al.</i> 1997
<i>rp</i> 132- <i>trnL</i>	<i>rp</i> 132F	CAG TTC CAA AA A AAC GTA CTT C	<i>trnL</i> (UAG)	CTG CTT CCT AAG AGC AGC GT	Shaw <i>et al.</i> 2007



a monophyletic group, and *Agave* sensu lato as its sister genus. Moreover, in their analyses, the three sections were paraphyletic or polyphyletic, *Clistocarpa* was sister to both *Chaenocarpa* and *Sarcocarpa*, and *Y. queretaroensis* was recovered in *Sarcocarpa* and not as sister species to all other *Yucca* species. Recently, Heyduk *et al.* (2016b) conducted an evolutionary study with 34 Agavoideae taxa, including 19 *Yucca* species. Their results showed low resolution at the subgeneric level and discrepancies between nuclear genes and chloroplast genome topologies. Nuclear gene topologies revealed *Yucca* forming two clades: one with *Y. brevifolia* Engelm (1871: 496) (*Clistocarpa*), and the other divided into two subclades: *Chaenocarpa* and *Sarcocarpa*. Conversely, the chloroplast genome topology also depicted two clades, but here, the first clade included both *Clistocarpa* and *Chaenocarpa*, while the second clade contained only *Sarcocarpa*. In both topologies *Y. brevifolia* was the sister species to the other yuccas.

To date, the research on *Yucca* has provided valuable insights into its evolutionary history and some characteristics. However, there is no consensus on the phylogenetic relationships of yuccas in previous studies. Therefore, it was necessary to work with a highly representative sampling and use tools that help us better understand how *Yucca* species are related to each other. With clearer relationships it is possible to explore the origin of some morphological characters of yuccas, the evolution of the variation of these characters, and how these species have adapted over time.

As a consequence, the aims of the present study on *Yucca* are to: 1) provide a molecular phylogenetic hypothesis based on both chloroplast and nuclear ITS data of a highly representative sample, 46 taxa constituting 80% of *Yucca* species, the most complete to date, 2) test former infrageneric classifications of *Yucca* and the monophyly of morphologically defined entities, and 3) investigate the evolution of some characters that have been used in the infrageneric classification of this genus, such as type and dehiscence of fruit, leaf margin and inflorescence type.

## Material and methods

### Taxon sampling

To obtain the DNA of the *Yucca* species, field trips were made to Mexico and the United States of America from 2016 to 2019. In many cases and whenever possible, we tried to visit the type localities and representative locations according to Matuda & Piña (1980), who identified where the most representative populations of each *Yucca* species occur in Mexico. Leaf tissue samples of *Yucca* and outgroup species were collected in silica gel for DNA isolation. Sampling was completed with material of the living collections of the Botanical Garden of the National Autonomous University of Mexico (Mexico City, Mexico) and the Desert Botanical Garden (Phoenix, Arizona, United States of America). Other samples were provided by the Huntington Botanical Gardens Herbarium (HNT), Mercer Botanic Gardens (MERCA) and private collectors. Species of the genera *Agave*, *Beaucarnea* Lemaire (1861: 57), *Beschorneria*, *Dasyllirion* Zuccarini (1838: 258) *Hesperaloe* and *Hesperoyucca* were used as the outgroup. Regarding the outgroup, *Hosta* Trattinnick (1812: 55) *Chlorophytum* Ker Gawler (1807: t. 1071), and *Anthericum* L. (1753: 310) have been identified as the closest relatives to the *Agave*-*Yucca* clade (Ji *et al.* 2023). However, we did not include them in this study because the markers used were not fully available in GenBank at the time of our analyses, and we required the selected data to be both comparable and consistent. Consequently, we opted to use *Beaucarnea* and *Dasyllirion* (Convallarioideae/Nolinoideae) to incorporate genera that are also closely related and have a strong representation in America, which we personally collected. The complete sampling list is shown in Table S1.

### DNA extraction, amplification, purification, and sequencing

The following protocol was developed for the molecular analysis of *Yucca* species. DNA was extracted from dried leaf material of 58 taxa, 49 of *Yucca* and nine species of the outgroup, with commercial kits (OMEGA Bio-tek, Norcross, Georgia, USA; QIAGEN, Germantown, Maryland, USA). To evaluate which regions would be useful to analyze the phylogenetic relationships of *Yucca*, 10 regions were used initially, three from the nucleus and seven from the chloroplast. For the latter, Shaw *et al.* (2007) was followed (Table 1). However, only four regions amplified: ITS 18S and 26S from the nucleus and *rps16exF-rps16exR*, *trnH-psbA* and *rpl32-trnL* from the chloroplast. Polymerase chain reactions (PCR) for each region were performed in 12.5 µl volume reactions, containing 2.75 µl nuclease free water, 6.25 µl KAPA2G Robust HotStart ReadyMix (Kapa Biosystems, Woburn, Massachusetts, USA), 0.75 µl of 10µM forward primer, 0.75 µl of 10 µM reverse primer, and 2.0 µl DNA template. Thermocycling conditions (Mastercycler Pro, Eppendorf, Westbury, New York, USA) consisted of an initial denaturation step of 3 min at 95°C, followed

by 35 cycles of 95°C for 15 s, 55°C for 15 s, and 72°C for 30 s; and a final elongation step of 1 min at 72°C. Purification was carried out by adding 0.025 µl exonuclease I and 0.125 µl shrimp alkaline phosphatase (Affymetrix, Santa Clara, California, USA), and incubating at 37°C for 30 min followed by 95°C for 5 min. Both forward and reverse sequences were obtained using 1/16 BigDye Terminator v. 3.1 (Life Technologies, Grand Island, New York, USA) cycle sequencing reactions and the same primers used for amplification. Thermocycling conditions were 94°C for 1 min, and 25 cycles of 94°C for 10 s, 50°C for 5 s, and 60°C for 30 s. Sequencing took place at the Molecular Biology laboratory of the Arizona State University (ASU) with an ABI 3730XL Hitachi sequencer from ThermoFisher (Carlsbad, California, USA).

## Data analyses

Sequences were obtained for each DNA region; however, only 248 had high quality, 52 of them corresponding to ITS 18S–26S, including 44 *Yucca* species and eight from the outgroup: *Dasyllirion* (1), *Agave* (2), *Beschorneria* (1), *Hesperaloe* (2) and *Hesperoyucca* (2). Of the three plastid regions, 72 sequences were obtained both for *trnH-psbA* and for *rpl32-trnL*, and 52 for *rps16*. However, complete sequences for all three chloroplast regions were obtained for 44 species only, of which 40 correspond to *Yucca* and four to the outgroup, *Beaucarnea* (1), *Beschorneria* (1) and *Hesperoyucca* (2). The sequences of each species were viewed and edited individually using Geneious Prime v. 2020.2.4 (<http://www.geneious.com>). Subsequently, sequences were aligned in a matrix for ITS, and the three regions of the chloroplast were aligned individually using the MUSCLE algorithm (Edgar, 2004) as implemented in Geneious, and then concatenated. In addition, sequences were corroborated with UGENE v. 38 (Okonechnikov *et al.* 2012). The evolutionary models for each region were calculated with jModelTest v. 2 (Darriba *et al.* 2012) under the BIC criterion (Bayesian Information Criterion).

## Phylogenetic hypothesis reconstruction

Maximum likelihood (ML) and Bayesian inference (BI) analyses were performed separately for the two matrices obtained, the ITS matrix and the one that included the three chloroplast regions. With the ITS matrix, ML analysis was carried out in RAxML v. 8 (Stamatakis, 2014) with 10000 bootstrap (BS) replicates, while BI analysis was carried out in MrBayes v. 3.2.2 (Ronquist & Huelsenbeck 2003), with two independent runs with four chains (two hot and two cold) for 20,000,000 iterations and resampling every 1000 generations, discarding 25% of the samples as burn-in. In both analyses, the evolutionary model used was GTR + G (General Time Reversible plus with a Gamma Distribution), and *Dasyllirion longissimum* Lemaire (1856: 91) was used to root the trees. For the chloroplast matrix, the partitions corresponding to each region were established, and the ML and BI analyses were performed with the same parameters and same software used for ITS. The models obtained for each region were F81 for *trnH-psbA*, TIM + I + G for *rpl32-trnL* and TPM3uf for exon *rps16* (Supplementary material, Table S2).

