

# Molecular evidence for the origin and evolutionary history of the rare American desert monotypic family *Setchellanthaceae*

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**Abstract** *Setchellanthus caeruleus*, which has disjunct populations in the north of the Chihuahuan Desert and in the Tehuacán-Cuicatlán valley, was selected to understand the evolutionary history of plants in this desert and its southerly relicts. This species constitutes the monotypic family Setchellanthaceae, which forms part of a group of plants that produce mustard-oil glucosides or glucosinolates. Molecular phylogenetic analyses based on DNA plastid sequences of plants of *S. caeruleus* from both areas, including representative taxa of the order Brassicales, were carried out to estimate the time of origin of the family (based on *matK+rcbL*) and divergence of populations (based on *psbI-K*, *trnh-psbA*, *trnL-trnF*). In addition, comparative ecological niche modelling was performed to detect if climate variables vary significantly in northern and southern populations. Analyses revealed that Setchellanthaceae is an ancient lineage that originated between 78 and 112 Mya during the mid-late Cretaceous—much earlier than the formation of the Chihuahuan Desert. The molecular data matrix displayed a few indel events as the only differences of plastid DNA sequences between northern and southern populations. It is suggested that due to climate changes in this desert in the Pliocene, populations of *Setchellanthus* remained in the Sierra de Jimulco and in Cuicatlán, in climatically stable locations. Ecological niche models of northern populations predict niches of southern populations and identity niche tests indicate that there are no differences in their ecological niches.

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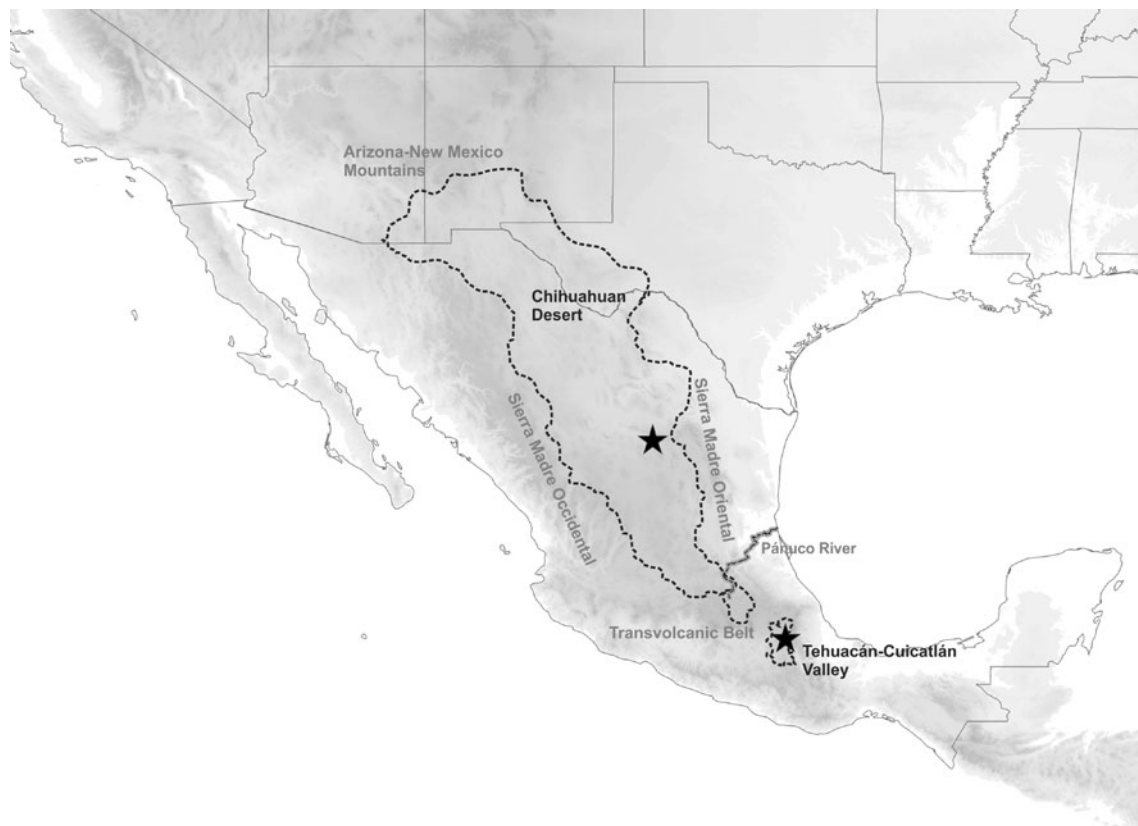
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## Introduction

The Chihuahuan Desert, a large upland arid area on the Mexican Plateau, is the most biologically diverse desert in North America (Jaeger 1957). This arid land is limited in the east by the Sierra Madre Oriental, in the west by the Sierra Madre Occidental, and in the north by the Arizona-New Mexico Mountains including western Texas (Fig. 1). A number of definitions and boundaries of the Chihuahuan Desert have been proposed based upon climate and vegetation factors, and variations appear mostly with respect to establishing southern limits. Based on the Martonne aridity indices, Schmidt (1986) determined that the southern limit of the Chihuahuan Desert is in Chihuahua. In contrast, delineations of the desert based on vegetation coincide in that its southern limit is in northern Queretaro (Henrickson and Johnston 1986; CONABIO 2000). Based on herpetological data, however, Morafka (1977) indicated that there are southerly relict Chihuahuan Desert zones in the Río Pánuco, in Hidalgo and adjacent areas. What is more, Shreve (1942) even proposed additional southern desert areas related closely to the more xeric vegetation of the Chihuahuan Desert, mainly in the Mesquital Valley in Hidalgo and in Tehuacán in Puebla and Oaxaca (see Fig. 1).

