MORPHOLOGICAL AND CYTOLOGICAL ANALYSES IN *CYLINDROPUNTIA* (CACTACEAE): THE TAXONOMIC CIRCUMSCRIPTION OF *C. ECHINOCARPA*, *C. MULTIGENICULATA*, AND *C. WHIPPLEI*

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ABSTRACT

A morphological and cytological study was conducted to elucidate the taxonomic circumscription of *Cylindropuntia multigeniculata* and its relationship to *C. echinocarpa* and *C. whipplei*. Morphological analyses included iterations of multivariate techniques including principle factor analysis, discriminant function analysis, and multivariate analysis of variance. Results suggested a geographic ring of two morphological clines where westernmost diploid populations of *C. whipplei* var. *whipplei* intergrade to the northeast with diploid populations of *C. multigeniculata* and intergrade to the south with tetraploid populations of *C. whipplei*. Primarily, results indicated that *C. multigeniculata* represents a group of populations with a number of distinctive morphological traits that suggest it is not of hybrid origin and that it is worthy of taxonomic recognition at the species level. Similarly, tetraploid populations of *C. whipplei* collectively possess a number of distinctive morphological traits, as well as a distinct chromosome number, and are worthy of taxonomic recognition at the varietal level as *C. whipplei* var. *enodis*.

KEY WORDS: *Cylindropuntia*, evolution, phytogeography, taxonomy, morphology

RESUMEN

Se realizó un estudio morfológico y citológico para elucidar la circunscripción taxonómica de *Cylindropuntia multigeniculata*, incluyendo sus relaciones con *C. echinocarpa*, y *C. whipplei*. El análisis morfológico incluyó varias técnicas multivariadas; análisis de componentes principales, análisis de funciones discriminantes y varianza de análisis multivariado. Los resultados sugieren un anillo geográfico de dos gradientes morfológicos en el cual las poblaciones diploides más al oeste de *C. whipplei* var. *whipplei* intergradúan al noreste con las poblaciones diploides de *C. multigeniculata*; e intergradúan hacia el sur con poblaciones tetraploides de *C. whipplei*. Primeramente, los resultados indican que *C. multigeniculata* representa un grupo de poblaciones con un numero de caracteres morfológicos distintos que sugiere no es de origen hibrido y es factible de reconocerse al nivel de especie. De la misma manera, las poblaciones tetraploides de *C. whipplei* colectivamente posee un numero de caracteres morfológicos distintos, así como un numero cromosómico diferente, y son también factibles de reconocerse taxonómicamente a nivel de variedad como C. *whipplei* var. *enodis*.

The study of systematics within *Cylindropuntia* has been complicated by such evolutionary processes as hybridization, apogamy, genetic drift, and polyploidy (Pinkava 2002). These processes were addressed using morphological and cytological techniques with the primary focus of shedding light on the evolutionary, geographical and taxonomic relationships of *C. echinocarpa* (Engelm. & J.M. Bigelow) F.M. Knuth, *C. multigeniculata* (Clokey) Backb., and *C. whipplei* (Engelm. & J.M. Bigelow) F.M. Knuth. The goal of the study was to ascertain which groups of populations possessed statistically different means for character values or groups of character values and whether these population groupings were correlated with geography and/or habitat type. The taxonomic rank of each group was then assessed based on the degree of its morphological uniqueness and which type locality population, if any, was contained within the group. Although multivariate statistical methods have been available for many decades, their use in morphological analyses in systematic botany has only recently gained popularity (Mraz et al. 2010; Sharma & Pandit 2011; Kaplan & Marhold 2012; Baker & Butterworth 2013; Diaz 2013; Othman et al. 2013; Sassone et al. 2013; Baker & Cloud-Hughes 2014; Jimenez-Mejias et al. 2014; Leveille-Bourret et al. 2014; Baker & Porter 2016).

The importance of sampling the degree of morphological variation within and among populations cannot be understated. DNA analyses have the distinct advantage of sampling genes that are little influenced by envi-

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ronmental factors. However, much of the inherent error caused by environmental influences during morphological sampling is resolved by adequate sample size. In addition, morphological studies have the clear advantage of having a direct application to the construction of taxonomic descriptions and keys. A more complete understanding of evolutionary and taxonomic relationships can be achieved through the combination of DNA, morphological, and other biosystematics techniques.