## Divergence time estimation

The phylogenetic tree obtained from the BI analysis of ITS was used to estimate divergence times in *Yucca* and its sister genera. For this purpose, a relaxed molecular clock analysis was carried out in BEAST v. 2 (Bouckaert *et al.* 2014), under an “uncorrelated relaxed clock” model with a log normal distribution and Birth-Death model as a tree prior. The root of the tree was calibrated with a mean of 62.49 Ma (million years), corresponding to Asparagales following Magallón *et al.* (2015), using a log normal distribution. The stem age of *Yucca* was set with a mean of 14.2 Ma, with a log normal distribution, corresponding to the fossil *Protoyucca shadissii* from the middle Miocene, which is closely related with the contemporary *Yucca* species (Tidwell & Parker, 1990). The analysis was run for 400,000,000 generations sampling every 20 000, 10% of which was discarded as burn-in. The molecular clock analysis was conducted in the CIPRES Science Gateway (Miller *et al.* 2010). Log outputs of the BEAST analysis were evaluated with Tracer v. 1.5 (Rambaut *et al.* 2018). Files containing the trees sampled for each execution of the MCMC run were analyzed with LogCombiner v. 1.7.5 (Helfrich *et al.* 2018, annotated using TreeAnnotator v. 1.7.5 (Helfrich *et al.* 2018), and finally visualized using FigTree v. 1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree/>).



## Ancestral character state reconstruction

The obtained phylogenetic framework was used to evaluate the evolution of morphological characters that have been employed to recognize sections and series within the genus *Yucca*, such as fruit type, caulescence, inflorescence type, and leaf margin.

To prepare the matrix of morphological characters, descriptions of the species were consulted in the relevant treatments of the Flora of North America (Hess & Robins, 2002), Flora Mesoamericana (García-Mendoza & Lot, 1994), as well as morphological studies and species protologues (Matuda & Piña, 1980; Clary and Simpson, 1995; Clary, 1995); Magallán-Hernández *et al.* 2014; Thiede, 2020). Each character was coded as binary: dry fruit = 0, fleshy fruit = 1; dehiscent fruit = 0, indehiscent fruit = 1; mainly acaulescent plants = 0, mainly caulescent plants = 1; leaf margin entire = 0, denticulate = 1, and inflorescence spike/raceme = 0, or panicle = 1.

Ancestral state reconstruction was conducted on time-calibrated trees to use tree branch lengths that represent absolute time rather than molecular change, as would be the case in a phylogram. This approach avoids assuming a close correlation between molecular change and morphological evolutionary rate. The analysis was performed on a random sample of 500 post burn-in topologies obtained with BEAST v. 2.0 (Bouckaert *et al.* 2014) to incorporate uncertainty in branch lengths and topology estimates (Pagel *et al.* 2004). The ancestral character states were analyzed for selected clades using the Pagel model implemented in BayesTraits v. 4.0 (Pagel *et al.* 2004). Initially, an ML analysis was conducted to derive empirical priors. After setting these priors (uniform distribution 0–10), a BI analysis was performed using a reversible-jump Markov Chain Monte Carlo (rjMCMC) for five million generations, sampling every 10,000 generations and discarding the first 25% as burn-in. The BI analysis was repeated to verify convergence using density plots. The convergence and mixing of the chains were confirmed in trace plots, and effective values sample sizes (ESS) were greater than 200. The results of BayesTraits were plotted on the tree using the R script described in Harrington & Reeder (2017), allowing us to graph the probability of each character state for each node and the probability of node no-existence, as described in Pagel *et al.* (2004).

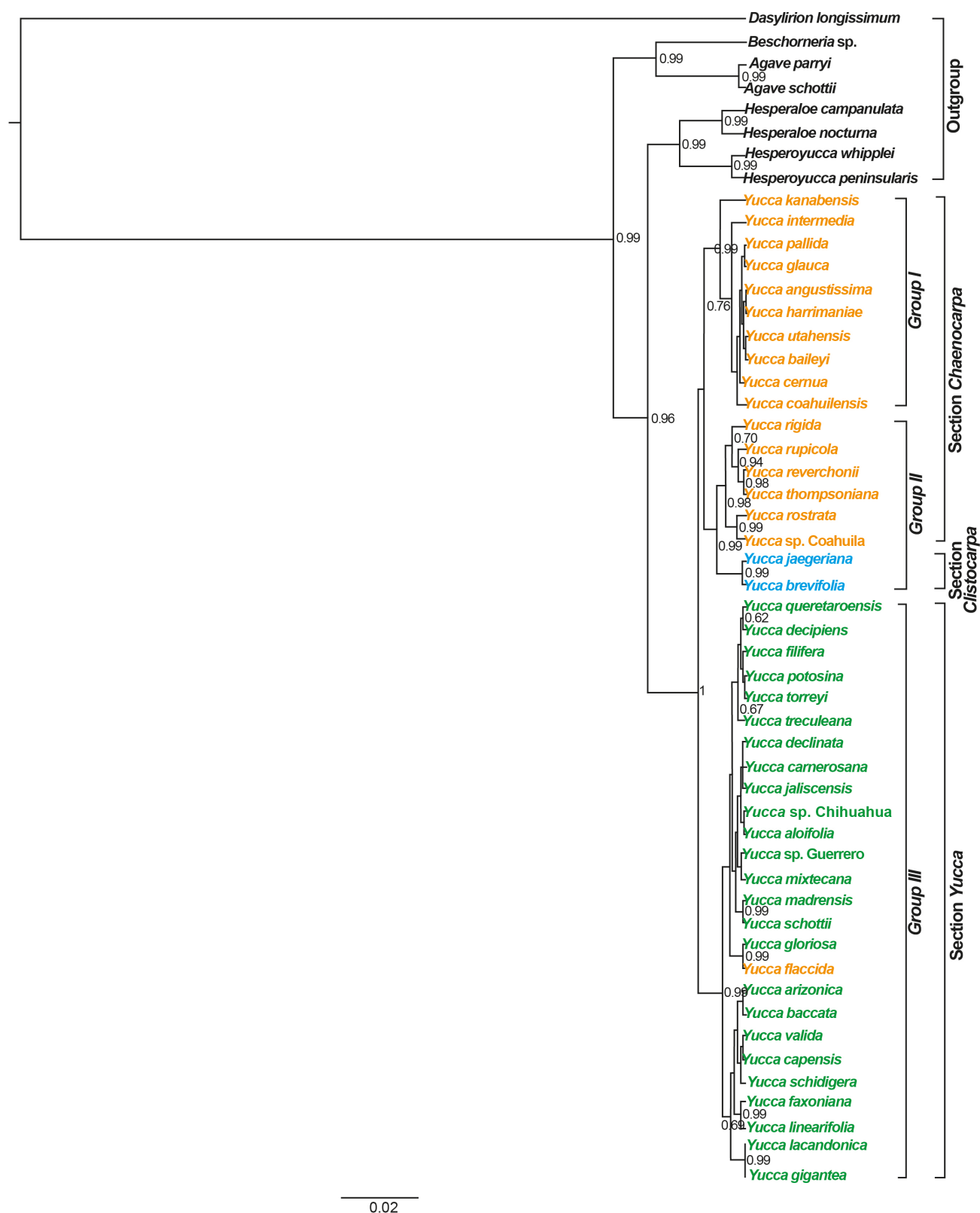
## Results

### Phylogenetic relationships based on ITS sequences

Phylogenetic relationships were reconstructed from an alignment that included 44 *Yucca* species and eight outgroup species (Figure 2). This matrix consisted of 752 aligned base pairs, with 127 variable sites, of which 86 were informative. The results of the ML and BI analyses were congruent in the earliest diverging nodes, and here were similar groupings in both trees; however, the support values for the nodes in ML were not as high as in the BI tree (Figure S1). The BI analysis converged to a posterior probability value of -2515.73, with an effective sample size (ESS) of 1251.71. *Agave* and *Beschorneria* were sister genera (bootstrap (BS)=99%, posterior probability (PP)=0.99), as were *Hesperaloe* and *Hesperoyucca* (BS=94%, PP=0.99). In both analyses, all *Yucca* species formed a single clade (BS=60%, PP=1.0). On the one hand, the ML topology showed yuccas to be grouped in three clades: the first one consisting of eight species of the sections *Clistocarpa* and *Chaenocarpa*, the second one with 26 species, 25 of the section *Yucca*, in addition to *Y. flaccida* Haworth (1819: 34), and the third comprised of 10 species of the section *Chaenocarpa*. On the other hand, the BI topology showed two clades, the first one with 18 species and the second one with 26, each consisting moreover of two groups. Finally, the series proposed by McKelvey (1938, 1947) were not recovered as monophyletic.

