

ON THE ORIGIN OF THE TWO PUTATIVE ALLOPOLYPOIDS, *OPUNTIA CURVISPINA* AND *O. MARTINIANA* (CACTACEAE): A CASE OF CRYPTIC SPECIATION IN PRICKLY PEAR CACTI

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ABSTRACT

The putative allopolyploid taxon, *Opuntia curvispina*, is distributed from northeastern Arizona and southern Nevada to southeastern California, where it often grows in close proximity to, or sympatric with, *O. chlorotica*, *O. engelmannii* and *O. phaeacantha*. Another putative allopolyploid, *O. martiniana*, grows sympatrically with *O. curvispina* in only one area north of the Hualapai Mts., northwestern Arizona. Both of these taxa have previously been treated as either nothospecies or mere spontaneous hybrids by previous researchers. My objectives were to determine the origins of both *O. curvispina* and *O. martiniana* to test previous hypotheses of hybrid origin, with *O. curvispina* putatively derived from *O. chlorotica* and *O. phaeacantha* and *O. martiniana* putatively derived from *O. chlorotica* and *O. engelmannii*. I also wanted to determine the overall extent of the ranges of the two taxa by expanding collections and verifying existing herbarium collections, as numerous specimens identified as *O. martiniana* and *O. curvispina* have been reported from outside of their expected ranges. My data indicate that *O. curvispina* indeed is partially derived from *O. chlorotica*, as well as another putative parent, likely *O. phaeacantha*, while *O. martiniana* appears to be most likely derived from *O. curvispina* and *O. macrorhiza*, two taxa not before implicated in the origin of *O. martiniana*. The hexaploid *O. phaeacantha* also appears to be non-monophyletic and may have been derived several times from different hybridization events. Careful morphological study of *O. martiniana* clearly separates that species from *O. curvispina*, although it may be considered cryptically different, a likely result of its partial putative origin from *O. curvispina*. Given the very broad distribution and dominance of *O. curvispina* in parts of its range, it is most appropriate to recognize this allopolyploid as a species, rather than as a nothotaxon, as previously designated. The unique feature of an obovate or urceolate style in *Opuntia martiniana* appears to be a transgressive trait rather than a synapomorphy, given that the taxon is derived from reticulation, and neither putative parent exhibits that character state. This work provides a framework for species recognition using DNA sequence data, morphological characters, geography and cytological information—a total evidence approach clearly needed for taxonomically difficult taxa, such as many within the genus *Opuntia*. A treatment of *O. curvispina* and *O. martiniana* is given with updated descriptions, and *O. martiniana* is neotypified.

RESUMEN

El alopoliploide putativo, *O. curvispina*, se distribuye desde el noreste de Arizona has el sur de Nevada y el sureste de California, donde crece cerca a o simpátricamente con *O. chlorotica*, *O. engelmannii*, y *O. phaeacantha*. Otro alopoliploide putativo, *O. martiniana*, crece simpátricamente con *O. curvispina* en solo un área al norte de las montañas Hailapai en la parte noroeste de Arizona. Ambos taxones han sido tratados previamente como notoespecies o simplemente híbridos espontáneos. Mis objetivos para este estudio fueron a determinar los orígenes de *O. curvispina* y *O. martiniana* para probar las hipótesis de que *O. curvispina* originó de hibridación entre *O. chlorotica* y *O. phaeacantha* y *O. martiniana* de *O. chlorotica* y *O. engelmannii*. También quería determinar la distribución actual de los dos taxones usando datos del herbario, ya que numerosos especímenes han sido identificados de ambos de lugares fuera de su rango esperado, y por trabajo de campo expandiendo las colecciones disponibles. Mis datos indican que *O. curvispina* si es parcialmente derivado de *O. chlorotica* y otro progenitor lo más probable que sea *O. phaeacantha*, mientras *O. martiniana* parece ser derivado de *O. curvispina* y *O. macrorhiza*, dos especies no previamente implicados en el origen del taxón. El hexaploide *O. phaeacantha* parece ser no monofilético y posiblemente ha sido derivado varias veces de diferentes eventos de hibridación. Estudio cuidadoso de morfología de *O. martiniana* claramente separa la especie de *O. curvispina* aunque se le podría considerar críptica, que posible tiene que ver con su origen parcial de *O. curvispina*. Dado la distribución amplia y dominancia de *O. curvispina* en partes de su rango, es más apropiado considerar este alopoliploide como una especie en lugar de nototaxon, como ha sido circunscrito previamente. El rasgo único del estilo o urceolado en *O. martiniana* parece ser un rasgo transgresivo en vez de sinapomorfía ya que el taxón es derivado de reticulación y ninguno de los progenitores exhibe este estado de carácter. Este trabajo provee una estructura para reconocer especies usando datos de ADN, morfología, geografía e información citológica—un análisis de evidencia total que se necesita para grupos taxonómicamente difíciles como en el género *Opuntia*. Se provee un tratamiento de *O. curvispina* y *O. martiniana* con descripciones actualizadas y se neotipifica *O. martiniana*.

KEY WORDS: Haplotypes, Hybridization, *Opuntia chlorotica*, Phylogenetics, Polyploidy

INTRODUCTION

Opuntia curvispina Griffiths was described by David Griffiths, originally as *O. curvispina* (Griffiths 1916), from material collected from between Nipton, CA, and Searchlight, NV. Crook and Mottram (1996) corrected the spelling of the specific epithet to *curvispina*, as per Art. 60.1, 60.8 of the code (Turland et al. 2018). Griffiths (1916) mentions that the species was more widespread, however, from CA through the AZ desert. Griffiths described the species as being arborescent with a single trunk and compared it directly to *O. chlorotica* Engelm. & J.M. Bigelow, however, noting the differences in spines, seeds, fruit and the overall “robustness” of the plant, with *O. curvispina* being described as more robust than *O. chlorotica* (see Fig. 1A–D, G–H). Parfitt (1980) studied the morphological and chromosomal variation in populations of *O. curvispina* from around Kingman, AZ, and concluded that the tetraploid species was of hybrid origin between *O. phaeacantha* Engelm. var. *major* Engelm. and *O. chlorotica*. Benson (1982) included *O. curvispina* within his concept of *O. phaeacantha* var. *mojavensis* (Engelm.) Fosberg (see below), and Pinkava (2003) recognized the taxon as a nothospecies, *O. xcurvispina*, owed to it being of hybrid origin, as suggested by Parfitt (1980).

Opuntia mojavensis Engelm. & Bigelow was described from west of what now is the Mojave National Preserve along Mojave Creek (River) in San Bernardino Co., CA, close to the town of Hesperia. Engelmann (1856) recognized *O. mojavensis* as a species but could not form a conclusive idea of where it belonged and suggested that it might even be a form of *O. phaeacantha*. Britton and Rose (1920) also recognized *O. mojavensis* as a species but noted the difficulty of determining the true relationship of the species based on such fragmentary material. This taxon was recognized at the varietal level within *O. phaeacantha* by Fosberg (1934). Benson (1982) followed this circumscription, and he considered *O. curvispina* to be a synonym of that taxon. Benson (1982) lectotypified *O. mojavensis* based on scant material from the original Bigelow collection, which consisted only of a few areoles with spines and a sterile fruit. Parfitt (1980) concluded that the description of *O. mojavensis* was too scant to determine the taxon to species (he had not seen the type at that time) but suggested that it most likely was not the same as that of *O. curvispina* based on the original description. He thus excluded the name in his treatment of *O. curvispina*. Parfitt (1980) concluded that the growth form as described by Engelmann was closer to that of *O. phaeacantha*, and the lectotype material of *O. mojavensis* at MO was later viewed and identified as *O. phaeacantha* by Pinkava and Parfitt.

Opuntia martiniana (L.D. Benson) Parfitt from north of the Hualapai Mountains in the Mohave Desert of northwestern Arizona (Fig. 2) is a small, erect shrub that was originally described as a variety of the Chihuahuan Desert species, *O. macrocentra* Engelm., by Benson (1950), and then transferred to a variety of a west coast hexaploid species, *O. littoralis* (Engelm.) Cockerell, as *O. littoralis* var. *martiniana* (L.D. Benson) L.D. Benson (Benson & Walkington 1965). Parfitt (1980) concluded that the tetraploid *O. martiniana* deserved specific status and was not conspecific with either *O. littoralis* or *O. curvispina*. Pinkava (2003) did not recognize *O. martiniana*, suggesting that the taxon was not distinct enough morphologically to separate from *O. curvispina*, but also mentioned that it could have been derived from hybridization between *O. chlorotica* and *O. engelmannii* Salm-Dyck ex Engelm. [instead of between *O. chlorotica* and *O. phaeacantha*, as in *O. curvispina* according to Parfitt (1980)]. Parfitt (1980) used the distinctive obovate style of *O. martiniana*, along with other more quantitative characters to distinguish the species from *O. curvispina* (see Fig. 1E–F, I).