Cylindropuntia multigeniculata was originally described as *Opuntia multigeniculata* Clokey from a limestone ridge just north of the town of Blue Diamond, Nevada (Clokey 1943). To date, only diploid, 2*n*= 22, individuals have been recorded for the taxon (Pinkava et al. 1998). Benson (1982) placed *O. multigeniculata* as a variety under *O. whipplei*. Recent treatments place *O. multigeniculata* under *Cylindropuntia* (Pinkava & Baker 2012). Within the past two decades, additional populations of *C. multigeniculata* have been discovered, some of which have fruits that are less spiny than those of the type locality, and some of which have fruits that are much spinier than those at the type locality. The populations with consistently spiny fruits, which occur in extreme southeastern Nevada and northwestern Arizona, have been traditionally assigned to *C. echinocarpa* (Pinkava 1999). Although spiny-fruited populations of *C. multigeniculata* occur sympatrically with those of *C. echinocarpa*, individuals with intermediate morphology between the two species are rare (Baker & Cloud-Hughes 2014). Based on morphological intermediacy of certain characters between *C. echinocarpa* and *C. whipplei*, Pinkava (1999, 2003) regarded *C. multigeniculata* as a hybrid between the two species. In contrast, Trushell (1985) reported that *C. multigeniculata* possessed at least some unique morphological characteristics that could not be explained by hybridization. Baker and Cloud-Hughes (2014) supported Trushell's findings; their discriminate function analysis (DFA) resulting in no individuals misclassified between *C. echinocarpa* and *C. multigeniculata*. This clearly supported the inclusion of the spiny-fruited western populations within *C. multigeniculata,* with no indication of hybrid origin as Pinkava (1999) suggested.

To date, only diploid individuals of *Cylindropuntia whipplei* have been recorded in the eastern portion of its range and in the western portion primarily north of the Colorado River. This distribution includes the type locality for the species, originally described under *Opuntia* as *O. whipplei* Engelmann & J.M. Bigelow. In lower elevations of western Arizona, to the south of the Colorado River, *C. whipplei* is known only as a tetraploid. Tetraploid individuals of *C. whipplei* have also been recorded just north of the Grand Canyon, near Mount Trumbull (Baker et al. 2009). The tetraploid individuals at the eastern end of their range and at higher elevations are similar in size and morphology to their diploid counterparts and are represented by the type *Opuntia hualpaensis* Hester. Tetraploid populations to the west at lower elevations possess a diminutive habit and smaller stems with fewer spines per areole and are represented by the type *Opuntia whipplei* var. *enodis* Peebles (*Cylindropuntia whipplei* var. *enodis* [Peebles] Backeb.), which has not been recognized by most authors (Benson 1982; Trushell 1985; Pinkava 1999). Pinkava and Baker (2012) resurrected the taxon based on fruit characters and ploidy level.

Cylindropuntia echinocarpa (2*n* = 22) was included in the present study as an outgroup and to assess its morphological relationship to the spiny-fruited forms of *C. multigeniculata*, as well as its role, if any, as a putative parent in the origin of *C. multigeniculata*. Populations of *C. echinocarpa* occur primarily in desert scrub from northern Sonora, north through western Arizona, southeastern California, southern Nevada and southern Utah. At present, there are no recognized varieties within *C. echinocarpa* (Pinkava 1999, 2003; Baker & Rebman 2012; Pinkava & Baker 2012).

METHODS

Study sites.—Twenty-one sites were visited between 31 Oct 1999 and 23 Jun 2003 (Fig. 1; Table 1). Twenty of these sites were revisited between 22 Jan 2013 and 20 Feb 2014. Sites were selected to include type localities, to represent the extent of geographic range of each potential taxon, and to include populations with adequate densities of individuals for statistical sampling. Sample sizes for each population ranged from 17 to 40 individuals, with all but one population having a sample size greater than 30. Individuals within each population were sampled from an area of approximately 1–2 km^2.