### Phylogenetic relationships based on chloroplast sequences

Phylogenetic relationships were reconstructed from an alignment that included 40 *Yucca* species and four outgroup taxa as mentioned above. The matrix consisted of 2547 aligned base pairs, of which 705 corresponded to *trnH-psbA*, 864 to *rpl32-trnL* and 978 to the *rps16* exon. Within these data, 163 variable sites were observed, 67 of which were informative. The topologies obtained by BI and ML were similar with respect to the relationships between the species. *Beschorneria* and *Hesperoyucca* were grouped together, and *Yucca* was recovered as monophyletic (PP=1). However, the sections and series were not recovered as monophyletic, and some major clades showed geographical patterns instead, mainly corresponding with the arid zones of the USA and Mexico, and another clade included species from the Mexican Transition Zone. Among them were the one made up of *Y. rigida* (Engelmann) Trelease (1902: 65) *Y. coahuilensis*, *Y. rostrata* Engelmann ex. Trelease (1902: 68), *Y. thompsoniana* Trelease (1911: 101), *Y. campestris*



**FIGURE 2.** ITS topology obtained through Bayesian Inference analysis, the numbers close to the nodes indicate the posterior probability values, only those greater than 0.6 are shown.

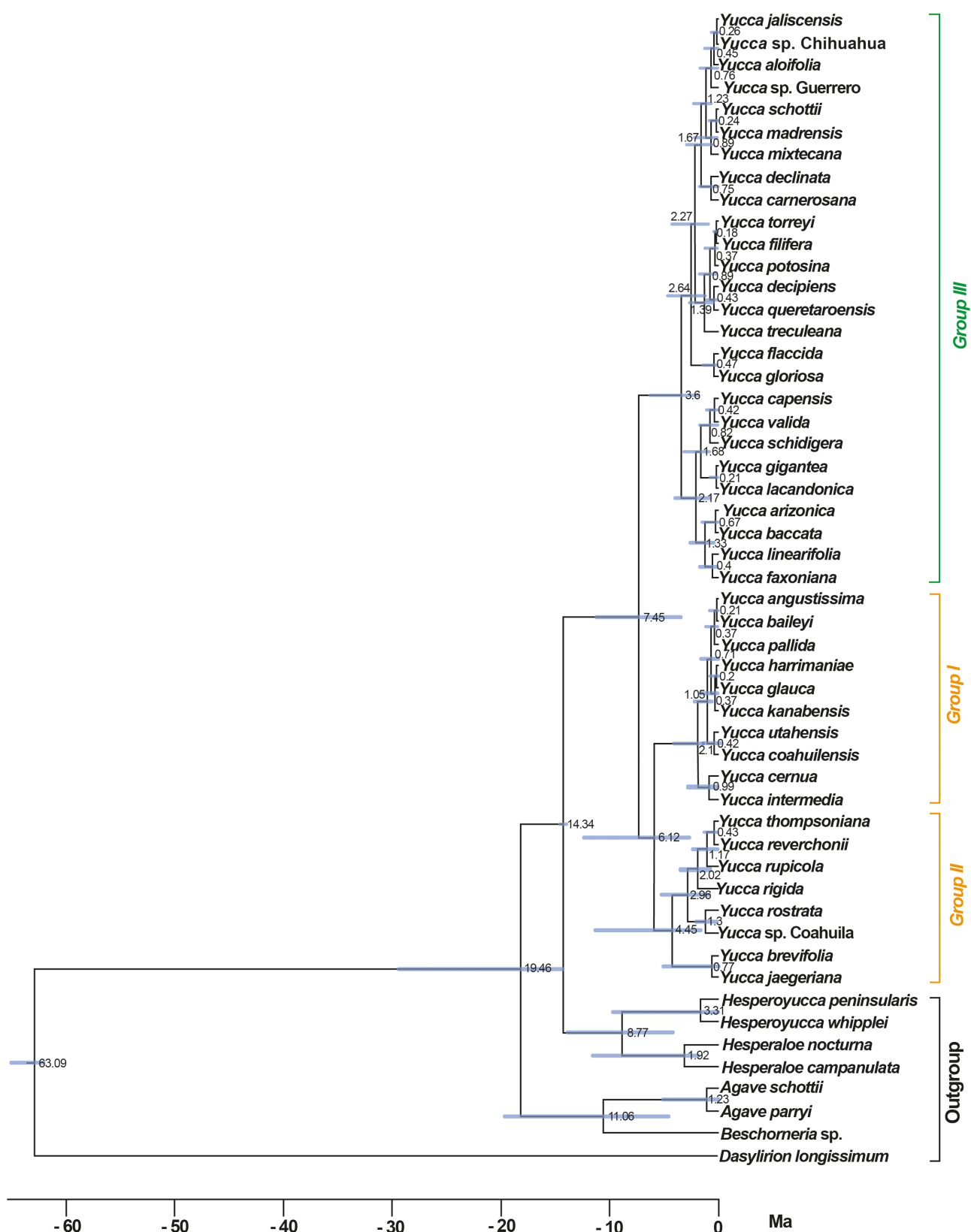


McKelvey (1947: 173), *Y. necopina*, *Y. intermedia* McKelvey (1947: 116), *Y. glauca* Nuttall (1813: 89), *Y. reverchonii* Trelease (1911: 102), *Y. baileyi* Wootton & Standley (1913: 114), *Y. sp. Coahuila*, *Y. lacandonica* and *Y. gigantea* Lem. (1859: 91). Except for the latter two, the afore-mentioned species occur in the north of the Chihuahuan desert and the Great Plains. Another clade consisted of *Y. valida* Brandege (1889: 208), *Y. capensis*, *Y. grandiflora* Gentry (1957: 51), *Y. baccata* Torrey (1859: 221), *Y. schidigera* Ortgies (1871: 110) and *Y. madrensis* Gentry (1972: 159), while another group includes *Y. jaegeriana* (McKelvey) Lenz (2007: 99), *Y. brevifolia*, *Y. utahensis* McKelvey (1947: 94), *Y. angustissima* Engelmann ex Trelease (1902: 58) and *Y. harrimaniae* Trelease (1902: 59). These species groups occur in the Mojave and Sonoran deserts, in addition to some parts of the Grand Canyon and the Colorado Plateau. Another subclade was made up of species that occur in the Chihuahuan desert and the Mexican Transition Zone like *Y. filifera* Chabaud (1876: 432), *Y. linearifolia*, *Y. potosina* Rzedowski (1955: 90), *Y. faxoniana* Sargent (1905: 121), *Y. sp. Chihuahua*, *Y. periculosa* Baker (1870: 1088), *Y. mixtecana* García-Mendoza (1998: 1) and *Y. torreyi* Shafer (1908: 157). Likewise, relationships with high PP values were recovered as *Y. lacandonica*-*Y. gigantea* (1), *Y. gloriosa*-*Y. flaccida* (1), *Y. periculosa*-*Y. mixtecana*, *Y. jaegeriana*-*Y. brevifolia* (0.99), and *Y. utahensis*-*Y. angustissima*-*Y. harrimaniae* (0.97). The BI topology can be found in the Supplementary material (Figure S2).