Numerous chromosome counts are available for both *O. martiniana* and *O. curvispina* (Pinkava et al. 1973; Parfitt 1980; Baker et al. 2009; Baker & Pinkava 2018), both of which have been consistently recorded as tetraploid ($2n=44$). Putative parental taxa *O. chlorotica*, *O. engelmannii* and *O. phaeacantha* have been recorded as diploid ($2n=22$) in the former and hexaploid ($2n=66$) in the two latter taxa. Other taxa occurring sympatrically or nearby to both *O. curvispina* and *O. martiniana* in the Hualapai Mts. consist of diploids (*O. basilaris* Engelm. & J.M. Bigelow) and tetraploids (*O. macrorhiza* Engelm. and *O. polyacantha* Haw. var. *erinacea* (Engelm. & J.M. Bigelow) B.D. Parfitt, although, diploid *O. macrorhiza* is known outside of Arizona (Majure et al. 2012a,b; Baker & Pinkava 2018).

Opuntia chlorotica and *O. phaeacantha* are part of the mostly Chihuahuan Desert *Macrocentra* clade [including *O. atrispina* Griffiths, *O. aureispina* (S. Brack & K.D. Heil) Pinkava & B.D. Parfitt, *O. chisosensis*



FIG. 1. Comparison of habit, cladodes and flowers of *Opuntia chlorotica*, *O. curvispina*, and *O. martiniana*. **A.** *O. chlorotica*, erect habit with single primary trunk (Majure 5729); **B.** cladode showing stramineous colored spines (Majure 6132); **C.** *O. curvispina* erect habit with single trunk (Majure 6087); **D.** cladode showing reddish-brown developing spines these aging amber colored as seen in lower cladode (Majure 5449); **E.** *O. martiniana* erect habit branching from the base of the plant; **F.** cladode showing yellowish-white spines (both from Majure 5539); **G.** longitudinal section of a flower of *O. chlorotica* showing unusual pinkish coloration around the ovary, reddish tepal bases and ovate style (Majure 5729); **H.** flower of *O. curvispina* showing ovate style (Majure 5547); **I.** and flower of *O. martiniana* showing obovate (urn-shaped) style (Majure 5545).

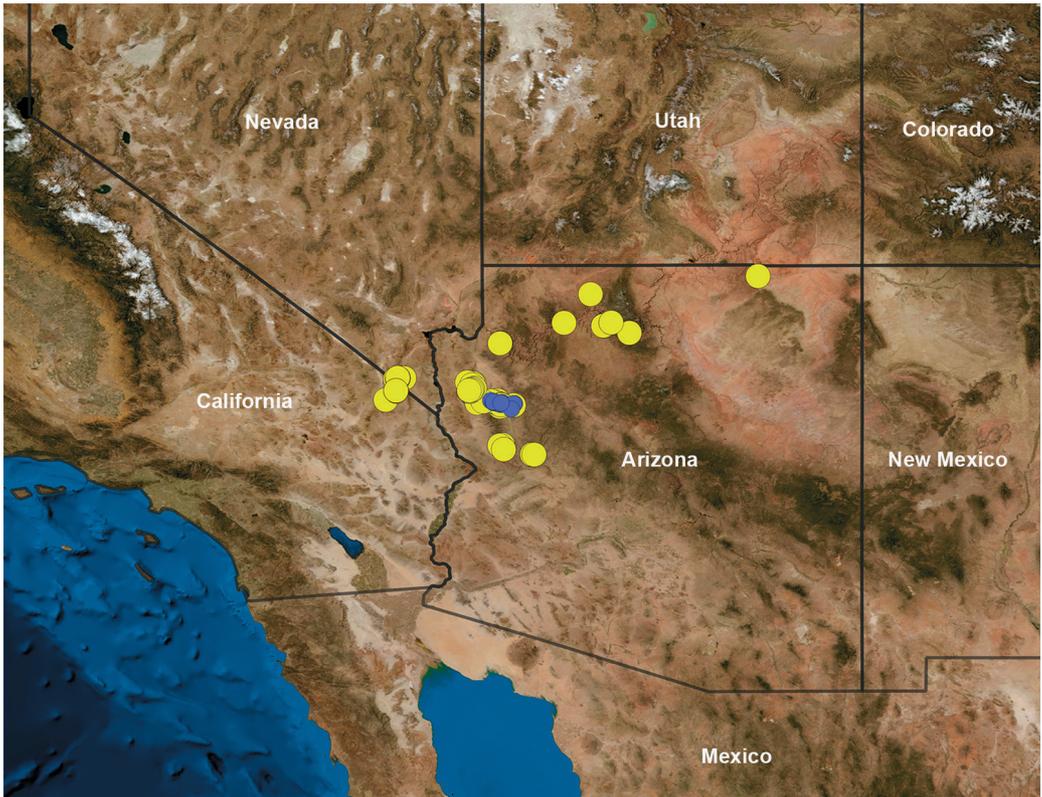


FIG. 2. Distribution of *O. curvispina* (yellow circles) and *O. martiniana* (blue circles) in the western United States.

(M.S. Anthony) D.J. Ferguson, *O. gosseliniana* F.A.C. Weber, *O. macrocentra*, *O. strigil* Engelm. and other taxa], whereas *O. engelmannii* is part of the *Scheerianae* clade, according to the results of Majure et al. (2012c). No samples of *O. curvispina* or *O. martiniana* have ever been analyzed phylogenetically. The one sample of “*O. martiniana*” analyzed by Majure et al. (2012c), although originally based on a collection of true *O. martiniana* (Hodgson 684, DES; see specimens examined), was a misidentified individual from the DBG living collection of *O. engelmannii* (DBG 1984 0579).

Populations of *O. curvispina* across its distribution have never been studied, as Parfitt (1980) only studied those populations north of and around the Hualapai Mts. I analyzed the morphological characters of populations of *O. curvispina* throughout its range from AZ to CA and incorporated material of *O. curvispina* and *O. martiniana* into phylogenetic analyses to determine whether or not these two taxa may have different evolutionary histories and whether or not they are indeed derived from hybridization among *O. chlorotica* and the other two putative parental species, *O. engelmannii* and *O. phaeacantha*.

MATERIALS AND METHODS

Taxon sampling, DNA extractions and sequencing.

Opuntia curvispina and *O. martiniana*, along with their putative progenitors, *O. chlorotica*, *O. engelmannii*, and *O. phaeacantha* (Parfitt 1980; Pinkava 2003) were sequenced for the plastid intergenic spacer *atpB-rbcL*, as well as the plastid genes *matK* and *ycf1* (Majure et al. 2012c). I incorporated data from previous studies (Majure et al. 2012a) for other species of the clades potentially involved here (*Humifusa*, *Macrocentra*, and

Scheerinae clades sensu Majure et al. 2012b). DNA extraction was carried out using a modified CTAB extraction buffer (Doyle & Doyle 1987) on either fresh or silica-dried tissue.

I directly sequenced nrITS (White et al. 1990) for a number of accessions of *Opuntia curvispina* (Majure 5552, 5567; WCH 23642) and *O. martiniana* (Majure 5539, 5561, 5829), as well as the other taxa sampled here, assuming that the most dominant copies of ITS directly sequenced for the hexaploids *O. engelmannii* and *O. phaeacantha* would be the copies present in the two putative progeny, *O. curvispina* and *O. martiniana*. All members of the *Scheeriana* clade share very similar copies of ITS when directly sequenced (Majure et al. 2012c, 2014). I used *O. basilaris*, *O. diploursina* Stock, N.Hussey & Beckstrom, *O. guatemalensis* Britton & Rose, and *O. polyacantha* as outgroup taxa.

One accession of *Opuntia martiniana* (Majure 5539) and one of *O. curvispina* (Majure 5440), from near the type localities for both, was genome-skimmed (Straub et al. 2012; Ripma et al. 2014; Majure et al. 2019, 2021), wherein ca. 1 µg of whole genomic DNA was extracted using a modified CTAB extraction and direct column cleaning of the supernatant (Neubig et al. 2014). Library building and paired-end sequencing on an Illumina HiSeq XTen platform was carried out by Rapid Genomics (Gainesville, FL, USA). Raw reads (150 bp) were reference-mapped to the three plastid loci used here, as well as to ITS1–2 for use in the detection of putative parental haplotypes. After reference-guided mapping of the nrDNA data, I extracted the two principal haplotypes recovered from both taxa, which were used for downstream phylogenetic and network analyses. Those were extracted using the create consensus feature in Geneious by implementing a 95% consensus sequence constraint. Given that two obvious haplotypes in both *O. curvispina* and *O. martiniana* were present at between 29–71% of the raw reads, the bases with disagreement were extracted as ambiguities (R and Y at positions 155 and 365 in my ITS dataset). Those were subsequently divided into the two primary haplotypes at that position based on comparison with my ITS dataset.

Phylogenetic analysis

I built two datasets, 1) all plastid data (*atpB-rbcL*, *matK*, *ycf1*) concatenated, and 2) ITS with directly sequenced products of *Opuntia curvispina* and *O. martiniana* and the two accessions of *O. curvispina* and *O. martiniana* (Majure 5440, 5539) with both haplotypes represented. I carried out a Maximum Likelihood analyses on both datasets using the RAxML (Stamatakis 2014) plugin in Geneious implementing the GTR+G model of evolution and carrying out 100 bootstrap pseudoreplicates. The ITS and plastid locus datasets are available on FigShare (Majure 2021).