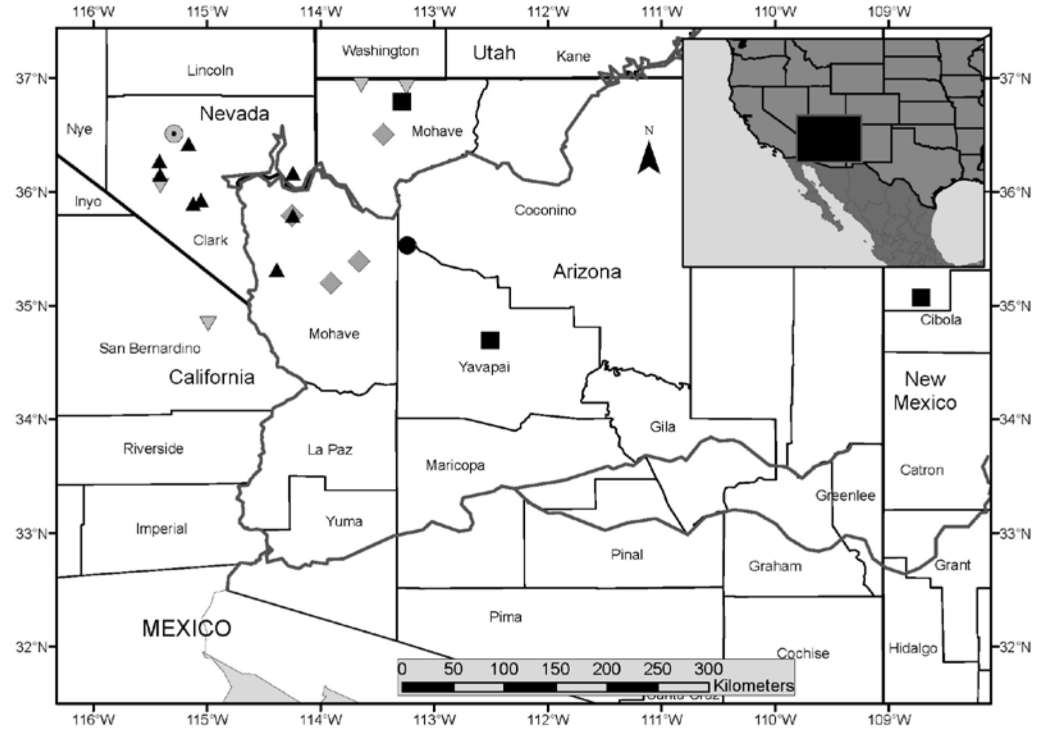

Fig. 1. Geographic locations of populations sampled for the *Cylindropuntia* morphological analysis. *C. echinocarpa* (gray triangles), *C. whipplei* var. *whipplei* (black squares), *C. whipplei* var. *enodis* (gray diamonds), *C. multigeniculata* (black triangles), and Black Gate Canyon population (gray circle with black center). Rivers and lakes are represented by broad gray lines.

Habitats varied significantly among populations, and, even within any single taxon, there was a considerable range in vegetation type, elevation, slope aspect and inclination, and substrate. The general habitats for populations of each taxon were as follows: *Cylindropuntia echinocarpa*: desert scrub below 1400 m; *C. whipplei* var. *whipplei*: pinyon-juniper woodland and mixed grasslands between 1520 and 2055 m; *C. whipplei* var. *enodis*: primarily Mojave desert scrub and disclimax desert grassland between 1160 and 1690 m; and *C. multigeniculata*: *Yucca brevifolia* woodland and Mojave Desert scrubland on rocky limestone, basalt, granite, and rhyolite substrates between 1035 and 1400 m.

Cytology.—Chromosome numbers were determined from young anthers, many of which were previewed in the field under 100× magnification using a compound microscope, fixed in a modified Carnoy's solution (95 % ethanol: chloroform: glacial acetic acid :: 3:3:1), transferred to 70% ethanol after 24 hours, and refrigerated. The anthers were squashed and stained in 45% acetocarmine and mounted in Hoyer's medium (Beeks 1955; Pinkava & Baker 1985). Chromosome number determinations were attempted from the first or second meiotic divisions of pollen mother cells. Multiple cells were examined for each determination.