The insularity of the Chihuahuan Desert has produced an area rich in endemic plant and animal species (e.g., Krings 2000; McCain 2003; Hafner and Riddle 2005; Riddle and Hafner 2006; Wilson and Pitts 2010a; Hernández and Gómez-Hinostrosa 2011; Sosa and De-Nova 2012). Moreover, it has been indicated that the Chihuahuan Desert might be regarded as the center of origin of desert biota



**Fig. 1** Distribution range of *Setchellanthus caeruleus* in the Chihuahuan Desert and in the Tehuacán-Cuicatlán Valley. Stars indicate the two areas where populations are found, in the north in the Sierra of Jimulco and in the South, in the Cuicatlán Valley

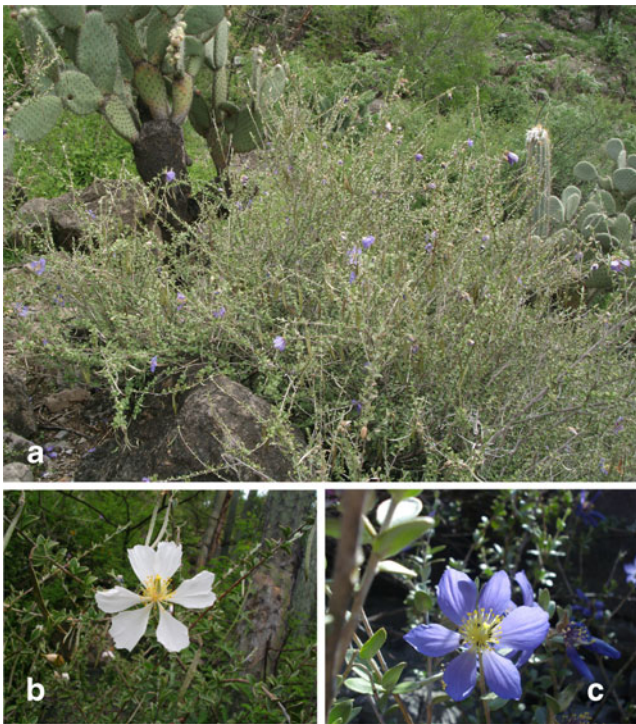
(Morafka 1977). With regard to plants, several groups are endemic to the Chihuahuan Desert (e.g., *Asta*, *Atriplex*, *Brickellia*, *Encelia*, *Holographis*, *Hyptis*, *Karwinskya*, *Larrea*; Shreve 1942; Sosa and De-Nova 2012). Some others extend their distribution and are found in the Chihuahuan Desert and in the Tehuacán-Cuicatlán valley (e.g., a small clade of the North American *Ephedra* lineage, Loera et al. 2012; a clade in *Leucophyllum*, Gándara and Sosa 2013; *Lindleya*, Henrickson 2012; *Hunnemannia* Ruiz-Sanchez et al. 2012; *Setchellanthus*, Iltis 1999). These arid lands are separated by an elevated mountain range: the Trans-Mexican Belt (see Fig. 1). Furthermore, the Tehuacán-Cuicatlán valley has also been identified as an ecological island given the high number of endemics (Dávila et al. 2002; Méndez-Larios et al. 2005; Sosa and De-Nova 2012).

To understand the evolutionary history of plants in the Chihuahuan Desert and its southerly relicts, *Setchellanthus caeruleus* Brandege was selected because it has disjunct populations in the north of the Chihuahuan Desert and in the Tehuacán-Cuicatlán Valley (Fig. 1). *S. caeruleus* is the sole species of Setchellanthaceae, a family that has received much attention because it forms part of a group of plants that produce mustard-oil glucosides or glucosinolates (Rodman et al. 1998; Mithen et al. 2010). Previously, the genus was considered part of Capparaceae. However, morphology (Iltis

1999), reproductive anatomy (Tobe et al. 1999), vegetative anatomy (Carlquist and Miller 1999), palynology (Tomb 1999) and molecular data (Karol et al. 1999) confirmed that it should be considered as independent. Setchellanthaceae is one of the families of the Brassicales—an order in which diversification began approximately 92 Mya (Magallón and Castillo 2009).

Plants of *Setchellanthus caeruleus* are shrubby, with longitudinally corky-ridged black branches, simple leaves and showy lavender-violet 5–7 merous solitary flowers with a small receptacle (Fig. 2). Populations of this species are infrequent, restricted in the north to mountains at the east of Durango and in Coahuila in the Sierra de Jimulco at elevations from 1,200 to 2,100 m. In the south they are restricted to the Tehuacán valley and plants have only been collected in two close areas in Cuicatlán at 760–1,950 m (Fig. 1).

The objectives of this paper were to perform molecular phylogenetic analyses based on DNA plastid sequences of plants of *Setchellanthus caeruleus* from the Chihuahuan Desert and from the Tehuacán-Cuicatlán valley including representative taxa of the order Brassicales. These analyses will estimate the time of origin of the family and of both groups of populations and determine whether they have diverged. In addition, comparative ecological niche modelling was



**Fig. 2** Habit (a) and flowers (b, c) of *S. caeruleus*

performed to find out if climate variables vary significantly between northern and southern populations.

## Methods

### Sampling

Although plants of *Setchellanthus caeruleus* from the Tehuacán–Cuicatlán valley and from the Chihuahuan Desert are infrequent, samples were collected (for northern populations 11 individuals were collected; for southern populations 12 individuals were collected). For representative taxa of the families of the order Brassicales, DNA sequences were downloaded from GenBank and a sample of *Carica papaya* was collected in Veracruz, Mexico. Morphological variation was observed on specimens from northern and southern populations of *Setchellanthus caeruleus* from the herbaria ENCB, IEB, MEXU, TEX and XAL (GenBank accession numbers are listed in Appendix I and vouchers and specimens in Appendix II).