### Divergence time estimation

Estimates of divergence times for *Yucca* species and closely related genera were obtained from BEAST analyses based on the ITS phylogeny (Figure 3). The ESS for all parameters exceeded values of 200, except for the mean value for uncorrelated clock, which was 167. Despite this exception, we considered the results for the divergence time estimations to be stable and informative. Results indicated that the stem group, consisting of *Agave-Beschorneria*, *Hesperaloe-Hesperoyucca* and *Yucca*, had an estimated age of 19.46 Ma (95% HPD: 28.51-14.24), corresponding to the Oligocene, the *Yucca* stem separated towards the middle Miocene at about 14.34 Ma (95% HPD: 14.64-14.2), and the separation of the crown group of the genus occurred 7.45 Ma (95% HPD: 11.31-3.48) ago, during the Pliocene. Within *Yucca* there were two main divergence events, the first happened 6.12 Ma (95% HPD: 9.84-3.08) and included groups I and II made up of *Y. angustissima*, *Y. baileyi*, *Y. pallida* McKelvey (1947: 57), *Y. harrimaniae*, *Y. glauca*, *Y. kanabensis* McKelvey (1947: 122), *Y. utahensis*, *Y. coahuilensis*, *Y. cernua* Keith (2003: 892), *Y. intermedia*, *Y. thompsoniana*, *Y. reverchonii*, *Y. rupicola* Scheele (1850: 143), *Y. rigida*, *Y. rostrata*, *Y. sp. Coahuila*, *Y. brevifolia* and *Y. jaegeriana*, here named the clade Rupicola, while the second event had an estimated age of 3.6 Ma (95% HPD: 6.06-1.6), and was represented by groups III and IV, or the clade Aloifolia, that includes *Y. jaliscensis* (Trel.) Trelease (1920: 92), *Y. sp. Chihuahua*, *Y. aloifolia*, *Y. sp. Guerrero*, *Y. schottii* Engelmann (1873: 46), *Y. madrensis*, *Y. mixtecana*, *Y. declinata* Laferrière (1995: 347), *Y. carnerosana* (Trelease) McKelvey (1938: 24), *Y. torreyi*, *Y. filifera*, *Y. potosina*, *Y. decipiens* Trelease (1907: 228), *Y. queretaroensis*, *Y. treculeana* Carrière (1858: 580), *Y. flaccida*, *Y. gloriosa* Linnaeus (1753: 219), *Y. capensis*, *Y. valida*, *Y. schidigera*, *Y. gigantea*, *Y. lacandonica*, *Y. arizonica* McKelvey (1935: 270), *Y. baccata*, *Y. linearifolia* and *Y. faxoniana*.

Within the clade Rupicola there were two main divergence events: the first event of this clade had an age of 4.45 Ma (95 % HPD: 7.46-1.92) and included three of the four species classified by McKelvey (1947) in series *Rupicolae* (*Y. rostrata*, *Y. rupicola* and *Y. thompsoniana*), that were recovered by Clary (1997) in her phylogenetic hypothesis, where also *Y. reverchonii* and *Y. rigida* were included. The latter species are known from the southwest of the USA and northern Mexico. Apart from the dry fruit, both species also share the corneous, yellowish, and denticulate leaf margin. A yet undescribed species from Coahuila was also included here. The node of 0.77 Ma (95 % HPD: 2.09-0.01) included *Y. brevifolia* and *Y. jaegeriana*, whose fruits are dry but indehiscent. The second divergence event occurred 2.1 Ma (95 % HPD: 4.25-0.52) and included species mainly from the Colorado Plateau and Great Basin, except for *Y. cernua*, *Y. pallida* and *Y. coahuilensis*, the first two being located in Texas, and the last one only known from the state of Coahuila in northern Mexico. Clade Aloifolia showed a divergence event at a node whose age was 2.17 Ma (95 % HPD: 3.21-0.59), where species located mainly in northwestern Mexico and southwestern USA in the Mojave and Sonoran deserts were related to each other, including *Y. schidigera*, *Y. valida*, *Y. capensis*, *Y. baccata* and *Y. arizonica*. *Yucca linearifolia* and *Y. faxoniana* occur at the limits of the Chihuahuan Desert and the north of the Sierra Madre Oriental. The exceptions were *Y. lacandonica* and *Y. gigantea* that inhabit the south of the Sierra Madre Oriental and the plains of the Gulf of Mexico. In another divergence event whose age was 2.27 Ma (95 % HPD: 2.79-0.36), there were mostly yuccas with fleshy fruit such as *Y. torreyi*, *Y. filifera*, *Y. potosina*, *Y. decipiens*, *Y. queretaroensis* and *Y. treculeana*, which are distributed primarily in the Chihuahuan Desert, a geographic region located from Texas to Hidalgo. Also included were additional species occurring in the Trans-Mexican Volcanic Belt, and *Y. gloriosa* and *Y. flaccida*, both of which are in the coastal area of the southeastern USA.

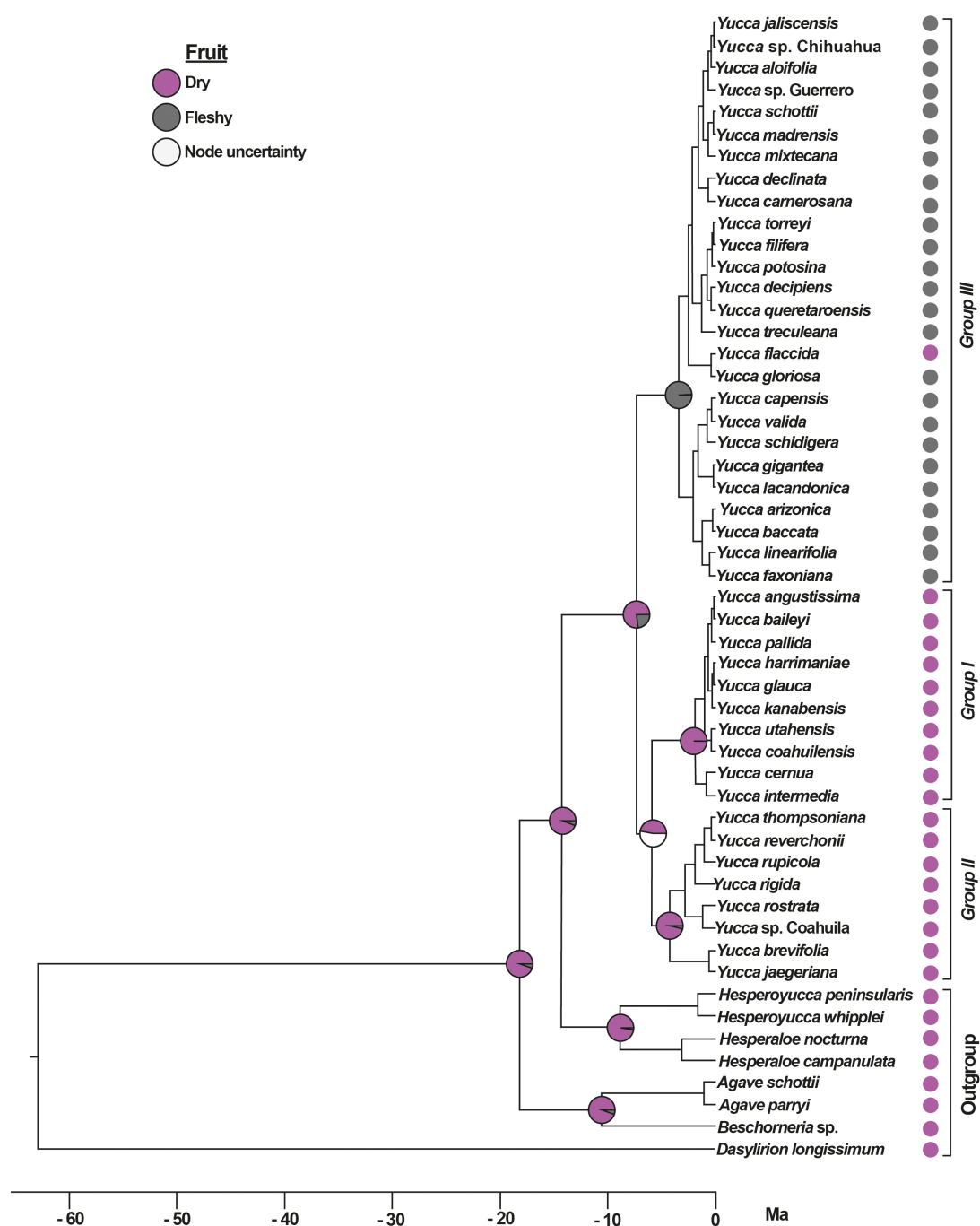


**FIGURE 3.** Chronogram obtained from the maximum clade credibility (mcc) tree in which the diversification times of *Yucca* species and some closely related genera can be observed. Numbers indicate mean divergence times and mean ages, and subsequent densities (HPD) higher than 95% are represented by blue bars.