To estimate pollen stainability, pollen was obtained from fresh flowers of *Cylindropuntia multigeniculata* (N = 128) in the field and stained in aniline-blue-lactophenol for 24 hours (Maneval 1936). A minimum of 500 pollen grains per individual were scored as staining or non-staining.

Morphological analysis.—Twenty-two continuous characters, including 11 stem characters and 11 fruit characters, were measured for 667 individuals (Table 2). An additional five characters were measured for 633 individuals, but only three of these characters were used in the analysis. The importance of these additional characters became apparent during a morphological analysis of *Cylindropuntia. acanthocarpa*, *C. chuckwallensis*,

Table 1. Locations of *Cylindropuntia* study populations, ploidy level, and herbarium specimen voucher(s). All specimens also represent vouchers for chromosome determinations. * Unpublished, † Chromosome number reported in Baker et al. (2009), ‡ Baker and Cloud-Hughes (2014), § Pinkava et al. (1998).

Pop. no.	Taxon	Ploidy	Locale	N	Latitude, longitude	Elevation (m)	Voucher(s) & *M. Baker* collection number
1	*C. echinocarpa*	diploid†	Near Blue Diamond, Clark Co., NV	29	36.052° -115.408°	1,035	*13670*†
2	*C. echinocarpa*	diploid*	South of St. George on the AZ Strip, Mohave Co. AZ	33	36.938° -113.639°	985	*16950.1** (ASU), *16950 2** (ASU)
3	***C. echinocarpa***	**diploid†**	**West of Needles, San Bernardino Co., CA, (neotype locality for *Opuntia echinocarpa* Engelm. & J.M. Bigelow)**	30	34.838° -114.985°	670	*13838*†
4	*C. echinocarpa*	diploid†	East of Hurricane Cliffs, Mohave Co., AZ	30	36.919° -113.240°	1,380	*16717.2*†
5	***C. whipplei* var. *whipplei***	**diploid†**	**Near Zuni, McKinley Co., New Mexico (lecto type locality for *Opuntia whipplei* Engelm. & J.M. Bigelow)**	30	35.072° -108.717°	2,055	*16670.2**, *16670.4*†, *16670.7**, *16670.9*†, 17829.3 ASU
6	*C. whipplei* var. *whipplei*	diploid†	Hurricane Cliffs, Mohave Co., AZ	35	36.795° -113.282°	1,615–1,630	*16715.1*†, *16715 2*†, *16715.3*†, *16715.4*†
7	*C. whipplei* var. *whipplei*	diploid* (†)	North of Prescott, Yavapai Co., AZ	34	34.697° -112.511°	1,520–1,530	*16721.1*†, *16721 2** (ASU, ASC), *16721.3** (ASU), *16721.4** (UNLV)
8	*C. whipplei* var. *whipplei* × *C. multigeniculata*	diploid*	Black Gate Canyon, Clark Co., NV.	30	36.514° -115.289°	1,770–1,860	*13960.1** (ASU)
9	***C. whipplei***	**diploid†, tetraploid†**	**Grand Canyon Caverns, Coconino Co., AZ (type locality for *Opuntia hualpaensis* Hester)**	**31**	35.532° -113.238°	1,650	16178.1† (*n* = 11), 16179.1† (*n* = 22), 16179.2† (*n* = 22)
10	*C. whipplei* var. *enodis*	tetraploid†	Little Hurricane Rim, Mohave Co., AZ	33	36.498° -113.447°	1,615	*16655.1** (ASU), *16712.1*†, *16712.2*†, *16712.3*†,*16712.4*†*16712.5*†
11	*C. whipplei* var. *enodis*	tetraploid†	SW of Valentine, Mohave Co., AZ	37	35.384° -113.662°	1,160	*15390.1*†,*15390.2*†,*15390.3*†
12	*C. whipplei* var. *enodis*	tetraploid†	White Elephant Wash, NE of Kingman, Mohave Co., AZ	38	35.787° -114.251°	1,350	*15368*†
13	***C. whipplei* var. *enodis***	**tetraploid†**	**North end of Hualapai Mountains, Mohave Co., AZ (type locality for *Opuntia whipplei* var. *enodis* Peebles)**	**39**	35.194° -113.906°	1,270	*11617*†, *13672*†, *15360*†, *15361*†
14	*C. multigeniculata* (typical)	diploid*	Gass Peak, Clark Co., NV	32	36.424° -115.161°	1,560	*15307.3** (ASU, RSA)
15	***C. multigeniculata* (typical)**	**diploid§**	**Blue Diamond, Clark Co., NV (type locality of *Opuntia multigeniculata* Clokey)**	**35**	**36.151°** **-115.415°**	**1,310**	***4969A*§, *11374A*§, *11374B*§**
16	*C. multigeniculata* (typical)	diploid	Black Mountain, Clark Co., NV	33	35.927° -115.052°	1,060	*18312.1**
17	*C. multigeniculata* (typical)	diploid‡	McCullough Range, Clark Co., NV	31	35.896° -115.124°	975	*15152*‡, *15153*‡, *15303*‡ (listed erroneously as 153033)