### DNA sequencing

DNA was extracted from dried leaf tissue using the CTAB method (Doyle and Doyle 1987). Three plastid DNA regions, *trnH-psbA*, *trnL-trnF* and *psbI-psbK*, were sequenced. The *trnH-psbA* and *trnL-trnF* regions were amplified with primers and protocols reported in Shaw et al. (2005) while *psbI-psbK*

was amplified using primers and protocols reported in Lahaye et al. (2008). In addition, sequences of the *matK* and *rbcL* plastid genes of representative taxa in the order Brassicales were utilized in analyses and sequences were downloaded from GenBank. Both *matK* and *rbcL* regions were sequenced for representative *S. caeruleus* specimens from the northern and the southern populations. The primers 1F, 714R, 674F and 1024R (Savolainen et al. 2000) were used for *rbcL* and the primers 3F-1R (Dunning and Savolainen 2010) for *matK*. PCR products were sequenced using BigDye Terminator Cycle Sequencing Kit v.3.1 (Applied Biosystems, Foster City, CA) on an ABI 310 automated DNA sequencer (Perkin Elmer, Applied Biosystems) following the manufacturer's instructions. All DNA fragments were sequenced in both directions. DNA chromatograms were proofed, edited and assembled using Sequencher 4.1 (Gene Codes, Ann Arbor, MI). Sequences were aligned automatically using MUSCLE v.3.6 (Edgar 2004) following a manual refinement using BioEdit v.5.0.6 (Hall 1999).

### Phylogenetic analyses

Two sets of phylogenetic analyses were performed, at family and at population level. For the first analysis, 76 taxa representative of families of the order Brassicales and of the order Malvales were selected. *Tapiscia sinensis* Oliv. and *Gerrardina foliosa* Oliv. (Huerteales) were considered as outgroups. For some cases in which both DNA regions (*matK* and *rbcL*) were not available for the same taxon, we assembled sequences from different species of the same genus to minimize missing data, following Campbell and Lapointe (2009). A substitution model for each DNA region was determined using the Akaike criterion in MrModeltest v.2.3 (Nylander 2004). Maximum likelihood analyses and Bayesian inference were performed. Maximum likelihood analyses were performed in RAxML v.7.0.4 (Stamatakis 2006) with an independent general time reversible model (GTR) and a gamma distribution for site rates classified in 25 categories for each data partition. We performed 100 independent searches starting from a different MP initial tree. The maximum likelihood tree was selected from the resulting trees on each search. Clade support was assessed with 1,000 replicates of a non-parametric bootstrap analysis. Bayesian inference was performed in MrBayes v.3.1.2 (Ronquist and Huelsenbeck 2003), with independent models for each data partition. Two independent runs with four Monte Carlo Markov chains (MCMCs, one cold and three incrementally heated) for  $1 \times 10^7$  generations, with trees sampled every 1,000th generation, were performed. The first 25 % of the sampled trees were discarded as “burn-in” using the software AWTY (Wilgenbusch et al. 2004). The remaining trees were used to build a 50 % majority rule consensus tree. For population level analyses, Bayesian

inference was performed as described above with the individuals of *Setchellanthus caeruleus* and the outgroup species, *Carica papaya* L. In addition, parsimony analyses were performed with the software TNT (Goloboff et al. 2003) using the "new technology" option. Support for internal branches was estimated with 1,000 replicates using TBR branch swapping, with ten random entry orders saving one tree per replicate utilizing bootstrap. For the population data matrix, gaps were coded with the simple gap method (Simmons and Ochoterena 2000) performed in SeqState (Müller 2005) and were included as additional characters in both Bayesian and parsimony analyses.

### Molecular dating

A relaxed molecular clock was performed in BEAST v.1.6.1 (Drummond and Rambaut 2007) to estimate dates for the origin of *S. caeruleus* and divergence between populations using the Brassicales family level dataset. An uncorrelated relaxed clock was drawn from a lognormal distribution, based in the ML tree as prior. The substitution model and clock model were unlinked and the Yule process prior of speciation was selected. Molecular substitution rates were determined under a GTR+G model with ten rate categories. To accommodate for calibration uncertainty, we implemented six restriction node dates with data obtained from the fossil record (see below). For each restriction, we implemented an exponential distribution as prior (Ho and Phillips 2009), setting the offset of the distribution to the minimum age reported for each fossil and a mean set to a proper date such that 95 % of the distribution covered 125 Mya, which corresponds to the oldest eudicot pollen found in the fossil record (Brenner 1996). We executed two runs of  $5 \times 10^7$  generations each and sampled every  $1 \times 10^3$  generations. Six dates obtained from the fossil record identified within the order Malvales and Brassicales allowed us to restrict dates for six nodes in our relaxed molecular clock: (1) the taxon fossil *Dressiantha bicarpellata* from the Turonian of New Jersey (Gandolfo et al. 1998) possess a set of characters now found in extant families of the order Brassicales (*sensu* APGII 2003). We placed this fossil date at the crown node of Brassicales (Core Brassicales), after the Tropaeolaceae/Akaniaceae divergence (see Gandolfo et al. 1998; Couvreur et al. 2010). (2) *Capparidoxylon holleisii*, a fossil wood from the early to the mid-Miocene in Bavaria, Germany (Selmeier 2005; see Beilstein et al. 2010) was assigned to Capparaceae after a comparison with four extant capparaceous genera and approximately 14 species of *Capparis* (Selmeier 2005), and we placed this as a possible date for the stem node leading to the Capparaceae divergence. (3) *Thlaspi primaevum*, from the Oligocene in southwestern Montana, USA (Becker 1961), has been placed in Brassicaceae by the possession of a superior ovary, bicarpellate construction and single style (Beilstein et al. 2010), and was assigned to

*Thlaspi* based on different characters such as the presence of multiple concentrically striate seeds within each locule (Becker 1961; Manchester and O'Leary 2010). Within Brassicaceae, striated seeds occur only in *Thlaspi* and *Alliaria*, and we used this fossil date to constrain the origin of the crown group leading to these taxa (Beilstein et al. 2010). Magallón and Sanderson (2001) and Magallón and Castillo (2009) report two fossils that allowed us to calibrate the crown and stem group of Malvales: (4) pollen grains from the Lower Maastrichtian (Wolfe 1976), and (5) *Craigia* sp. and *Tilia* sp. from the Late Eocene of North America (Manchester 1999), respectively. (6) Finally, *Akania* sp. is the oldest fossil assigned to this genus, found in the Danian (Paleocene) from Palacio de los Loros flora in Argentina (Iglesias et al. 2007; Beilstein et al. 2010) and we used it as a crown date, to calibrate the split between Akaniaceae and Tropaeolaceae.