Morphological data

Morphological data were gathered from *Opuntia curvispina* and *O. martiniana* from plants in the field, as well as material grown at DBG and associated herbarium specimens (see specimens examined) based on commonly used morphological features in *Opuntia* (Pinkava 2003; Majure et al. 2017).

RESULTS

Phylogenetic analyses

Plastid sequences of *Opuntia curvispina* were most phylogenetically related to *O. chlorotica*, although, a clade resolved of the two taxa was not well supported (Fig. 3A). Surprisingly, plastid sequences of *O. martiniana* accessions showed a close relationship with *O. macrorhiza* s.l. (Fig. 3A) and *O. phaeacantha* (in part), except for one accession that was more closely related to *O. chlorotica* (Majure 5548). Directly sequenced products of ITS for *O. curvispina* were nearly identical to *O. chlorotica*, while most directly sequenced products for *O. martiniana* again closely aligned to a clade composed of *O. macrorhiza* and *O. phaeacantha*, in part (note: ITS products of diploid and tetraploid *O. macrorhiza* exhibit putative plesiomorphy and are nested within a subclade of the *Macrocentra* clade rather than with other members of the *Humifusa* clade, as with plastid data; see Fig. 3B). As in the plastid data, one directly sequenced accession of *O. martiniana* for ITS (Majure 5548) also showed a closer relationship with *O. curvispina*/*O. chlorotica* rather than with other *O. martiniana*/*O. macrorhiza*. *Opuntia phaeacantha* was resolved as closely related to *O. engelmannii*, as well as with *O. macrorhiza*, in our

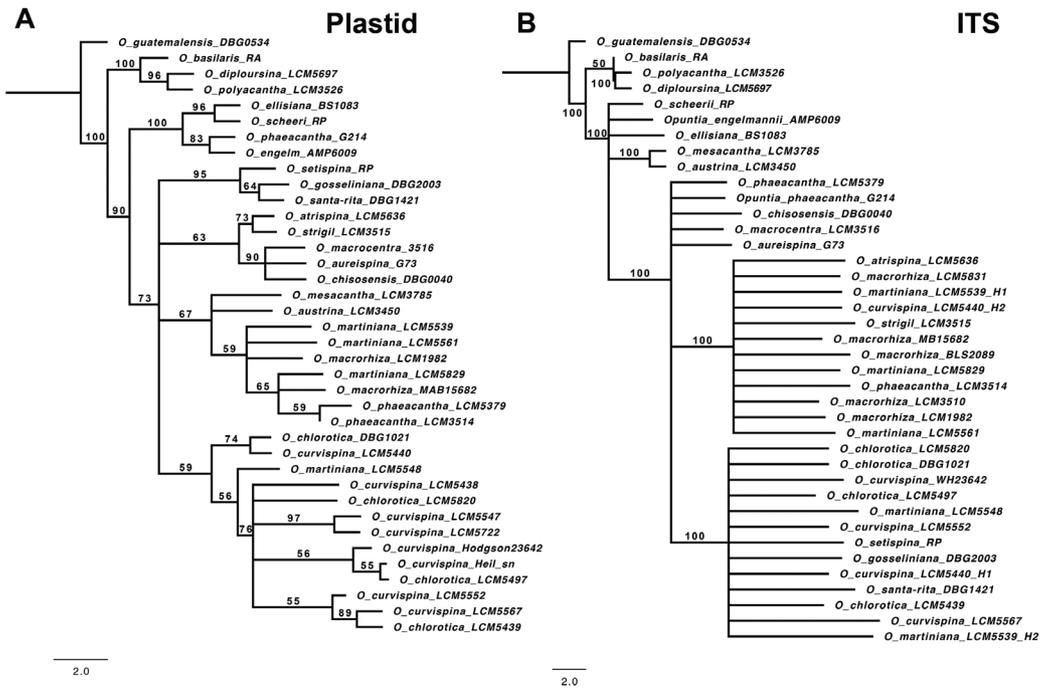


FIG. 3. Plastid and ITS phylogenies including *Opuntia curvispina*, *O. martiniana* and *O. phaeacantha*. Plastid data place *O. curvispina* in a clade with *O. chlorotica* and *O. martiniana* and *O. phaeacantha* in a clade with *O. macrorrhiza*. *Opuntia phaeacantha* also is resolved in a clade with *O. engelmannii*. Members of the *Nopalea* and *Xerocarpa* clades were used as outgroups.

analysis of plastid data (Fig. 3A), and one accession was resolved with *O. macrorrhiza* with ITS data, while the other two were unresolved with other members of the *Macrocentra* clade (Fig. 3B).

The two reference-mapped accessions, one of *Opuntia curvispina* (Majure 5440) and the other of *O. martiniana* (Majure 5539), exhibited two, clear haplotypes with mutations at 155 and 365 bp, reflecting haplotypes consistent with the *O. chlorotica* group and *O. macrorrhiza* group, based on my ITS dataset and phylogenetic analysis (Fig. 3B). Percentage differences between minority haplotype base pairs varied based on the number of reads mapped per site but were close in percentage across both sites within one species. For the raw reads mapped of the *O. martiniana* accession used (Majure 5539), at base pair 155, 44.8% of the raw reads showed the minority haplotype base pair (G), and at base pair 365, 38.4% of the raw reads exhibited the minority haplotype (T). The raw reads mapped from my accession of *O. curvispina* (Majure 5440) exhibited 29% of the minority haplotype base pair (A) at base pair 155 and 28.8% of the minority haplotype base pair (C) at base pair 365.

Morphological characters

Opuntia curvispina shares the tree-like growth form of *O. chlorotica*, and the spine color (golden brown or reddish-yellow) seen in many individuals of *O. curvispina*, also likely reflects parentage from *O. chlorotica*. However, the darker colors in the spines likely reflect parentage of *O. phaeacantha*, which tends to have brownish or brownish-red spines. Although *O. curvispina* is erect, it often produces horizontally spreading branches, which seem to also suggest influence from *O. phaeacantha*, a low-shrubby species that forms chains of horizontally spreading stem segments along the ground surface.

Opuntia curvispina is generally a much larger plant than *O. martiniana* (0.4–1.2 vs. 0.4–0.5 m tall), although both taxa are erect shrubs, which in general contrasts with either *O. phaeacantha* or *O. macrorrhiza*. Spines of *O. curvispina* tend to be dark brownish-red or reddish-yellow (or golden-brown), while those of *O.*

martiniana are yellow, or whitish-yellow (cream) with reddish-brown banding towards the base. The size of the cladodes in *O. curvispina* are larger than those of *O. martiniana* and exhibit more areoles per cladode face (36–46 vs. 24–34) or per diagonal at midstem (6–9 vs. 4–6). The styles of *O. curvispina* and *O. martiniana* are markedly different, with those of *O. curvispina* ovate and those of *O. martiniana* obovate or urceolate. The fruit of *O. curvispina* in general are barrel-shaped, while those of *O. martiniana* are clavate. See the descriptions of both species below for further differences.

DISCUSSION

Opuntia curvispina is clearly very closely related to *O. chlorotica*, as seen in morphology (Fig. 1A–D), as well as my topologies derived from plastid and nuclear DNA data (Fig. 3A–B). The robust, erect, tree-like growth form and densely spiny trunk (generally) of *O. curvispina* appears to be derived directly from *O. chlorotica*, which also reaches impressive sizes, up to 1.9–2 m or so tall in some specimens (Majure 5439, Majure 5820; DES). The accumulation and extension of trichomes in the areoles of *O. curvispina*, also is a common feature seen in *O. chlorotica*, and in general, although a variable character, the rounded shape of the cladodes also unites both species. The robustness and length of the spines apparently is a character derived, in part, from the other parent of *O. curvispina*, which appears to be *O. phaeacantha* based on my phylogenetic topologies and the often, chaining cladodes and horizontally radiating branches (although above the ground level) of *O. curvispina*. Likewise, the reduced number of cladode and pericarpel areoles suggests a close relationship with *O. phaeacantha*, as do the darker, brownish-red to reddish-yellow spines, as opposed to the translucent yellow spines of *O. chlorotica*, although spine color varies from nearing *O. chlorotica* to *O. phaeacantha* in different individuals of *O. curvispina*. The one accession of *O. curvispina* for which genome skimming data were available was from a population in southern Nevada, however, there are no known populations of *O. macrorhiza* near that population, although *O. phaeacantha* does occur there (see Majure 5441; DES), providing further support for *O. phaeacantha* as one of the putative parents of *O. curvispina*. The barrel-shaped fruits of *O. curvispina* are likely derived from both *O. chlorotica* and *O. phaeacantha*, both of which often form barrel-shaped fruits.

Although the occasional slight pinkish hue at the base (adaxially) of the inner tepals in the flowers of *O. curvispina* has been cited as a character derived directly from *O. phaeacantha*, which normally exhibits inner tepals with reddish bases (Parfitt 1980; Pinkava 2003), this character is not straightforward. I have noticed several specimens of *O. chlorotica* with reddish or pinkish tepal bases (Majure 5544, 5729; also noted by Pinkava 2003), a color that often even extends into the ovary wall, as noticed in longitudinal section (Fig. 1G). The reddish tepal bases are a common feature in the *Macrocentra* clade, from which *O. chlorotica* is derived (see Majure & Puente 2014), so it is no surprise that the species may occasionally show this coloration, albeit not nearly as conspicuously as in *O. macrocentra* or other close relatives (e.g., *O. aureispina*, *O. azurea* Rose, *O. chisosensis*).