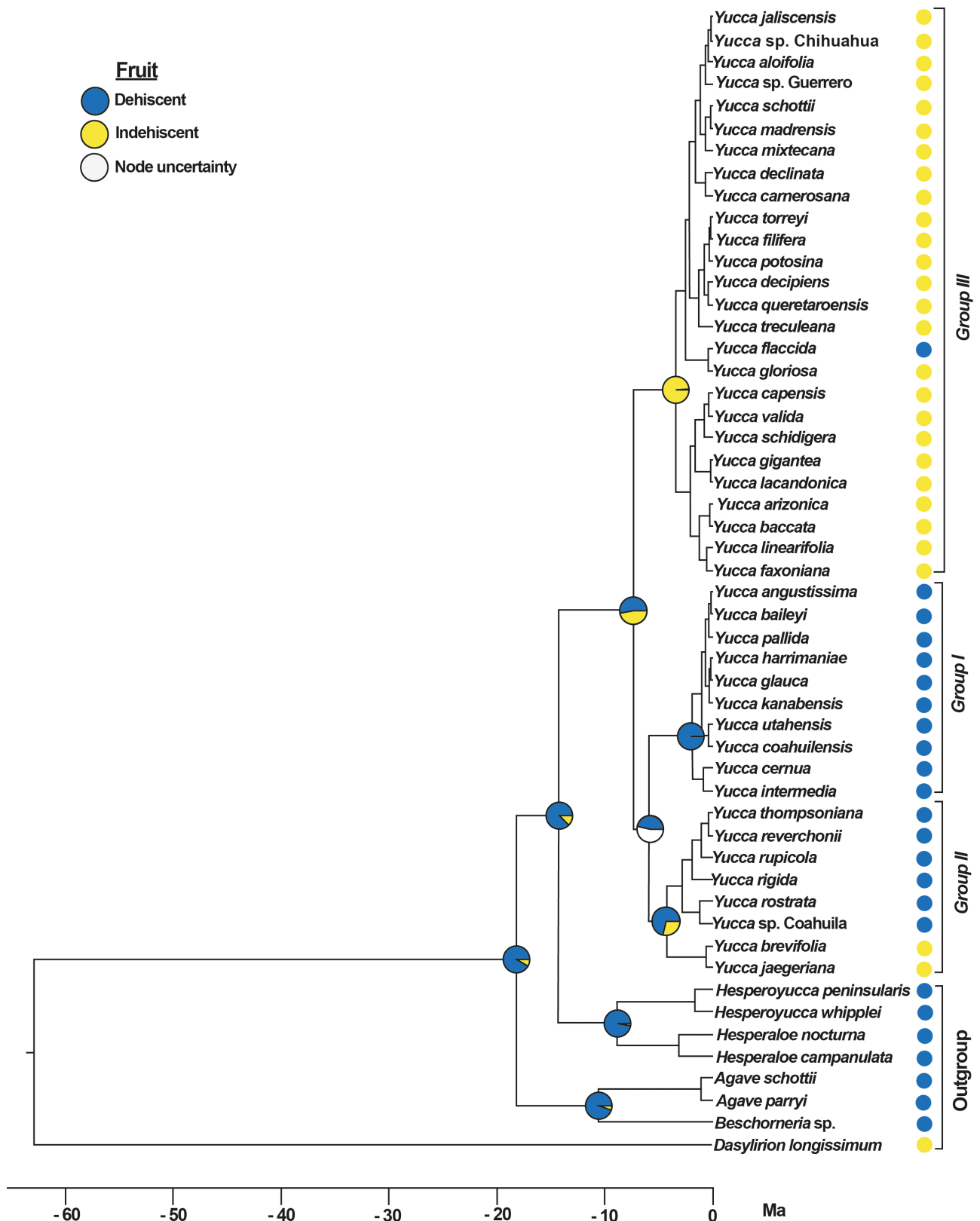


## Ancestral character state reconstruction

Ancestral state reconstruction with respect to the fruit and dehiscence types corresponded with the main clades obtained in the phylogenetic hypothesis (Figures 4 and 5). In contrast, the caulescence, leaf margin and inflorescence type varied between the major clades in the phylogenetic trees (Supplementary material, Figures S3, S4 and S5). In *Yucca*, the ancestral character state was the dry fruit while the fleshy fruit originated later. The ancestral character state for fruit dehiscence was the dehiscent fruit. The ancestor of *Agave*, *Beschorneria*, *Hesperaloe*, *Hesperoyucca* and *Yucca* had dry fruit (PP=0.93), as well as the clade containing *Agave-Beschorneria* (PP=0.93), and that of *Hesperaloe-Hesperoyucca* (PP= 0.97). The node including *Hesperaloe-Hesperoyucca-Yucca*, which also was the stem group, had a posterior probability (PP) of 0.92 for dry fruit, while the *Yucca* crown group had a PP of 0.77. Subsequently, this character state diversified into two large groups: in one of them (clade Rupicola) the dry fruit remained (PP=0.45), while the second group (clade Aloifolia) changed to fleshy fruit. Therefore, the probability of dry fruit in the latter clade was 0.5; *Y. flaccida*, which reverted to dry fruit, can be found in this group.



**FIGURE 4.** Ancestral state reconstruction of the fruit type indicated on the tree of maximum clade credibility obtained by Bayesian inference in BEAST. The circles indicate the probability of the fruit type at each node.



**FIGURE 5.** Ancestral state reconstruction of the fruit type dehiscent indicated on the tree of maximum clade credibility obtained by Bayesian inference in BEAST. The circles indicate the probability of the fruit type dehiscent at each node.

With respect to fruit dehiscent, the ancestor of *Beschorneria*-*Agave*-*Hesperaloe*-*Hesperoyucca* and *Yucca* had a probability of 0.91 of dehiscent fruit, 0.85 for the stem group, and it was uncertain with a probability of 0.56 for the crown group. The probability for the node corresponding to clade *Rupicola* was 0.44. This clade consisted of two subclades with mainly species with dehiscent fruits: the first one with PP of 0.99, while the second one, where *Y.*



*brevifolia* and *Y. jaegeriana* with indehiscent fruits can be found, had a probability of 0.69. The node of clade Aloifolia had a probability of 0.1 of its ancestor presenting dehiscent fruits; except for *Y. flaccida*, the remainder of the species have indehiscent fruits.

In relation to caulescence, the ancestor of *Beschorneria-Agave-Hesperaloe-Hesperoyucca* and *Yucca* had a posterior probability of 0.78 of being mainly acaulescent plants, 0.56 for the stem group, and 0.99 for the crown group. In clade Rupicola, the node corresponding to Group I had a probability of 0.71, whereas the probability in Group II is 0.2. For clade Aloifolia, the probability was 0.1. With respect to leaf margin, the node probability values were 0.43 for a mainly entire leaf margin in the ancestor of *Beschorneria-Agave-Hesperaloe-Hesperoyucca* and *Yucca*, 0.72 for the stem group, 0.76 for the crown group, 0.96 corresponding to Group I, 0.2 corresponding to Group II, and 0.98 for clade Aloifolia. Finally, the inflorescence was the character state that varied least; the node values were 0.74 for the ancestor of *Beschorneria-Agave-Hesperaloe-Hesperoyucca* and *Yucca*, 0.92 for the stem group, 0.96 for the crown group, 0.54 for Group I, 0.99 for Group II, and 1.0 for Group III with respect to the type II inflorescence which is a panicle. However, inflorescence type I, which is a spike/raceme, presented a higher posterior probability (0.73) corresponding to the node of *Agave-Beschorneria* (Figure S5). The posterior probabilities presented by specific characters and character states in the studied clades are summarized in Table S3.