### Ecological niche models

Ecological niche models of northern and southern populations of *Setchellanthus caeruleus* were generated from georeferenced herbarium collections at ENCB, IEB, MEXU, TEX and XAL (included in Appendix II). We used climate variables from the WorldClim database (Hijmans et al. 2005) with a resolution of approximately 1 km<sup>2</sup> for estimating the ecological niche models with the maximum entropy algorithm MaxEnt v.3.3.2 (Phillips et al. 2006; Phillips and Dudik 2008). We selected this software because it performs well with small sample sizes (Hernández et al. 2006; Pearson et al. 2007), like the rare populations with only a few individuals of *S. caeruleus*. To measure the degree to which the models produced differed from what would be expected by chance, and to obtain a confidence measure for the ENMs (see Lobo et al. 2008), we used AUC (the area under the receiving operating characteristic curve); values near 1 indicate high predictive capacity and values near 0 indicates models with low predictive capacity (Phillips and Dudik 2008). To evaluate differences of the ecological niches of the northern and southern populations, we used a set of comparative similarity measures and statistical tests estimated with a graphical interface called ENMTools (Warren et al. 2010) that interacts with MaxEnt (Phillips et al. 2006; Phillips and Dudik 2008). This approach measures Schoener's (1968) similarity *D* and a measure derived from the Hellinger distance called *I* (Warren et al. 2008). Values of these measures range from 0 to 1, with values close to 1 indicating high similarity between niches, whereas values close to 0 indicate niche dissimilarity. This analysis also included two quantitative tests of niche evolution. The identity test asks whether the ENMs generated from two populations are significantly identical comparing the observed *D* and *I* values with a null distribution of *D* and *I* measures generated by pooling the occurrence points of two or more species or populations. In addition, the background test asks whether

ENMs of two or more species are more or less similar than expected by chance considering their available environment (as opposed to the exact locations in which the species occur). Based on our errors of omission and commission, we assumed the area with a prediction threshold  $\geq 0.3$  of the ENMs generated with MaxEnt (Phillips et al. 2006; Phillips and Dudik 2008) as the background area. For both tests, we used 100 pseudoreplicates and significance for both analyses was tested with confidence levels of 0.05 and 0.01.

## Results

### Phylogenetic analyses

The concatenated chloroplast *rbcL* and *matK* DNA matrix of the representative species of the families of the order Brassicales included a total of 74 taxa. Additionally, we included the *rbcL* and *matK* sequences of an individual from the Chihuahuan Desert and of an individual from the Tehuacán Cuicatlán Valley of *S. caeruleus* in this dataset. The matrix comprised 2,250 aligned sites, from which 993 were variable and 692 were parsimony informative. Bayesian inference and maximum likelihood analyses yielded topologically identical trees, with most of the nodes highly supported by posterior probabilities and bootstrap values (Fig. 3). The monophyly of Brassicales is confirmed in both ML and Bayesian trees. The first clade to diverge within Brassicales includes the Akeniaceae and Tropaeolaceae (*Bretschneidera sinensis* Hemsl. and *Tropaeolum majus* L. respectively). Following this split, and within the so-called Core Brassicales, the next clade to diverge includes the representative species of Moringaceae and Caricaceae. *S. caeruleus* diverged after this clade, and is sister to the remaining Brassicales families (Limnanthaceae, Koeberliniaceae, Bataceae, Tovaricaceae, Pentadiplandraceae, Gyrostemonaceae, Resedaceae, Capparaceae, Cleomaceae and Brassicaceae) (Fig. 3).

The population matrix of the concatenated *trnH-psbA*, *trnL-trnF*, and *psbI-psbK* plastid regions included a total of 25 terminals and 1,543 sites, from which 252 were variable and 12 were parsimony informative. Our alignment revealed low variability at the nucleotide substitution level; however, several indel events characterized the northern and/or the southern individuals and their inclusion as coded characters in further Bayesian and parsimony analyses allowed us to evaluate genetic divergence between the northern and the southern populations (the data matrix is included as Fig. 1 of the Electronic Supplementary Material). Bayesian analyses retrieved a topology where northern and southern populations were recovered as monophyletic with moderate support values (not shown). Parsimony analyses retrieved two most parsimonious trees (L=892, CI=0.992, RI=0.998); the consensus tree also shows two clades with good support values, corresponding

to the northern individuals of *S. caeruleus* and the other including the southern individuals (Fig. 4).