Opuntia martiniana appears to be derived from a form of *O. macrorhiza* and either *O. chlorotica* or *O. curvispina* but is apparently not partially composed of *O. engelmannii*, as previously suggested (Pinkava 2003). It appears most likely that *O. curvispina*, rather than *O. chlorotica*, is the other parent of *O. martiniana*. Both *O. curvispina* and *O. macrorhiza* are tetraploids, so the fusion of their reduced gametes would lead directly to tetraploidy. Likewise, morphological characters of *O. martiniana* are more closely aligned with those of *O. curvispina* (growth form, spines) rather than to *O. chlorotica*, and the existence of morphotypes that are nearly identical to *O. curvispina*, other than the short, compact growth form, areole numbers and floral characters (Majure 5548) either suggests that *O. martiniana* and *O. curvispina* form hybrids or more likely that *O. martiniana* has formed multiple times from reciprocal crosses of *O. macrorhiza* and *O. curvispina*. Directly-sequenced ITS products of *O. martiniana* from accession Majure 5548 were nearly identical to those of *O. chlorotica* and *O. curvispina*, as were the plastid data. However, the other three accessions of *O. martiniana* sequenced here (Majure 5539, 5561, 5829) showed the opposite pattern, being more closely related to *O. macrorhiza* both with plastid and ITS data. All four accessions can be easily identified to *O. martiniana* based

on their growth form and other vegetative characters (e.g., areole number), as well as floral characters (e.g., expanded styles).

The small size of *Opuntia martiniana*, as compared to *O. curvispina* or *O. chlorotica*, likewise supports the phylogenetic data that *O. macrorhiza* s.l. is likely the other parent of *O. martiniana*. The clavate fruits of *O. martiniana* also are a character state exhibited by *O. macrorhiza*, and the reduced numbers of areoles per cladode face also suggest parentage involving *O. macrorhiza* (see also Majure et al. 2017). The erect growth form of *O. martiniana* is certainly derived from *O. curvispina* (and inadvertently *O. chlorotica*).

Opuntia martiniana only occurs north of the Hualapai Mountains, where it grows sympatrically with *O. curvispina* and *O. macrorhiza*, as well as *O. chlorotica*, *O. engelmannii* and *O. phaeacantha*, *O. basilaris* and *O. polyacantha*. The occurrence of both *O. curvispina* and *O. macrorhiza* growing together in this area likely accounts for the presence of a putative hybrid between the two, *O. martiniana*, as the two putative progenitors do not co-occur at any other known locations. Oddly, although Parfitt (1980) studied in detail the populations of *O. curvispina* from the area north of the Hualapai Mts., he never mentioned that it co-occurred there with *O. martiniana*.

Benson's (1950) and Benson and Walkington's (1965) concept of *O. martiniana* (as *O. littoralis* var. *martiniana*) was quite broad and included material, such as the pentaploid *O. charlestonensis* Clokey (Baker et al. 2009; Baker & Cloud-Hughes 2014; Baker & Pinkava 2018), as well as other populations of *O. curvispina*, based on specimens seen by myself (Benson 10100, RSA), which were misidentified as *O. martiniana*, and the distribution given by the authors, such as in the New York Mountains, where *O. curvispina* is abundant and often forms the dominant species in the area. Benson and Walkington (1965) also mentioned that *O. martiniana* "shaded" into *O. macrorhiza* and *O. phaeacantha*, but this seems likely an opinion based on the very broad circumscription of the taxon by those authors.

Opuntia phaeacantha is a very widespread and polymorphic species, which is often seen growing and potentially hybridizing with other taxa (Pinkava 2003), including *O. engelmannii* (e.g., Baker et al. 2009; Majure et al., pers. obsv.). This species has been recorded as a hexaploid across its range (Pinkava 2002; Powell & Weedon 2004; Majure et al. 2012a; Baker & Pinkava 2018), and results presented here provide some evidence that what is currently considered to be one taxon could actually have been derived multiple times from different parents, including *O. macrorhiza* s.l., *O. engelmannii* and perhaps some member of the *Macrocentra* clade, such as *O. macrocentra*. It is clear considering the range and tremendous morphological variation in *O. phaeacantha* that the taxon is a species complex, which is in urgent need of major taxonomic study and revision, including determining parentage of the multiple morphotypes currently considered to be part of *O. phaeacantha* s.l.

Minority haplotypes

Based on reference mapping of raw reads of ITS of one accession of *O. martiniana* and one of *O. curvispina*, there was a very clear minority haplotype for both taxa, with the minority haplotype for *O. martiniana* (represented at around 42% of raw reads) most closely related to the *O. chlorotica* group and the minority haplotype for *O. curvispina* most closely related to the *O. macrorhiza* group (represented at an average of 29% of raw reads). This asymmetry in haplotype abundance is expected given the reported frequency of concerted evolution in ITS through gene conversion or unequal crossing over (Feliner & Roselló 2007), but this also suggests that the paternal (pollen donor) parent involved in each of these two separate hybridization events represents the minority haplotype in both cases. So there appears to be preferential concerted evolution towards the maternal parent in both scenarios involved here. This has also been found in *Miscanthus* (Hodkinson et al. 2002), although bidirectional homogenization has been found in *Oryza*, and other groups show ranges of patterns, including chimera formation and total homogenization of haplotypes towards one parent or the other (Bao et al. 2010). Although many more accessions should be accessed, the low percentage of the minority haplotype in *O. curvispina* could perhaps reflect an older age of origin for that taxon, or at least higher rates of recombination, driving more rapid concerted evolution of ITS towards the maternal parent. This may provide

further evidence to support *O. curvispina* as a putative parent of *O. martiniana*, if indeed *O. curvispina* has an older origin.

Speciation

Although *Opuntia curvispina* has been treated as a nothospecies (Pinkava 2003), the acceptance of *O. curvispina* as an actual species appears warranted considering the wide range of the taxon (i.e., from Navajo Co., AZ to San Bernardino Co., CA covering an area of over 57,550 km; see specimens examined and Fig. 2) and the cohesive phylogenetic signal among populations. That cohesive genetic structure could also indicate a degree of decreased evolutionary rates in the species cannot be ruled out, nor can the propagation of the species via agamospermy, and thus a lack of recombination. However, decreased DNA polymorphism could also be a result of recent formation and polyploidy in general, and the loci used here show very little sequence divergence for this very young group of species, which likely evolved at the end of the Pliocene/beginning of the Pleistocene (see Majure et al. 2012b). *Opuntia curvispina* does readily form fertile fruit with viable seeds (seeds have been germinated from several populations—Majure, unpubl. data) and becomes a dominant prickly pear in specific areas, especially in parts of northwestern AZ (Mohave Co.), southern NV (Clark Co.) and through to southeastern CA (Mojave National Preserve; Fig. 2).

Opuntia martiniana, although restricted to a small area just north of the Hualapai Mountains (Fig. 2) and clearly derived from hybridization, also warrants specific status. The taxon exhibits unique morphological characters, e.g., obovate styles with a stigmatic band of tissue below the stigma lobes—likely a transgressive trait (Stebbins 1959) rather than a synapomorphy, as the taxon is clearly non-monophyletic—and is obviously not purely clonal, as it has likely arisen multiple times based on data presented here. *Opuntia martiniana* produces viable seed—seeds have been germinated at the University of Florida, Dept. of Biology greenhouse. However, as in *O. curvispina*, it is unknown whether or not *O. martiniana* produces any seed via agamospermy, but fertile fruit with viable seeds are readily produced throughout the population(s) of this taxon.

Specific morphological characters can be used to identify *Opuntia martiniana* from *O. curvispina*, however, *O. martiniana* could be considered morphologically cryptic. Only very careful observation of vegetative and reproductive characters allows for the differentiation of the two species. Young, but reproductive, *O. curvispina* can oftentimes fall within the size range of *O. martiniana*. Cryptic speciation in *Opuntia* and relatives may actually be more common than is known (see Majure et al. 2013; Majure et al. 2017), however, we most often lack the detailed phylogenetic and basic biological information, such as chromosome number determinations, to detect it. I do not suggest that all minor morphological variants of a taxon be recognized as separate species, especially in *Opuntia* where morphological variation within a given taxon abounds, however, there are specific instances where morphotypes do deserve recognition. In the case of *O. martiniana*, it is clear from these data that the taxon was derived separately from *O. curvispina*, and likewise, based on morphological grounds should be recognized as a separate species. The transgressive trait of having an obovate style is a clear qualitative character that can be used to separate *O. martiniana* from *O. curvispina*—such a clear character is not often present in cryptic species.

Species concepts

Opuntia curvispina and *O. martiniana* are polyploid derivatives that are morphologically separable from their putative progenitors, as well as one another, with both taxa exhibiting their own suite of unique morphological characters. Both taxa produce fertile fruit with viable seeds, juveniles of both taxa have been seen in the field, and seeds of both taxa have been germinated at the University of Florida. Populations of *O. curvispina* are sometimes vast and form the dominate prickly pear species in certain areas, such as west of the Cerbat Mts. (Mohave Co., AZ) or in parts of the New York Mts. of the Mojave National Preserve (San Bernardino Co., CA). The large population of *Opuntia martiniana* consists of hundreds of individuals over a ca. 100 km area, although a census of the entire population has not been carried out. Therefore, it is clear that the taxon is also not merely a spontaneous hybrid. We can thus recognize these two taxa under a morphological phenetic species concept (Judd 2007), as well as under a diagnostic species concept (Wheeler & Platnick 2000), given their morphological separability.