## Discussion

### Phylogenetic relationships based on ITS do challenge the current *Yucca* classification

According to the phylogenetic hypothesis obtained from ITS, *Beschorneria* and *Agave* are sister genera (PP=0.94) and *Hesperoyucca* is a genus distinct from *Yucca*, containing three species: *H. newberryi* (McKelvey) Clary (2001: 845), *H. peninsularis* (McKelvey) Clary (2001: 845) and *H. whipplei* (Torrey) Baker ex Trelease (Figure 2). However, McKinney & Hickman (1993) considered that the latter two species are synonyms, and they only recognized *H. whipplei*. *Hesperoyucca* is more related to *Hesperaloe* (PP=0.99), as already shown by previous authors (Clary, 1997; Flores-Abreu *et al.* 2019; Jiménez-Barron *et al.* 2020).

The ITS-based phylogenetic hypothesis supports that *Yucca* is a monophyletic genus; however, neither the sections nor their series proposed by Engelmann (1873) and McKelvey (1938, 1947) were recovered as monophyletic groups. This may be due to the characters on which previous classifications were based, being mainly foliar, such as the presence or absence of filaments and teeth of the leaf margin, and reproductive, such as the length of the pistil and floral tube. With respect to these characters, Verhoeck-Williams (1975), for the tribe Poliantheae, and Rocha *et al.* (2006), in *Agave*, showed that such convergences are related to dry environments and dry periods alternated with rainy periods, while the variation in the length of the floral tube and the pistil are likely more related to the mainly arid and semi-arid habitats in which many *Yucca* species occur.

In the tree (Figure 2), *Yucca* is made up of two highly supported clades, each one having undergone two divergence events, one leading to dry fruits and the other one to fleshy fruits. The first clade contains 18 species with dehiscent or indehiscent dry fruits, here recognized as the clade Rupicola, consisting of two subclades: the first one including 10 species distributed in the southwestern USA, most with filiferous leaf margin, except *Y. cernua* characterized by corneous leaf margin. The second group consists of eight species, distributed in the southwestern USA and northern Mexico, all with denticulate leaf margin. These phylogenetic relationships are similar to those presented by Clary (1997).

The second clade, which we here recognize as Aloifolia, includes 26 species with indehiscent-fleshy fruit (except *Yucca flaccida* which has a dry fruit), and is well-represented in Mexico as there are 24 native species distributed here. The species with the northernmost distribution are *Y. baccata* and *Y. gloriosa*; the first one occurs in the southwest of the USA and northern Mexico in Sonora and Chihuahua, while the second one is endemic to the southeast of the USA, along the Atlantic Coast from North Carolina to Louisiana. On the other hand, the southernmost species are *Y. gigantea* and *Y. lacandonica*. *Yucca aloifolia* is a special case as it is a cultivated species with a broad distribution, from the southeastern USA to Nicaragua. Species with occurrence in both Mexico and the USA are *Y. aloifolia*, *Y. arizonica*, *Y. baccata*, *Y. faxoniana*, *Y. madrensis*, *Y. treculeana* (Hess & Robins, 2002), and *Y. carnerosana* (McKelvey, 1938). In general, the species of this clade are arborescent plants growing in xerophytic scrub (*Y. filifera*, *Y. mixteca*), temperate or tropical forests (*Y. baccata*, *Y. capensis*), to rainforests and cloud forests (*Y. gigantea* and *Y. lacandonica*). This clade also consists of two subclades, the first one with 17 species occurring from the Mexican Plateau, north-

central Mexico, and the east coast of the USA, to northernmost North Carolina. The second subclade consists of nine species distributed from California to Baja California, Sonora, Chihuahua, and Nuevo León in Mexico, except for *Y. lacandonica* and *Y. gigantea* distributed in southeastern Mexico and Guatemala.

The ancestor of the clade *Aloifolia* had a fleshy fruit, which was maintained in the remainder of the species of this group, except for *Y. flaccida*. This is probably due to hybridization processes, as is the case of *Y. gloriosa*, which is a young hybrid between *Y. aloifolia* and *Y. filamentosa* according to Heyduk *et al.* (2021). Additionally, introgression has been mentioned by Hanson (1993) and Arteaga *et al.* (2020) in *Y. schidigera*, *Y. baccata*, *Y. capensis* and *Y. valida*, phenomena that are facilitated by pollinators, in addition to gene duplication, a process that has been documented in Agavoideae (McKain *et al.* 2012).

The first highly supported (PP=0.98) subclade of *Rupicola* consists of *Y. sp.* Coahuila (a yet undescribed species from the state of Coahuila), *Y. rostrata*, *Y. rigida*, *Y. rupicola*, *Y. reverchonii*, and *Y. thompsoniana*, and is characterized by dry fruits, acaulescent or caulescent habit, and finely toothed leaf margins with a yellowish line. However, in this study neither *Y. pallida* nor *Y. cernua* are included in this subclade, which contrasts with the results obtained by Clary (1997), who also recorded that the taxa included in the series *Rupicolae* (McKelvey, 1947) possess an extra 27 bp indel in the ITS 1 and 2 region, which is absent in the other species of the genus. To date, we have not observed this indel in our ITS, 18 S and 26 S alignments in any of our study species. A second highly supported (PP=0.99) subclade of *Rupicola* consists of *Y. jaegeriana* and *Y. brevifolia*, which are considered to be a single species, as already proposed by several authors (Reveal 1977; Hess & Robins, 2002; Thiede, 2020), although others have considered these as two separate species (Lenz, 2007; Smith *et al.* 2021).

Rentsch & Leebens-Mack (2012) and more recently, Heyduk *et al.* (2021), based on the comparison of complete chloroplast genomes, confirmed that *Y. gloriosa* is a young hybrid between cultivated *Y. aloifolia* and *Y. filamentosa*, which had already been pointed out by Trelease (1902), and may explain the close phylogenetic relationship between *Y. gloriosa* and *Y. flaccida* that we recovered. Hess & Robins (2002) mentioned that *Y. filamentosa* has expanded its range due to its wide cultivation and that this species and *Y. flaccida* are probably the same species. Although they are similar in some morphological characters, *Y. filamentosa* and *Y. flaccida* differ in their habitat, and we therefore agree with Clary (1997) and Ward (2011) that they are two distinct species. Nonetheless, the widespread cultivation of these closely related species, along with the evidence of hybridization, is a likely explanation for the relationship we observed.

*Yucca baccata* and *Y. arizonica* also form a highly supported group (PP=0.99). Clary (1997) and W. Hodgson (pers. comm.) consider them as different species; however, Hess & Robins (2002) and Thiede (2020) consider *Y. arizonica* a synonym of *Y. baccata*. The first authors divided *Y. baccata* into two subspecies, *Y. baccata* subsp. *baccata* and *Y. baccata* subsp. *brevifolia*, and synonymized *Y. arizonica* with the latter. The separation of both subspecies is because the first subspecies is characterized by mainly acaulescent and less frequently caulescent habit, 1-6 stems, aerial or underground, generally measuring less than 0.3 m, and curly and thick leaf margins, while the second subspecies is characterized by 1-24 well-developed aerial stems, frequently branching, and sometimes reaching 2 m high, and its filiferous leaf margins.

A tool that could improve our understanding of the phylogenetic relationships between *Yucca* species is phylogenomics, which has the advantage of dealing with ILS (Incomplete Lineage Sorting), whereby gene histories do not necessarily follow the speciation history. This is particularly useful in groups where radiation may have been quite rapid (Meleshko *et al.* 2021; Kandziora *et al.* 2022). Moreover, phylogenomics incorporates large data sets in which events such as substitutions, insertions, fissions, gene fusions, deletions, among others, provide information that the use of only a few regions does not provide, especially if the molecular region used does not vary enough to resolve phylogenetic relationships in several taxonomic categories, including at the species level (Wysocki *et al.* 2015; Yu *et al.* 2018).