### Molecular dating

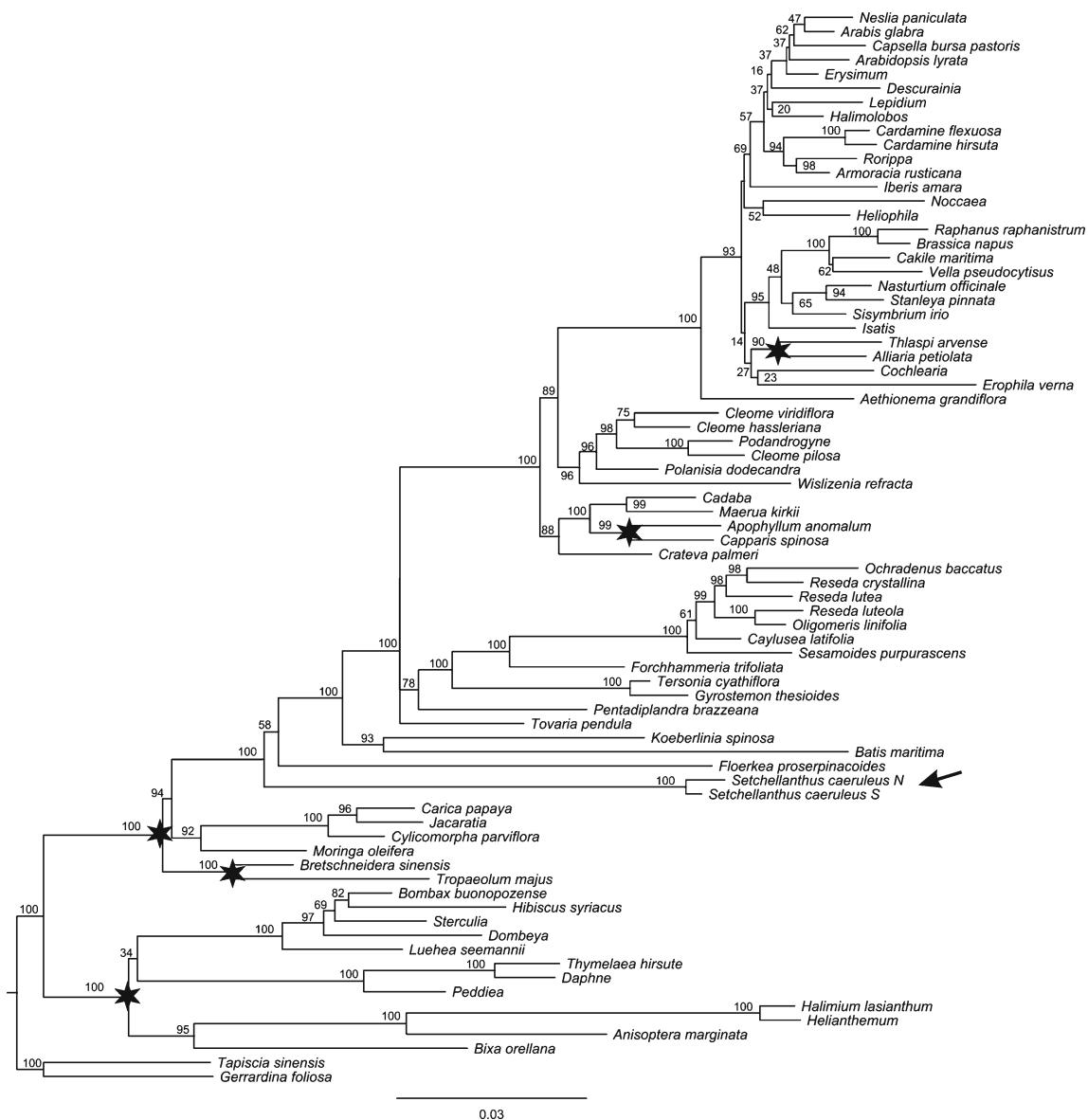
Resulting dates for the origin of crown and stem groups of the main Brassicales families are included in Table 1 and the resulting chronogram is shown in Fig. 2 of the Electronic Supplementary Material. Our dating analyses revealed an ancient origin of *S. caeruleus*, between 78 and 112 Mya during the mid- to late-Cretaceous. Given the genetic divergence among northern and southern *S. caeruleus* populations confirmed with results of the population level phylogenetic analyses described above, as well as the observed divergence in *matK* and *rbcL* sequences from representative individuals (results not shown), we considered the inclusion of a northern and a southern specimen of *S. caeruleus* in our family level dataset to obtain possible estimates for the ages of the split of populations. Our results indicate that the divergence among northern and southern populations occurred very recently, at around 2 to 13 Mya, during the late Miocene or even the Pliocene (see also Fig. 2 of the Electronic Supplementary Material).

### Ecological niche modeling analyses

Given that we found no evidence of morphological variation among northern and southern populations of *S. caeruleus*, we performed an ecological niche model comparison approach to detect variation at an ecological level. The ecological niche models for the northern and southern populations of *S. caeruleus* are shown in Fig. 5. Both models can reach the distribution of the other, and particularly for northern populations, models reach portions of the Tehuacán Valley with high probability values (Fig. 5). The measured niche overlap between both populations was  $I=0.424$  and  $D=0.149$ . Results of the identity and background tests are summarized in Fig. 3 of the Electronic Supplementary Material. According to the identity test, the true calculated  $I$  and  $D$  values are much lower than the randomized distribution of pseudoreplicates, both with significance levels of  $P>0.01$  and  $P>0.05$ . The background test result could not reject the null hypothesis, indicating a similarity of ecological niches (Fig. 3 of Electronic Supplementary Material).

## Discussion

Results of Bayesian inference and maximum likelihood analyses corroborated that *Setchellanthus* is independent, not part of Capparaceae. Moreover, Iltis (1999) had previously assigned this genus in its own family. *Setchellanthus* was retrieved as the sister taxon to a large group comprising