Other putative hybrids with *Opuntia chlorotica*

There are several other putative hybrids with *Opuntia chlorotica*. Material from parts of southwestern Arizona near Peña Blanca Lake exhibits spine and epidermal characters that appear to be intermediate between *O. chlorotica* and *O. santa-rita*, although, that population grows in mountainous rocky, habitat, which seems more suitable for *O. chlorotica*. Likewise, M. A. Baker discovered putative hybrids ($2n=66$) between *O. chlorotica* and *O. engelmannii* near Congress, AZ (reported in Baker & Pinkava 2018). These two populations are currently under study to determine their hybrid status.

Distribution

Based on more recent collections and observations, the distribution of *Opuntia curvispina* clearly ranges broadly as far east as northeastern AZ in Navajo County near Kayenta west to southeastern CA (see Pinkava 2003 in part) in the Mojave National Preserve and as far south as Nothing in Mohave Co., AZ (Majure pers. obs., see Additional Specimens Examined; Fig. 2). Numerous collections of *O. curvispina* have been made from in and around the Grand Canyon (see Hodgson 10600, Hodgson 23642, Hodgson 23643), although, there do not appear to be large populations of the species from those areas, with often only one or a few individuals recorded. Likewise, only a few individuals were seen near Nothing, AZ (Majure 5815), and apparently very few individuals are known from the Navajo County population (Heil & Clifford s.n.). I consider that these outlying populations could potentially have been introduced through either recent or historical translocations by humans. The large fruit of *O. curvispina* could have been utilized by Native Americans as a food stuff, as was common for prickly pear fruit in the desert Southwest and elsewhere, aiding in long-distance dispersal from its more likely sites of origin in the core Mohave Desert. However, given that *O. curvispina* is likely derived from *O. chlorotica* and *O. phaeacantha*, two taxa that are frequently found co-occurring, it could be that the taxon has formed multiple times in different areas throughout its current distribution. Population level markers, such as microsatellite data, may be necessary to ascertain if *O. curvispina* has been generated through recurrent hybridization between its putative parents at different sites.

KEY TO *O. CURVISPINA*, *O. MARTINIANA*, RELATIVES AND/OR SYMPATRIC SPECIES

1. Erect shrubs, mostly with a single, primary trunk, 0.6–2 m tall; cladodes mostly orbicular; central spines translucent yellow or translucent reddish-brown or golden-brown.
 2. Central spines translucent yellow (stramineous), mostly flattened at the base, 0.50–0.81 mm in width at base, longest, 8.27–36.1 mm; areoles 8–12 in a diagonal row at midstem, seeds 2.8–4.6 mm long _____ ***O. chlorotica***
 2. Central spines translucent reddish-brown or golden-brown, mostly cylindrical in cross section or flattened at the base, 0.83–1.37 mm in width at base, longest 40.6–71 mm; areoles 6–9 in diagonal row at midstem, seeds 3.2–5.7 mm long _____ ***O. curvispina***
1. Erect or sprawling shrubs, generally branching from the base, 0.3–1.5 m tall; cladodes orbicular, obovate or elliptical; central spines white, chalky yellow, or dark reddish-brown or white and brown banded.
 3. Sprawling or spreading shrubs; inner tepals yellow with reddish, reddish-pink or reddish-brown bases.
 4. Plants mostly cespitose or spreading; pads cross-wrinkling in winter _____ ***O. macrorhiza***
 4. Plants spreading, with pad surfaces perpendicular to ground surface; pads remaining turgid during winter _____ ***O. phaeacantha***
 3. Erect shrubs; inner tepals mostly yellow throughout.
 5. Cladodes elliptical or obovate; glochids forming pin cushion structure, dispersed throughout the areole; spines stout, not easily dislodged from the areole; style ovate _____ ***O. engelmannii***
 5. Cladodes mostly rotund or obovate; glochids forming dense fascicle in the adaxial portion of the areole; spines fragile, easily dislodged from the areole; style obovate _____ ***O. martiniana***

TAXONOMIC TREATMENT

Opuntia curvispina Griffiths, Bull. Torr. Bot. Gard. 43:88. 1916. TYPE: UNITED STATES. NEVADA. [Clark Co.]: between Nipton and Searchlight, Apr 1912, *Griffiths 10530* (LECTOTYPE designated by Benson (1982), on 4 sheets: US-2576150A–2576153A1, seen online; ISOLECTOTYPES: ASU1, POM-313376A-B1).

Description.—Erect shrubs to small trees, 0.4–1.2 m tall, generally forming a primary trunk up to 10 cm in diameter at base. Branches spreading or more commonly erect. Cladodes glabrous, green to yellow-green, obovate, orbicular or elliptical, 12.5–20 × 14–20.7 cm, areoles per cladode face 36–46 and areoles per diagonal row at midstem, 6–9; cladodes glaucous grey-green during development turning chlorotic-green when

mature. Spines 1–9 per areole, composed of both centrals and radials, the central spines red, brownish-red or reddish-yellow, generally forming a “crow-foot” pattern in uppermost (i.e., youngest cladodes), thus the spines spreading, 4–6 per areole, 28–71 mm long, radial spines white or occasionally with dark banding, deflexed along the cladode surface, 1–5 per areole, 3–16 mm long, yellow to amber, aging brown, areoles producing large tufts of trichomes that protrude from the cladode conspicuously, especially as the cladode ages (as in *O. chlorotica*). Pericarpel barrel-shaped, 23–66 × 14–24 mm, inner tepals obovate, 27–43 × 19–32 mm, entirely yellow or faintly pinkish or greenish at the base adaxially, filaments thigmonastic, whitish-green, anthers yellow, style ovate, 13–27 mm long, whitish-cream, stigma lobes green, 6–10. Fruit barrel shaped, juicy, 27–51 × 17–36 mm, dark red to reddish-pink, pericarpel areoles 23–45. Seeds lenticular, 3.23–5.77 mm wide including funicular girdle, girdle and funicular envelope glabrous. $2n=44$.

Habitat and Distribution.—Mohave Desert, as well as along the Colorado River in parts of the Grand Canyon. *Opuntia curvispina* occurs mostly in granitic soils, but also over basalt and limestone soils in the Mojave National Preserve. Associate species include: *Aristida adsencionis*, *Atriplex canescens*, *Bouteloua barbata*, *B. eriopoda*, *Canotia holacantha*, *Juniperus arizonica*, *J. osteosperma*, *Cirsium mohavense*, *Coleogyne ramossissima*, *Coryphantha vivipara*, *Cylindropuntia acanthocarpa*, *C. echinocarpa*, *C. leptocaulis*, *C. whipplei*, *Dasyochloa pulchella*, *Grusonia parishii*, *Echinocereus coccineus*, *E. engelmannii*, *E. mojavensis*, *Ephedra nevadensis*, *Ericameria cuneata*, *Eriogonum fasciculatum*, *Fallugia paradoxa*, *Ferocactus acanthodes*, *Pleuraphis jameisii*, *P. rigida*, *Krameria erecta*, *Larrea tridentata*, *Menodora scabra*, *Muhlenbergia porteri*, *Nolina microcarpa*, *Opuntia basilaris*, *O. chlorotica*, *O. engelmannii*, *O. erinacea*, *O. martiniana*, *O. phaeacantha*, *Parkinsonia microphylla*, *Pectis papposa*, *Phorodendron californicum*, *Prunus fasciculatus*, *Psilostrophe cooperi*, *Rhus trilobata*, *Scutellaria mexicana*, *Salvia dorrii*, *Stipa speciosa*, *Thamnosma montana*, *Yucca baccata*, *Y. brevifolia*, *Y. jaegeriana*, *Y. schidigera*, *Senegalia greggii*, and *Ziziphus obtusifolia*.