### Chloroplast-based phylogeny

The chloroplast DNA within Agavoideae shows little variation or phylogenetic utility at the species level. An example of this is seen in the study carried out by Jiménez-Barron *et al.* (2021), who recorded 3670 bp in four regions of the chloroplast for *Agave* and closely related genera, only 29 variable sites, with only 19 of these being informative. Even with complete chloroplast genome sequences, there is no better resolution among different groups of Agavoideae where gene duplications have also been documented (McKain *et al.* 2012).

*Yucca* is a genus of recent divergence in which diversification has been most active from the last 4 Ma to the present, with most of its species having ages estimated at just 1 Ma or less. This rapid and recent radiation obscures

phylogenetic relationships, especially if the phylogeny is based on cpDNA, since due to its uniparental inheritance, the information is more sensitive to the processes of introgression, hybridization, and gene flow, among others. These last two processes in *Yucca* are facilitated by its pollinators. For example, hybridization between geographically close species such as *Y. schidigera* and *Y. baccata* (Hanson, 1993), and relationships with pollinators have been documented in *Yucca*, e.g., *Y. capensis* and *Y. valida* that both are pollinated by *Tegeticula baja* Pellmyr & Balcazar-Lara (2008: 306) (Arteaga *et al.* 2020), and even in cultivated yuccas as *Y. aloifolia* and *Y. filamentosa* (Heyduk *et al.* 2021).

The recovered relationships in this hypothesis could also be associated with biogeographic and climatic events, as according to Althoff *et al.* (2012), these can cause allopatric divergence in species of *Yucca* and its pollinators as the mutualistic interaction spreads across the landscape. Their results show that within the subclade with dry and fleshy fruits, the *Yucca* species and its known *Tegeticula* Zeller (1873: 232) pollinators are mainly allopatric.

The low genetic variation in chloroplast DNA is consistent across various plant groups (Reginato *et al.* 2016; Chang *et al.* 2021; Loeuille *et al.* 2021), including Agavoideae (Jiménez-Barrón *et al.* 2020). However, this phenomenon in *Yucca* and other members of Agavoideae could be explained by stabilizing selective pressure, which favors the conservation of genes essential for critical metabolic processes such as photosynthesis and light regulation. In various plant groups primarily adapted to arid and semi-arid environments, including Agavoideae such as *Yucca* or *Agave*, efficient chloroplast functionality is crucial, and any change that compromises these processes would be unfavorable in an evolutionary context. On the other hand, gene duplications are common in the nuclear genome and allow the evolution of new functions (Li *et al.* 2016; Panchy *et al.* 2016). However, the chloroplast genome appears to be more evolutionarily constrained due to its critical role in photosynthesis and the environmental stress faced by these plants. In *Yucca* and other Agavoideae, the high conservation of chloroplast DNA limits the number of informative sites and complicates phylogenetic resolution at infrageneric levels.

## Divergence times and character evolution

The estimated ages are in general agreement with other related publications, e.g., Good-Ávila *et al.* (2006) estimated that the *Yucca* stem age is approximately 17.2 Ma. Smith *et al.* (2008) registered the divergence times of 9.52 Ma for the stem group and 6.41 Ma for the crown group of *Yucca*, while those obtained by Jiménez-Barrón *et al.* (2021) are 14.2 Ma and 8.52 Ma, respectively. It should be noted that the same fossils were used to calibrate. However, our sampling was broader for *Yucca*, as the studies of Good-Ávila *et al.* (2006) and Jiménez-Barrón *et al.* (2021) focused on *Agave*. Smith *et al.* (2008) stated that the *Yucca* crown group sections diversified rapidly at 4 to 6 Ma, and that the highest rates of lineage formation occurred at 3 to 4 Ma.

According to our results, the ages of the nodes grouping the two main subclades range from 6.12 (clade Rupicola) to 3.6 Ma (clade Aloifolia), and most of the *Yucca* species are of recent divergence, with some overlapping in their distribution. For example, the divergence time between *Y. valida* and *Y. capensis* is only 0.42 Ma (95 % HPD: 1.04-0.01), or *Y. gigantea* and *Y. lacandonica* separated 0.21 Ma (95 % HPD: 0.65-0.0) ago, among other species that are geographically close. Most of speciation events for the extant taxa of *Yucca* occurred during the Pliocene-Pleistocene. In the Pliocene, the climate became drier and colder in contrast with the more humid and warmer climate recorded during the Miocene (Steinhorsdottir *et al.* 2021); large extents of forests were replaced by more arid environments, including those of the United States of America and northern Mexico (Metcalf *et al.* 2000), where yuccas are established more frequently. In the Pleistocene, fluctuations in precipitation, seasonality, and temperature prevailed, and in addition the Trans-Mexican Volcanic Belt presented more intense activity (Metcalf, 2006; Mastretta-Yanes *et al.* 2015). The divergence times give an idea of how diversification could have occurred in yuccas and, consequently, also of their morphological evolution.

According to the reconstruction of ancestral states, the first *Yucca* plants were caulescent, with leaves with mainly entire margins, inflorescence simple or branched, and dry and dehiscent fruit (Figures 4–5; Supplementary material Figures S3, S4 and S5). Fossil evidence found by Tidwell & Parker (1990) suggests that more than 14 Ma ago *Yucca*-related plants like *Y. brevifolia* were arborescent and had stiff leaves. Today, most species of the genus have conspicuous single or branched stems that vary in height (Thiede, 2020). These characteristics are also present in other Agavoideae, including *Furcraea* and less frequently in *Agave* and *Beschorneria* (García-Mendoza, 2011). It is possible that, as has happened with other plants, the variation in the height and branching of the stems is a response to climatic and orographic changes that have occurred from the Plio-Pleistocene to the present, since such characteristics provide them with physiological resistance to frost and drought and reduce herbivory to a certain extent (Lusk *et al.* 2016). Acaulescent *Yucca* species reach reproductive maturity before the stem fully elongates, either due to slower development or an early cessation of growth. In both cases, retaining the leaves in a rosette minimizes exposure to heat



and reduces water loss. In contrast, species develop stems that allow for greater water transport and storage, which structurally and functionally contribute to their ecological success. Further studies under different ontogenetic scenarios would be valuable to better understand the evolution of the stem and other characters development in *Yucca*.

The presence of fleshy and dry fruit in one family as well as the shifts from one to the other type occur frequently in angiosperms (Bremer & Eriksson, 1992; Pabón-Mora & Litt, 2011; Wang *et al.* 2015). For example, the fleshy fruit has originated more than 20 times in monocots and at least eight times in Asparagales (Givnish *et al.* 2005). This may be due to various factors, from changes in dispersal (Bremer & Erikson, 1992) to gene duplication, which has favored new functions leading to more diversity in the structures of carpels and fruits (Scutt *et al.* 2006). According to our results, the dry fruit is ancestral in *Yucca*, while the fleshy one evolved afterwards, which agrees with the proposal by other authors (Trelease, 1902; Weber, 1953; Clary, 1997). This change from dry to fleshy fruit, similar to that of caulescence, could be explained by the ontological changes where the mature fruit before completion of lignification forms dehiscence lines (Dardick & Callahan, 2014). From the perspective of adaptation, the transition to fleshy fruits in yuccas of recent divergence reflects the environmental and ecological pressures these plants are exposed to. Similarly, the fruit development is halted before dehiscence is reached, so that the fruit structure is optimized to facilitate disperser attraction; ensuring their reproductive success in specific environments.

With respect to the changes in dispersal where there is a relationship between the fruit type and the disperser, two main patterns have been observed in *Yucca*. In the current species of the genus that have dehiscent capsules (clade Rupicola), the seeds are thin and are dispersed by the wind (Trelease, 1902; Weber, 1953; Dodd & Linhart, 1994), although it has been documented that animals may occasionally intervene (Geluso *et al.* 2022); moreover, these are distributed in open environments (Weber, 1953). The species with dry and indehiscent fruit (*Y. brevifolia* and *Y. jaegeriana*), and those with fleshy indehiscent fruits (clade Aloifolia) have thick seeds whose dispersal takes place by animals, mainly rodents (Vander Wall *et al.* 2006; Waitmann *et al.* 2012; Borchert & DeFalco, 2016), or even birds and bats (Lenz, 2001). Although most of this group of plants are distributed in xerophytic scrubs and some of them also in open environments, by means of zoochory the distance at which their seeds are moved is close to 100 m (Borchert & DeFalco, 2016).