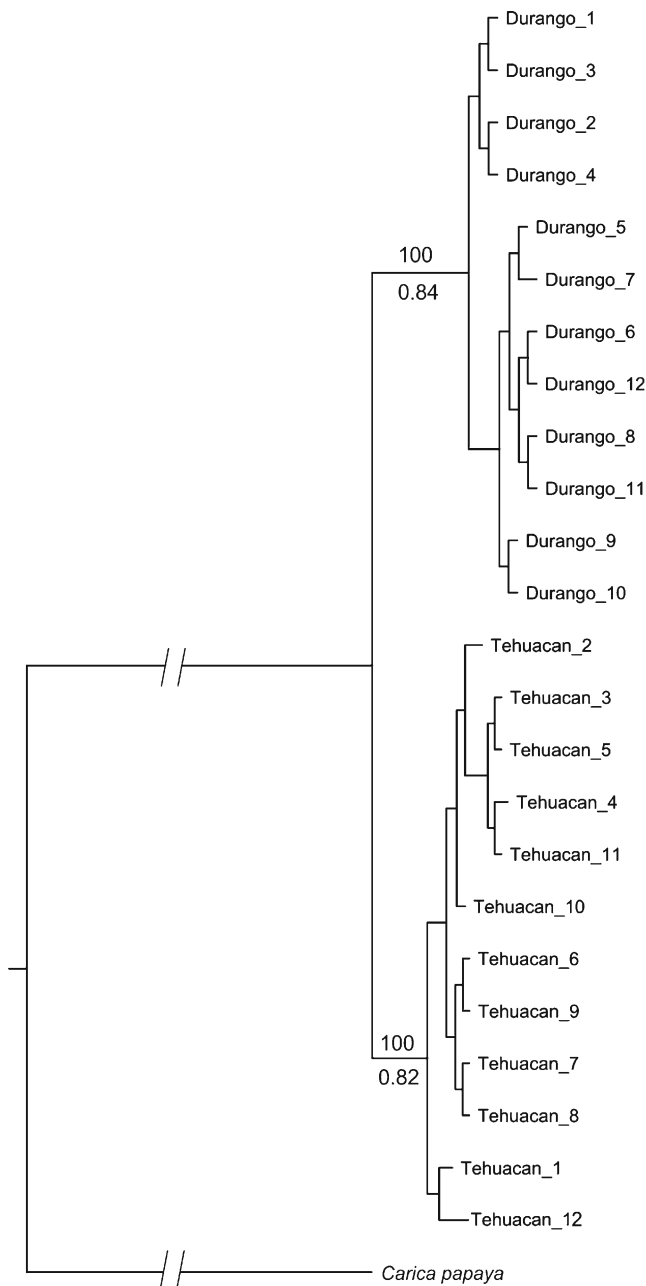
**Fig. 3** Maximum likelihood tree with 74 representative species of the families of the orders Brassicales and Malvales. Asterisks indicate nodal age constraints for the relaxed molecular clock analyses

the rest of the families of the Core Brassicales, largely agreeing with the latest glucosinolate-producing plants phylogeny based in molecular and morphological data (Ronse de Craene and Hanson 2006).

Our estimated dates for the origin and diversification of the order Brassicales are older than previous reported dates. Wikström et al. (2001) dated the origin of stem Brassicales to 94–85 Mya, with a diversification of the crown group at 76–70 Mya. Wang et al. (2009), using a Bayesian relaxed approach, obtained dates of 89 Mya for the stem and 73 crown groups respectively, while Magallón and Castillo (2009) estimated ages of 91.9 and 92.1 Mya for relaxed and constrained penalized likelihood for the age of stem

Brassicales, and 65.8 and 66 Mya for the crown group. However, it is necessary to mention that these dates were obtained for phylogenies constructed for larger groups such as the angiosperms or Rosids, and thus have a broader taxonomic sampling at higher taxonomic levels.

Couvreur et al. (2010) used a dataset of eight molecular markers covering the three genomes to date the origin of Brassicaceae. They obtained dates for the crown Brassicaceae [37.6–(24.2)–49.4 Mya] agreeing with our estimates. Beilstein et al. (2010) used a similar approach to date the origin and diversification of *Arabidopsis*. They dated the origin of crown Brassicaceae at 64.2–45.2 Mya with a stem group date of 76.5–54.4 Mya, comparable to our dates. Recently, Carvalho and



**Fig. 4** Consensus tree of two most parsimonious trees of analyses based on the Chihuahuan Desert and Tehuacán-Cuicatlán Valley populations of *S. caeruleus* and *Carica papaya* as the outgroup taxon

Renner (2012) estimated the time of origin of the Caricaceae at 43.1 Mya, resembling our results of 38.1 Mya for the origin of this family, which belongs to Brassicales.

Hence Setchellanthaceae is an ancient lineage that originated between 78 and 112 Mya during the mid-to-late Cretaceous. The Chihuahuan Desert, like the other North American deserts is of very recent origin, no older than the late Miocene or early Pliocene, 7–5 Mya (see the review by Wilson and Pitts 2010b). Axelrod (1958, 1979) suggested that plants endemic to the North American deserts evolved

earlier than the deserts and that the evolution of a xeric flora started as early as 99–65 Mya. These dates coincide with the time of origin of *Setchellanthus*. Divergence of other Chihuahuan Desert endemics is, however, posterior, like in the poppy *Hunnemannia* (12.8 Mya, Ruiz-Sanchez et al. 2012) or in the borage *Tiquilia* (33 Mya, Moore and Jansen 2006).

Our population molecular data matrix, including individuals of *Setchellanthus* from the Chihuahuan Desert and from the Tehuacán-Cuicatlán Valley did not display polymorphisms at the nucleotide substitution level. Furthermore, we tested the most variable molecular markers suggested by Shaw et al. (2005) and they did not exhibit variation either. Variation consisted of several indel events, which were coded to perform further Bayesian and parsimony analyses. Moreover, our observations on the specimens from these localities found no morphological differences, suggesting that both groups of populations are relicts of a more ample distribution of *Setchellanthus* in arid lands, corroborating the relationship of the southerly arid lands with the main Chihuahuan Desert proposed by Shreve (1942). Although the history of the Trans-Mexican Volcanic Belt was dynamic and occurred in several stages, it has been estimated that it emerged from 3 to 20 Mya (Ferrari et al. 2012), i.e., later than our estimated time of origin for *Setchellanthus*.