Additional Specimens Examined. **ARIZONA. Coconino Co.:** Kanab Canyon, above Kanab Creek, Kanab Creek Wilderness Area, along Ranger Trail 41, at mouth of Jensen Canyon 36.614983°N, -112.619083°W, 1036m, 13 Sep 1997, *Hodgson 10600* (ASU, DES); Grand Canyon National Park, along trail from Royal Arch Canyon to rappel site, 36.191897°N, -112.447643°W, 9 61m, 29 Oct 2008, *Hodgson 23642* (DES); Grand Canyon National Park, along trail from Royal Arch Canyon to rappel site, 36.19456°N, -112.451028°W, 961m, 29 Oct 2008, *Hodgson 23643* (DES). **Mohave Co.:** Rattlesnake Hill, 7.5' USGS topographic Quadrangle; 12S 02 33 150mE 38 99 100mN, 35.201°N, 113.931°W; T21N R16W, middle of S.13, 1160 m, along I-40, E of Kingman, 1.8 km W of Rattlesnake Hill, 10 Oct 1999, *Baker & Trushell 13630* (ASU); Burnt Mill Ranch (Gold Basin), White Hills, 64 km NNW of Kingman, 13 km SE of Senator Mt., 35.7507°N, -114.2155°W, 1115 m, 30 Oct 1999, *Baker 13647* (ASU, RSA); Aquarius Mts., 43 km ESE of Kingman, along Willow Creek, 1.7 km NE of Mesquite Thicket Spring, 35.141°N, -113.551°W, 1155 m, 18 Feb 2008, *Baker 16646* (ASU); bajada of Hualapai Mt., 11 km E of Kingman, 2.8 km ESE of Rattlesnake Hill, 35.1935°N, -113.8824°W, 19 Sep 2008, *Baker 16726* (ASU); between Peacock and Hualapai Mts.; N bajada of Hualapai Mt., 24 km ESE of Kingman, 11 km ESE of Rattlesnake Hill, 35.1741°N, -113.7853°W, 1285 m, 2 Feb 2012, *Baker 17524* (ASU, US); 30 mi NW of Alamo Crossing of the Bill Williams River, Rawhide Mts., 28 Mar 1940, *Benson 10100* (RSA); Along rt. 93 ca. 1.5 mi NW of Nothing, Arrastra Mt. NE 7.5 quad. sect. 12, NW 1/2, T13N, R11W, 9 Jun 1980, *Butterick, Hillyard & Hodgson 687* (DES); Hualapai Nation, along Quartermaster Point Rd., E of Hualapai Ranch, 35.967417°N, -113.80045°W, 1427 m, 16 Mar 2019, *Hodgson 31973* (DES); N of Hualapai Mts., off of Sweetwater Rd. (D-W Ranch Rd.), just S of Interstate 40, 35.16044°N, -113.82751°W, 1392 m, 18 Mar 2015, *Majure 5547* (DES); Cerbat Mts., just E of Hwy. 93, N of Chloride, 35.44388°N, -114.24004°W, 1208 m, *Majure 5551* (DES); flats below Cerbat Mts., just E of Hwy. 93 and S of Mineral Springs Rd., 35.31248°N, -114.19437°W, 1021 m, *Majure 5552*, N of Hualapai Mts., off of Sweetwater Rd. (D-W Ranch Rd.), just S of Interstate 40, 35.16799°N, -113.84251°W, 1391 m, 19 Mar 2015, *Majure 5560*, ca. 3 mi NW of Nothing, off of Hwy. 93S, 34.48691°N, -113.38610°W, 846 m, 19 Mar 2015, *Majure 5567* (DES, FLAS); ca. 3 mi NW of Nothing, off of Hwy. 93S, 34.48691°N, -113.38610°W, 846 m, 20 Aug 2015, *Majure 5815* (DES, FLAS); N of Hualapai Mts., off of Sweetwater Rd. (D-W Ranch Rd.), just S of Interstate 40, 35.15994°N, -113.82855°W, 1393 m, 20 Aug 2015, *Majure 5819* (DES, FLAS); N of Hualapai Mts., off of Sweetwater Rd. (D-W Ranch Rd.), just S of Interstate 40, 35.18246°N, -113.87736°W, 1314 m, 20 Aug 2015, *Majure 5824* (DES, FLAS); vicinity of Chloride in route to Mural, 35.41006°N, -114.17969°W, 9 Apr 2016, *Majure 6087* (DES, FLAS); N of the Hualapai Mts. at Rattlesnake Hill, just E of Kingman and S of I-40, 35.19751°N, -113.93054°W, 20 Jul 2016, *Majure 6174* (DES, US); *ibid*, *Majure 6174* (DES, US); N of the Hualapai Mts., off of D-W Ranch Rd., just E of Kingman and S of I-40, 35.15084°N, -113.79541°W, 20 Jul 2016, *Majure 6180* (DES, US); ca. 18.2 air km W of Wikiup off of Chicken Springs Rd., 34.61298°N, -113.77535°W, 999 m, 30 Sep 2016, *Majure 6213* (DES, FLAS); off of Alamo Rd. ca. 5 km S of junction with Chicken Springs Rd., just N of junction with Coyote Wells Rd., 34.55394°N, -113.76081°W, 879 m, 30 Sep 2016, *Majure 6226* (DES, FLAS); along Alamo Rd., ca. 1.7 km S of junction with Chicken Springs Rd., 34.57266°N, -113.78078°W, 30 Sep 2016, *Majure 6228* (DES, FLAS); along Alamo Rd., ca. 2.3 km N of junction with Chicken Springs Rd., 34.60493°N, -113.81310°W, 937 m, 30 Sep 2016, *Majure 6230* (DES, FLAS); Chico Mine Rd., 1 mi E of Rte. US 93, 29 May 1971, *M.G. McLeod 452* (DES, ASU); Chico Mine Rd., 1 mi E of Rte. US 93, 29 May 1971, *M.G. McLeod 454* (DES, ASU); Chico Mine Rd., 1.2 mi E of U.S. 93, north of Kingman, 4 Sep 1971, *M.G. McLeod 927* (ASU); S of Santa

Claus, Chico Mine Rd., 0.2 mi E of Hwy 93, 17 Apr 1977, *B. Parfitt & W.D. Clark 2140* (ASU); 0.25 mi S of abandoned dwelling, S of mine tailing on Chico Mine Rd., S of Santa Claus, 24 May 1977, *B.D. Parfitt & W.D. Clark 2169* (ASU); S of Santa Claus, 1.8 mi S of Chico Mine Rd. on major N-S gravel rd., 24 May 1977, *B.D. Parfitt 2172* (RSA, ASU); 1.3 mi S of Chloride on a major gravel rd., 3.8 mi N of Mineral Park Rd., 24 May 1977, *B.D. Parfitt & W.D. Clark 2173* (ASU); 100 meters E of Hwy 93, 3.3 mi N of the jct. with Hwy 68, near mile post 64, 26 May 1977, *W.D. Clark & B. Parfitt P2221* (ASU); Cerbat Mtns., S of Chloride, 4.7 mi E of Hwy 93 on Chico Mine Rd., S of Duval Mine Tailings, 26 May 1977, *B.D. Parfitt & W.D. Clark 2223* (ASU); Old Hwy. 93, 6.1 mi SE of its jct. with Peacock Mt. Rd, 24 Jun 1977, *B. Parfitt & E. Lehto 2253* (ASU); of Santa Claus, 150 m E of major north-south gravel Rd., 0.7 mi S of Chico Mine Rd., 26 June 1977, *B.D. Parfitt & E. Lehto 2276* (ASU); S of Santa Claus, 0.5 mi S of Chico Mine Rd. on N-S running Rd., 1 May 1978, *B.D. Parfitt 2540* (ASU); Western bajada of the Cerbat Mtns., S of Santa Claus, 0.5 mi S of Chico Mine Rd. along gravel road E of and parallel to Hwy 93, 1 May 1978, *B. Parfitt & G. Brown 2541* (ASU); SE of Santa Claus, SE of mine tailing in Cerbat Mts near end of Chico Mine Rd. SW1/4 S6 T22N R17W, 7 Jun 1978, *B.D. Parfitt 2581* (ASU); Along Hwy I-40 E of Kingman, 2.5 mi W of Hualapai Mt. Rd. and 0.3 mi E of Rattlesnake Wash. T21N R15W S14, 16 Jul 1980, *B.D. Parfitt 2616* (ASU); 0.6 mi S of the jct. of Hwy 93 and Peacock Mt. Rd., 17 Mar 1979, *B. Parfitt & D.J. Pinkava 2702* (ASU); 0.4 mi SE of Peacock Mt. Rd. on old Hwy 93 (Kingman Rd.) T20 N R14W S6 NW1/4, 24 May 1979, Butterwick, *B. Parfitt & D. Hillyard 5003* (ASU); 1 mi E of US 93 on Chico Mine Rd., 7 Apr 1972, *Pinkava, Brown, McLeod 1075* (DES, ASU); 1.5 mi S of Duval Mine Rd. on Chico Mine Rd., 7 Apr 1972, *Pinkava, Brown, McLeod 1079* (DES, ASU); vicinity of Duval Mine, along Rt. 93, 7 Apr 1972, *Pinkava, Brown, McLeod 1082* (DES, ASU); near small wash 0.25 mi from Duval Mine Rd. on Chico Mine Rd., 7 Apr 1972, *Pinkava, Brown, McLeod 1083* (DES); Chico Mine Rd. 1 mi E of US 93 (12 mi NW of Kingman), 7 Apr 1972, *Pinkava, Brown, McLeod 1083* (DES, ASU); Chico Mine Rd., 0.8 mi E of its junction with Hwy. 93, 27 Apr 1974, *Pinkava & Reeves 11974* (ASU); Rt. 93, vicinity of Duval Mine, 7 Apr 1972, *McLeod et al. 1088* (DES, ASU); *ibid*, *McLeod et al. 1089* (DES, ASU); *ibid*, *McLeod et al. 1090* (DES, ASU). **Navajo Co.:** Navajo Nation; Todicheenie Bench of Skeleton Mesa; ca. 1 mi WNW of Adahchijyahhi Canyon, 36.85500°N, 110.41800°W, 1807 m, *Heil & Clifford s.n.* (ASU). **CALIFORNIA. San Bernardino Co.:** Mojave National Preserve, just S of Castle Peaks, 35.32668°N, 115.17197°W, 1469 m, 13 May 2015, *Majure 5710* (DES); Mojave National Preserve, just S of Castle Peaks, 35.33278°N, -115.17664°W, 1489 m, 14 May 2015, *Majure 5715* (DES); Mojave National Preserve, just S of Castle Peaks, 35.33064°N, -115.17632°W, 1475 m, 14 May 2015, *Majure 5717* (DES); Mojave National Preserve, off of Hart Mine Rd., ca. 10 km SE of Ivanpah, 35.28766°N, -115.21802°W, 1438 m, 14 May 2015, *Majure 5720* (DES); Mojave National Preserve, New York Mts., off of New York Mt. Rd., 35.20986°N, -115.31232°W, 1638 m, 14 May 2015, *Majure 5722* (DES); about 7.1 km E of Vanderbilt in straight line, on dirt road 2.7 km N of Hart Road starting 9.29 km from Ivanpah Road along Hart Rd., Mohave National Preserve, 35.32669 -115.17194, 805m, 13 May 2015, *Puente 5012* (DES); Mohave National Preserve, Castle Peak Mountains, 35.33417 -115.17743, 1381m, 14 May 2015, *Puente 5020* (DES); Mohave National Preserve, Castle Peak Mountains, 35.33417 -115.17743, 1482m, 14 May 2015, *R. Puente 5024* (DES); Mohave National Preserve. New York Mountains, 35.33417 -115.17743, 1634m, 14 May 2015, *Puente 5031* (DES). **NEVADA. Clark Co.:** Piute Valley, 5.3 km NE of Crescent Peak, 14 km WNW of Searchlight, 35.501°N, -115.071°W, 1295 m, 21 Oct 2008, *Baker 16754.1* (ASU); *ibid*, *Baker 16754.2* (ASU); 8.2 air km E of Nipton, CA in E-W mountain range just S of Hwy. 164 (Nipton Rd.) and W of Crescent Peak, 35.46403, -115.16131, 1381 m, 28 Oct 2014, *Majure 5438* (DES, FLAS); 8.2 air km E of Nipton, CA in E-W mountain range just S of Hwy. 164 (Nipton Rd.) and W of Crescent Peak, 35.46813, -115.15845, 1397 m, 28 Oct 2014, *Majure 5440* (DES, FLAS); 8.2 air km E of Nipton, CA in E-W mountain range just S of Hwy. 164 (Nipton Rd.) and W of Crescent Peak, 35.45442, -115.18436, 1279 m, 28 Oct 2014, *Majure 5449* (DES, FLAS); McCullough Mts., 0.7 mi N of Hwy. 68, N of powerline towers, 2.7 mi E of California, at end of jeep trail, 25 May 1977, *Parfitt 2215* (RSA); about mi W of Searchlight along Joshua Tree Hwy. (164), 0.5 km N of freeway along Powerline Rd, 35.49490 -115.15850, 1381m, 13 May 2015, *Puente 5011* (DES).