The inflorescence is a character where it was useful to know the ancestral state in the genus. Both in the stem and crown groups, the ancestral state is a panicle; however, some changes have occurred in this character, particularly in Group III in which more than half of the species show racemes and spikes. In *Agave* the type of inflorescence has been used to subdivide it into subgenera *Littaea* and *Agave* (Gentry, 1982), while in *Yucca* the inflorescence has not been used as a taxonomic character to classify it into sections and series. However, at the species level it allows for the recognition of some species. Some of the characters and character states used in the inflorescence are very branched or little branched, shape (ellipsoidal or conical), presence or absence of trichomes (glabrous or pubescent), including their erect or pendular orientation (Matuda & Piña, 1980). However, in our results, due to the type of branching, we did not observe correspondence with the main clades obtained in the phylogenetic hypothesis.

Concerning leaf margin, this was used by Halpin & Fishbein (2013) and Baker (1880) to classify yuccas into three groups: *Serrulatae*, *Integrifoliae* and *Filiferæ*. According to this classification, the species of the first group have leaves whose margin is toothed, in the second it is entire (although in adult leaves, they may be scarcely filiferous, and in juvenile leaves they may be serrate at the base), and the third presents abundant filaments. Our reconstruction of ancestral states shows that the margin is variable between the clades. It was only observed that in Group III of the clade Rupicola all the species have a denticulate margin; in the rest of the clades both leaves with entire and dentate margins are present. Clary (1997) recognized a clade of yuccas with denticulate leaves whose species partially correspond to those of Group III of the present study. In the clade Aloifolia, most members have margins without teeth; the exceptions are *Y. aloifolia*, *Y. gloriosa*, *Y. gigantea* and *Y. lacandonica*, which are distributed in environments with higher humidity than the other species of the clade. However, some species that live in arid environments also have leaves with teeth such as *Y. linearifolia* and *Y. queretaroensis*. In future research, it would be of great interest to explore the morphological evolution of the characters analyzed in this study, as well as others, such as floral traits, under ontogenetic and adaptive scenarios.

## Conclusions

Within the subfamily Agavoideae and especially in the ABK clade, the ITS marker has given congruent results, as it separates well at the generic level, but lacks sufficient resolution at the species level. As shown in our results in which

*Yucca* is recovered as a monophyletic group, *Hesperoyucca* is a separate genus from *Yucca* and is more closely related to *Hesperaloe*, as well as to *Agave* and *Beschorneria*. However, probably due to the recent evolutionary history of the genus at the species level, questions remain unanswered, especially in those that are phylogenetically related but geographically distant, as well as in those that are sympatric but not closely related.

Regarding the chloroplast regions analyzed, they exhibit low variability and insufficient resolution, recovering *Yucca* as monophyletic but not the sections and series within the genus. However, these chloroplast data reveal geographical patterns at the species level, likely influenced by hybridization events that obscure other phylogenetic relationships.

According to our study, the evolution of morphological traits provides additional context for understanding *Yucca* diversification. The fruit type and its dehiscence are consistent with the main phylogenetic clades, supporting the ancestral condition of the dry fruit in the genus. The divergence towards fleshy fruits may reflect changes in dispersal strategies and climatic events of the Pliocene-Pleistocene, possibly coupled with gene duplication events. In contrast, traits such as caulescence and inflorescence type appear to be homoplastic, evolving independently multiple times.

Future research on *Yucca* phylogenetic relationships should address the limitations of traditional markers by employing high resolution genomic approaches, such as Single Nucleotide Polymorphisms (SNPs), Genotyping by Sequencing (GBS) and Restriction-site Associated DNA Sequencing (RAD-seq). Additionally, phylogenomic analyses using coalescent-based methods would be particularly beneficial, as these approaches are better suited to handle incomplete lineage sorting (ILS), where gene genealogies do not completely align with the evolutionary history of the species.

To further enhance our understanding of the evolutionary and morphological history of *Yucca*, we recommend incorporating data on biogeography, fruit evolution, and previously overlooked characters such as floral traits and anatomical features. Special attention should also be given to recently described taxa as well as to those whose previous studies such as *Yucca cernua* and *Y. flaccida* may require further verification, as possible identification errors could have occurred.

Finally, some recently described taxa should be included as well. The characters used for the classification are not necessarily homologous; as shown in many studies morphological characters used in classification instead are homoplastic, such as the ovary position in some monocot families (Rudall, 2002), or the sori in grammitid ferns (Ranker *et al.* 2004). To determine homologous characters or potentially informative features that have been regarded as having no taxonomic value or have been ignored, we need to use a methodology to codify them and determine the homology of morphological characters as proposed by Tórres-Montúfar *et al.* (2018).

## Data availability statement

All sequences generated for this study are deposited in the NCBI GenBank under accession numbers shown in Supplementary Table S1.

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**Supplementary Materials.** The following supporting information can be downloaded at the DOI landing page of this paper:

**FIGURE S1.** ITS phylogeny inferred through Maximum Likelihood analysis, the numbers close to the nodes indicate the bootstrap values, only those equal to or greater than 60 are shown.

**FIGURE S2.** Chloroplast phylogeny inferred through Bayesian analysis, the numbers close to the nodes indicate the posterior values, only those equal to or greater than 60 are shown. The rectangles indicate the geographical area occupied by the species.

**FIGURE S3.** Ancestral state reconstruction of the caulescence indicated on the tree of maximum clade credibility obtained by Bayesian Inference in BEAST. The circles indicate the probability of the caulescence type at each node.

**FIGURE S4.** Ancestral state reconstruction of the leaf margins indicated on the tree of maximum clade credibility obtained by Bayesian Inference in BEAST. The circles indicate the probability of the margin leaf type at each node.

**FIGURE S5.** Ancestral state reconstruction of the inflorescence indicated on the tree of maximum clade credibility obtained by Bayesian Inference in BEAST. The circles indicate the probability of the inflorescence type at each node.

**TABLE S1.** Taxons used in molecular analyses. DBG= Desert Botanical Garden, Phoenix, Arizona, USA, FEZA=Herbarium of the Faculty of Higher Studies of Zaragoza; HNT= Huntington Botanical Gardens Herbarium; MERCA= Mercer Botanic Gardens; RSA= Rancho Santa Ana Botanic Garden. In Voucher herbarium w/n=without number. Section and serie according McKelvey (1938, 1947). In GenBank accession numbers P\* =accession number assignment in process.

**TABLE S2.** Evolution models of each analyzed region of the chloroplast.

**TABLE S3.** The probabilities that a specific character or state of character were present in a common ancestor at a specific node of the phylogenetic tree. Clades: **ABK** (Agavoideae Bimodal Karyotype: *Beschorneria*, *Agave*, *Hesperaloe*, *Hesperoyucca* and *Yucca*); **SG**: *Yucca* Stem Group; **YCG**: *Yucca* Crown Group; **YFF**: *Yucca* Fleshy Fruit; **YDF**: *Yucca* Dry Fruit; **YDF I**: *Yucca* Dry Fruit I (Group I); **YDF II**: *Yucca* Dry Fruit II (Group II); **H-H**: *Hesperaloe*-*Hesperoyucca*; and **B-A**: *Beschorneria*-*Agave*. **Characters**: **DF**=Dry fruit, **FF**=Fleshy fruit, **DhF**=Dehiscent fruit, **IhF**=Indehiscent fruit, **AMA**=Plants mainly acaulescent, **CMA**=Plants mainly caulescent, **MLE**= Margin leaf mainly entire, **MLD**=Margin leaf mainly denticulate, **IT1**=Inflorescence spike/raceme, and **IT2**=Inflorescence panicle.