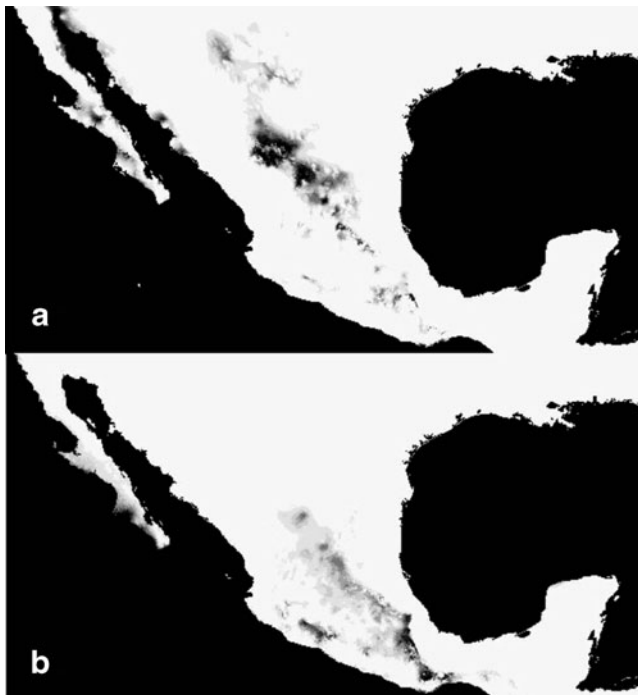
During the early Pliocene, the Chihuahuan Desert, along with the other North American deserts, attained its maximum extension, but it shrank during the moist late Pliocene and during the Pleistocene pluvial intervals (Riddle and Hafner 2006). In the Pleistocene, the desert was much more mesic, dominated by *Pinus* and *Juniperus* forests (van Devender 1990). Fossil arthropod remains from packrat middens found in the Chihuahuan Desert indicate that at least some refugia for desert biota also existed during this time (Miller 1977; Elias et al. 1995). Thus, we propose that, due to these climate changes, populations of *Setchellanthus* remained only in the Sierra de Jimulco and in Cuicatlán. Wilson and Pitts (2012) found a climatically stable location in the Chihuahuan Desert during the Pleistocene that corresponds to northern localities of *Setchellanthus*.

Comparative studies of ENMs have been used to assess whether environmental requirements have diverged between the species or populations located on the either side of biogeographic boundaries (e.g., Graham et al. 2004; Kozak and Wiens 2006; Jezkova et al. 2009; see Glor and Warren 2010), and have proven a powerful tool in species delimitation when morphological divergence is absent (Hawltischek et al. 2011). Here, we used an ENM comparative framework that can be utilized to test for the presence of significant environmental variation across biogeographic boundaries (Glor and Warren 2010). On the one hand, ecological niche modelling of northern and southern populations of *Setchellanthus caeruleus* indicates that they do not fully occupy their potential distribution area,

**Table 1** Results of molecular dating for representative taxa in families of orders Brassicales and Malvales

Taxon	Stem group date of origin (Mya)	Crown group date of origin (Mya)
Huerteales	105.64 – 155.91	49.78 – 140.91
Malvales	104.87 – 152.98	77.34 – 125.37
Brassicales	104.87 – 152.98	90.20 – 128.04
Akaniaceae -Tropaeolaceae	61.70 – 82.37	
Core Brassicales	90.20 – 128.04	89.30 – 124.64
Moringaceae	54.93 – 112.10	
Caricaceae	54.93 – 112.10	13.27 – 38.61
<i>Setchellanthus caeruleus</i>	78.48 – 112.18	2.70 – 13.82
Limnanthaceae	76.36 – 109.92	
Koerberliniaceae	52.05 – 89.24	
Bataceae	52.05 – 89.24	
Tovariaceae	61.34 – 88.90	
Pentadiplandraceae	54.47 – 82.11	
Gyrostemonaceae	47.30 – 74.62	4.82 – 20.90
Resedaceae	36.90 – 62.11	19.90 – 36.87
Resedaceae (Including <i>Forchhammeria</i> )	47.30 – 74.62	36.90 – 62.11
Capparaceae	49.33 – 70.61	35.60 – 63.42
Cleomaceae	32.50 – 57.77	24.68 – 48.51
Brassicaceae	45.73 – 65.96	36.36 – 51.63
<i>Arabidopsis</i>	11.06 – 22.99	

suggesting also the hypothesis of refugia. On the other hand, ecological niche models from the northern populations predict areas of the southern populations. What is more, *I* and *D*



**Fig. 5** a,b Ecological niche models obtained for *S. caeruleus* populations. a Chihuahuan Desert, b Tehuacán-Cuicatlán Valley

measures indicate that niches of these groups of populations do not differ, reflecting an early ecological divergence. Thus, despite the large distance and the mountain ranges separating them, both groups of populations have not yet achieved a complete ecological divergence.

In conclusion, *Setchellanthus* is an ancient lineage, which originated between 78 and 112 Mya during the mid-to-late Cretaceous prior to formation of the Chihuahuan Desert. Due to climate changes in the Pleistocene, populations remained in Durango-Chihuahua and Tehuacán-Cuicatlán. Although they are separated by mountain ranges and by large distance, populations have not diverged ecologically, morphologically and genetically.

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## Appendix 1

Species considered in phylogenetic analyses for Setchellanthaceae and their GenBank accession numbers (*matK* and *rbcl*).