Opuntia martiniana (L.D. Benson) Parfitt, Syst. Bot. 5:416. 1981. *Opuntia macrocentra* Engelm. var. *martiniana* L.D.

Benson. Cacti Ariz. ed. 2, 64. 1950. *Opuntia littoralis* (Engelm.) Cockerell var. *martiniana* L.D. Benson, Ann. Missouri Bot. Gard. 52:270. 1965. TYPE. ARIZONA. MOHAVE CO.: N of Hualapai Mts., off of Sweetwater Rd. (D-W Ranch Rd.), just S of Interstate 40, 35.16798°N, 113.84248°W, 1396 m, 11 May 2015, *L.C. Majure 5685* (neotype, designated here: DES; ISONEOTYPE: FLAS).

Note.—Type material not found: ARIZONA. MOHAVE CO.: Kingman Rd. on the N side of Hualapai Mountain, Alt. 5000 ft, 30 Mar 1940, *L. Benson 10169* (HOLOTYPE: POM 274107, on two sheets, not found; ISOTYPE: ARIZ, not found).

Description.—Erect shrubs 0.4–0.5 m tall, generally with a short trunk and multiple branches produced from the base. Cladodes glabrous, orbicular, elliptical, or occasionally obovate, 7.3–17.7 × 8.4–15.5 cm, areoles per cladode face 24–34 and areoles per diagonal row at midstem, 4–6; cladodes glaucous grey-green during development turning pale green or yellow-green when mature. Spines 3–11 per areole, composed of both centrals and radials, the central spines yellowish or whitish-yellow with reddish-brown banding towards the base, generally forming a densely fascicled pattern, the spines erect to slightly spreading (peripheral central spines), 2–8 per areole, 25–69 mm long, radial spines small, white, produced at the base of the areole and mostly deflexed against the surface of the cladode, 1–3 per areole, 6–18 mm long, glochids yellow, aging brown. Pericarpel mostly clavate (narrowed at the base), or uncommonly barrel-shaped, 38–55 × 17–20 mm, inner tepals obovate, 36–50 × 26–34 mm, entirely yellow or yellow with a faint reddish-pink base, filaments thigmonastic, whitish-green, anthers yellow, style urn-shaped (narrowed at the base and widest in the middle

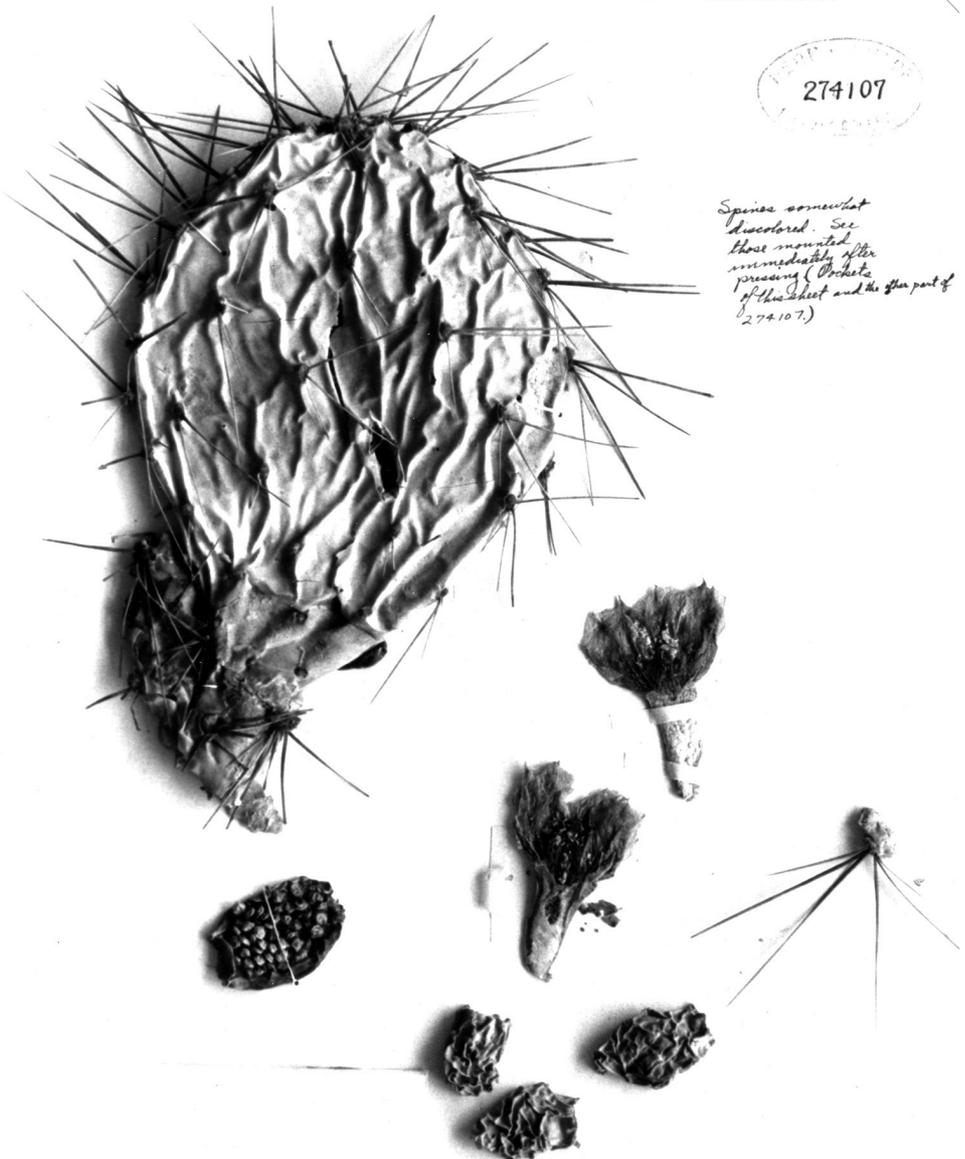
then abruptly narrowing to the apex), 19–22 mm long, whitish-cream, the wide, middle portion appearing stigmatic and pollen grains often adhering to it, stigma lobes green, 7–9. Fruit clavate or rarely barrel shaped, not conspicuously juicy at maturity, 27–71 × 17–29 mm, reddish-pink or yellowish-green, or yellowish-green with a faint pinkish hue, pericarpel areoles 14–24. Seeds lenticular, 4.45–6.62 mm wide including funicular girdle, girdle and funicular envelope glabrous. $2n=44$.

Habitat and Distribution.—Mohave Desert restricted to the north end of the Hualapai Mts. and the immediate surrounding areas (see Fig. 2). *Opuntia martiniana* occurs in granitic soils on the lower northern slopes of the Hualapai Mts. and in the surrounding desert vegetation. Associate species include: *Aristida purpurea*, *Baccharis pteronioides*, *Berberis haematocarpa*, *Bouteloua curtipendula*, *Canotia holocantha*, *Castilleja confusa*, *Ceanothus pauciflorus*, *Ericameria nauseosa*, *Cylindropuntia acanthocarpa*, *C. whipplei*, *Dasyochloa pulchella*, *Echinocereus coccineus*, *E. engelmannii*, *Eriodyction angustifolium*, *Eriogonum wrightii*, *Euphorbia incisa*, *Glandularia gooddingii*, *Gutierrezia microcephala*, *G. sarothrae*, *Juniperus californica*, *Menodora scabra*, *Nolina microcarpa*, *Opuntia basilaris*, *O. chlorotica*, *O. curvispina*, *O. engelmannii*, *O. erinacea*, *O. phaeacantha*, *Penstemon*, *Pinus edulis*, *Prosopis velutina*, *Quercus turbinella*, *Scutellaria mexicana*, *Stephanomeria pauciflora*, *Senegalia greggii*, *Yucca baccata*, *Zinnia grandiflora*.

The type specimens of *Opuntia martiniana* have not been located. Apparently, the holotype was lost in shipment from SD to RSA (M. Baker, pers. comm.). Attempts to find the type at RSA have been unsuccessful (M. Nazaire, pers. comm.). A photograph of the type was taken while on loan at ASU by M. Baker and is figured here (Fig. 4), although, as the photograph was not mentioned in the protologue and thus is not original material, it cannot be used as a lectotype for the species (see Staples & Prado 2018 for a discussion on the use of photographs as types). It is clear that Benson collected material for what he designated as the type on multiple occasions. *Opuntia martiniana* is merely in bud in March (Majure pers. obs.) during the supposed time of collection of the type, however, the type material consists of one mature cladode, one developing cladode, flowers and fruit. The species does not flower until May–June, and mature fruit are only encountered during a short window of time in later summer (i.e., July–August) before dropping off the plants or being eaten by local fauna (Majure pers. obs.). As the photograph of the type clearly shows that Benson derived the type specimen from materials collected at least three different times, which he had done for other species as well (see Majure et al. 2017), it would have been necessary to lectotypify the species (see Fig. 4). However, as the types of this species was lost, as mentioned above, I have chosen to neotypify the species based on a more complete, and extant, collection in flower collected on 11 May 2015 (see above, *Majure* 5685).

Benson (1950) mentioned that *Opuntia martiniana* had also been collected in other locations other than north of the Hualapai Mts. One specific collection he mentions, *Benson 10100*, from north of Lake Alamo, actually is of *O. curvispina* not *O. martiniana* (see also *Majure* 6213, 6226, 6228, 6230 under *O. curvispina* above).

Additional Specimens Examined. **ARIZONA. Mohave Co.**: Hualapai Spring, 7.5° USGS Zone 12 02 46 310ME 38 95 730mN, 35.1742°N, -113.7853°W, 1285 m, pass between Peakcock and Hualapai Mts., N bajada of Hualapai Mt., 24km ESE of Kingman, 11 km ESE of Rattlesnake Hill, 6 Jan 2009, *Baker* 16788 (ASC, ASU); *ibid*, 26 May 2009, *Baker* 16925 (ASU); *ibid*, 24 Sep 2010, *Baker* 17235 (ASC, ASU, RSA, SRSC); *ibid*, 27 May 2011, *Baker* 17353 (ASC, ASU); 10 mi on Hualapai Mt. Park Rd., 1–2 Jun 1977, *BLM s.n.* (ASU); Hualapai Mt. Rd., just W of D-W Ranch on N side of Hualapai Mts., sec. 6 NE 1/4, T20N, R15W, Rattlesnake Hill quad., 7 Jun 1980, *Butterick, Hillyard & Hodgson H684* (DES); N of Hualapai Mts., off of Sweetwater Rd. (D-W Ranch Rd.), just S of Interstate 40, 35.16044°N, 113.82751°W, 1392 m, 18 Mar 2015, *Majure* 5539 (DES); *ibid*, *Majure* 5545 (DES); *ibid*, *Majure* 5548 (DES, ASU); N of Hualapai Mts., off of Sweetwater Rd. (D-W Ranch Rd.), just S of Interstate 40, 35.16044°N, 113.82751°W, 1393 m, 20 Aug 2015, *Majure* 5821 (DES, FLAS); N of Hualapai Mts., off of Sweetwater Rd. (D-W Ranch Rd.), just S of Interstate 40, 35.18512°N, 113.88940°W, 1311 m, 18 Mar 2015, *Majure* 5549 (DES, ASU); N of Hualapai Mts., off of Sweetwater Rd. (D-W Ranch Rd.), just S of Interstate 40, 35.16750°N, 113.84298°W, 1405 m, 20 Aug 2015, *Majure* 5822 (DES, FLAS); N of Hualapai Mts., off of Sweetwater Rd. (D-W Ranch Rd.), just S of Interstate 40, 35.16799°N, 113.84251°W, 1391 m, 19 Mar 2015, *Majure* 5561 (DES); N of Hualapai Mts., off of Sweetwater Rd. (D-W Ranch Rd.), just S of Interstate 40, 35.18246°N, 113.87736°W, 1314 m, 20 Aug 2015, *Majure* 5829 (DES); off of DW-Ranch Rd. just S of Interstate 40, 35.18684°N, 113.89517°W, 1303 m, 20 Jul 2016, *Majure* 6177 (DES, US); 35.15064°N, 113.79444°W, 1355 m, 20 Jul 2016, *Majure* 6179 (DES, US); Off of Old Hwy. 93, 35.12736°N, 113.71266°W, 1162 m, 20 Jul 2016, *Majure* 6181 (DES, US); 2.8 mi SE of the junction of Peacock Mt. Rd. and Old Hwy. 93, on Old Hwy. 93, 24 Jun 1977, *Parfitt* 2251 (ASU); 0.6 mi NW of the junction of Peacock Mt. Rd. and Old Hwy. 93, on Old Hwy. 93, 50–100 m E of hwy., 25 Jun 1977, *Parfitt* 2256 (ASU); 0.5 mi NW of Hwy. 93, Peacock Mt. Rd., along dirt rd. of El Paso gas line, 25 Jun 1977, *Parfitt* 2260 (ASU); 0.7 mi NW of Peacock Mt.



274107

Spines somewhat discolored. See those mounted immediately after pressing (Pockets of this sheet and the other part of 274107.)

Opuntia martiniana (L. Benson) Parfitt

Bruce D. Parfitt 1980

Type Collection--Second Sheet No. 274107
HERBARIUM OF POMONA COLLEGE Sheet 1

Opuntia macrocentra Engelm.

Var. *Martinii* L. Benson

State Arizona County Mohave

Locality Hualpai Mountain

Mt. Range Hualpai Alt. 5000 ft.

Drainage Area Soil

Veg. Type Slope

Lyman Benson No. 10,169 Date March 30 1940
Kingman Road on north side of Hualpai Mountain.

FIG. 4. Lost type of *O. martiniana* showing the discordant elements apparently collected at different times of the year.

Rd. on Old Hwy. 93 through foothills of Hualapai Mt., 1 May 1978, *Parfitt 2542* (ASU); Foothills of Hualapai Mt. Old Hwy. 93 one mi W of its junction with Peacock Mt. Rd., 5 Jun 1978, *Parfitt 2545* (ASU); 0.1 mi S of jct. of Hwy. 93 and Peacock Mt. Rd., 17 Mar 1979, *Parfitt 2701* (ASU); Hualapai Mt. foothills, Hualapai Mt. Rd. 1.8 mi S of Hwy. I-40, 18 May 1980, *Parfitt 2838* (ASU); intersection of DW Ranch Rd., Getz Ranch Road and BLM 7127, 21 May 2014, *Thibault 933* (HNT); *ibid*, *Thibault 935* (HNT).

APPENDIX 1

Taxa newly sequenced for this study (data are available on FigShare; Majure 2021). Herbarium acronyms follow Thiers (2017+).

Opuntia atrispina Majure 5636 (DES); *Opuntia chlorotica* Majure 5439, 5497, 5820 (DES); *Opuntia curvispina* Heil s.n. (plastid only, ASU), Majure 5438 (plastid only, DES), 5440, 5547, 5552, 5567, 5722 (plastid only, DES); Hodgson 23642 (DES); *Opuntia diploursina* Majure 5697 (DES); *Opuntia gosseliniana* DBG 1999-0038-21 (DES); *Opuntia macrorhiza* s.l. Snow 2089 (FLAS), Majure 1982 (MISSA), 3510 (FLAS), 5831 (DES), Baker 15682 (ASU, FLAS); *Opuntia martiniana* Majure 5539, 5548, 5561, 5829 (DES); *Opuntia phaeacantha* Majure 5379 (DES, FLAS).

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