Malvales: *Anisoptera marginata* AJ581409, Y15144. *Bixa orellana* FM179929, AF022128. *Bombax buonopozense* AY321171, AF022118. *Daphne bholua* FM179927, AF022132. *Dombeya spectabilis* AY321173, AY082354. *Halimium lasianthum* GQ281698, GQ281670. *Helianthemum scopulicola* DQ092970, Y15141. *Hibiscus syriacus* EF207270, AY328174. *Luehea seemannii* GQ982036, GQ981791. *Peddiea africana* FJ572800, AJ745176. *Sterculia tragacantha* AY321178, FJ976172. *Thymelaea hirsuta* EU002191, Y15151. Huertales: *Gerrardina foliosa* FM179924, AY757086. *Tapiscia sinensis* EU002190, AF206825. Brassicales: *Bretschneidera sinensis* AY483220, M95753. *Batis maritima* AY483219, M88341. *Aethionema grandiflora* AF144354, AY167983. *Alliaria petiolata* AF144363, FJ395597. *Arabidopsis lyrata* AF144342, XM2888312. *Arabis glabra* AF144333, DQ310542. *A Armoracia rusticana* FN597648, AF020323. *Brassica napus* AB354273, AF267640. *Cakile maritima* GQ424577, AY167981. *Capsella bursa-pastoris* HQ619802, FN594844. *Cardamine flexuosa* AB248011, D88905. *Cardamine hirsuta* HQ619803, HQ619739. *Cochlearia danica* AF174531, FN594827. *Descurainia sophia* GQ424581, FN594838. *Erophila verna* HQ619804, HQ619740. *Erysimum handel-mazzettii* DQ409262, AY167980. *Halimolobos jaegeri* DQ406763, FN594846. *Heliophila variabilis* GQ424588, AM234933. *Iberis amara* GQ424589, FN594828. *Isatis tinctoria* AB354278, FN594830. *Lepidium perfoliatum* DQ406766, GQ436651. *Nasturtium officinale* AY483225, AF020325. *Neslia paniculata* DQ406767, DQ310541. *Raphanus raphanistrum* AB354265, GQ184382. *Rorippa islandica* DQ406770, AF020328. *Sisymbrium irio* AF144366, AY167982. *Noccaea cochleariformis* GQ424598, FN594826. *Thlaspi arvense* AF144360, FN594829. *Vella pseudocytisus* GQ248209, GQ248705. *Apophyllum anomalum* AY483227, AY483264. *Cadaba virgata* EU371753, AM234931. *Capparis spinosa* AY491650, AY167985. *Crataeva palmeri* AY483229, AY483265. *Maerua kirkii* AY483229, AY483265. *Wislizenia refracta* AY483230, AY483266. *Forchhammeria trifoliata* AY483235, AY483271. *Carica papaya* AY483245, AY483277. *Cylicomorpha parviflora* AY042564, M95671. *Jacaratia digitata* AY461575, AF405244. *Cleome hassleriana* AY461574, AF405245. *Cleome pilosa* AY491649, M95755. *Cleome viridiflora* AY483231, AY483267. *Podandrogyne decipiens* AY483232, AY483268. *Polanisia dodecandra* EU371815, AY483269. *Gyrostemon thesioides* AY483234, AY167984. *Tersonia cyathiflora* FJ212199, FJ212210. *Koerberlinia spinosa* AY483238, L22441. *Floerkea proserpinacoides* AY483222, L14600. *Moringa oleifera* EU002178, L12679. *Pentadiplandra brazzeana* AY483223, L11359. *Caylusea latifolia* AY483239, U38533. *Ochradenus baccatus* GQ891209, GQ891229. *Oligomeris linifolia* GQ891194, GQ891210. *Reseda lutea* AY483240,

AY483272. *Reseda luteola* AY483241, AY483273. *Reseda crystallina* FJ212206, FJ212219. *Sesamoides purpurascens* FJ212200, FJ212212. *Setchellanthus caeruleus* FJ212208, KC778754; FJ212221, KC778756; *Tovaria pendula* AY483242, M95758. *Tropaeolum majus* AY483224, AB043534.

## Appendix II

Specimens of *Setchellanthus caeruleus* utilized for morphological observations and for ecological niche models. Specimens utilized for molecular analyses and their GenBank accession numbers are also indicated.

### Northern populations

*Coahuila*: I. M. Johnston 11478 (XAL), Coahuila, Jimulco, 103.2°W, 25.183°N; J. Valdés Reyna, H. H. Iltis, K. Karol & E. Blanco 149963 (TEX), Coahuila, Torreón, 103.64° W, 25.51°N; M. Engleman (XAL). Durango: Durango, Lerdo, 103.64°W, 25.52°N; Durango: D. S. Correll & I. M. Johnston 20012, 149968 (TEX), Durango, Lerdo, 103.65°W, 25.21°N; H. H. Iltis & A. Lasseigne, 100 (XAL) Durango, Lerdo, 103.71°W, 25.43°N; H. H. Iltis & A. Lasseigne, 149964 (TEX) Durango, Lerdo, 103.71°W, 25.45°N; E. Gándara 3046 (XAL), Durango, Mapimí, 25.67°W, 103.87°N; E. Gándara 3050 (XAL), Durango, Lerdo, 25.43°W, 103.70°W; H. Sánchez-Mejorada 2576 (MEXU), km 101 carretera Gómez-Palacio a Ceballos; J. Henrickson 23145 (TEX), Durango, ca. 16 air miles SW of Torreón, upper road to Microondas Sapioris 103.45°W, 25.26°N. DNA plastid sequences (*psbA-trnH*, *trnL-trnF* and *psbI-psbK*). Population 1: E. Gándara 3046 (XAL) (KC778733 - KC778736; KC778769 - KC778772; KC867730 - KC8737) Population 2: Gándara 3050 (XAL) (KC867721 - KC867728; KC86772 - KC867719; KC778749 - KC778752).

### Southern populations

*Oaxaca*: C. A. Purpus 3400 (UC), Mesa de Coscomate; F. González-Medrano F-1552 (MEXU), Oaxaca, Cuicatlán, 96.99°W, 17.81°N; J. G. Sánchez-Ken 220 (MEXU), Oaxaca, Cuicatlán, 97.01°W, 17.81°N; F. González-Medrano F-1168 (MEXU), Oaxaca, Teotitlán, 97.06°W, 18.09°N; P11767 (XAL), 97.48°W, 18.31°N; A. Valiente Banuet 900 (MEXU), 97.48°W, 18.32°N; 149967 (TEX), Puebla, San José Miahuatlán, 97.22°W, 18.17°N; M. Castañeda-Zárate & W. Colorado-Durán 432–444 (MEXU), Puebla, Zapotitlán de las Salinas, 18.31°W, 97.47°N; J. Rzedowski 33229 (MEXU), 17 km SW de Tehuacán, carretera a Huajuapán de León; J. Panero

(3465) (MEXU) ca. 5 km from Axusco. DNA plastid sequences (*trnH-psbA*, *trnL-trnF* and *psbI-psbK*): Population 1: M. Castañeda-Zárate & W.B. Colorado-Durán 432–436 (MEXU) (KC778722 - KC778726; KC778758 - KC778762; KC778738 - KC778743). Population 2: M. Castañeda-Zárate & W. B. Colorado-Durán 437–444, (KC778727 - KC778730; KC778763 - KC778767; KC778744 - KC778748) A. Valiente Banuet 900 (MEXU) (KC78731 - KC8732; KC8767 - KC778768).

*Carica papaya* N. P. Moreno 101 (XAL), Xalapa, Veracruz (KC867738, KC867729, KC867720).

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