Pop. no.	Taxon	Ploidy	Locale	N	Latitude, longitude	Elevation (m)	Voucher(s) & *M. Baker* collection number
18	*C. multigeniculata* (typical)	diploid*	La Madre Mountain, Clark Co., NV	34	36.272° -115.417°	1,475	*15304** (ASU, RSA, UNLV)
19	*C. multigeniculata* (spiny-fruited)	diploid*	White Elephant Wash, NW of Kingman, Mohave Co., AZ	31	35.794° -114.242°	1,360	*13650.1** (ASU), *15214** (ASU)
20	*C. multigeniculata* (spiny-fruited)	diploid‡	Black Mountains, Mojave Co., AZ	31	35.317° -114.387°	1,156	*15257*‡ (ASC, ASU), *15258*‡ (ASU), *15262*‡ (ASU), 17670.2*
21	*C. multigeniculata* (spiny-fruited)	diploid*	NNE of Bonelli Peak, Clark Co., NV	39	36.166° -114.243°	1,035	*15305.1** (UNLV), *15305.3** (ASU)

Table 2. Description of characters measured. Plant height and width were measured only once per individual and were included only for descriptive purposes. The characters used in the morphological analyses were measured three times for each individual.

Abbreviation	Character	Description
PLANT H	plant height	height of plant from soil level to the tallest stem, excluding spines
PLANT W	plant width	width of plant at its widest point, excluding spines
BRANG	branch angle	angle between one or more of the main trunks and the primary branches, a divaricate branch equaling 90°
INTL	trunk internode length	length along a main trunk between two trunk nodes (identified by one or more living branches or branch scars). Trunk nodes in this sense are not synonymous with stem nodes, which are thought to be homologous with areoles.
WHORL	number of stems per whorl	number of stems at one trunk node, which often occur in whorls ≥ three
STEML	stem length	mean length of three stem segments
STEMDIA	stem diameter	mean diameter at mid-stem of three stem segments
TUBL	stem tubercle length	mean length of topmost penultimate tubercle from each of three stem segments
TUBW	stem tubercle width	mean width of topmost penultimate tubercle from each of three stem segments
TUBH	stem tubercle height	mean height of topmost penultimate tubercle from each of three stem segments
NOCSP	central spine number	mean number of central spines from each of three mature areoles of the tubercles described above
NORSP	radial spine number	mean number of radial spines from each of three mature areoles of the tubercles described above
CSPL	central spine length	mean length of longest central spine from each of three mature areoles as described above
RSPL	radial spine length	mean length of longest radial spine from each of three mature areoles as described above
CSPTH	central spine thickness	mean thickness of longest central spine from each of three mature areoles as described above
SHTH	central spine sheath thickness	mean thickness of the sheath of the longest central spine from each of three mature areoles as described above
FRTL	fruit length	mean length of three fruits
FRTW	fruit width	mean width of three fruits measured at widest portion
UTUBL	fruit uppermost tubercle length	mean length of the uppermost tubercle from three fruits
UTUBW	fruit uppermost tubercle width	mean width of the uppermost tubercle from three fruits
UTUBH	fruit uppermost tubercle height	mean height of the uppermost tubercle from three fruits
LTUBL	fruit lowermost tubercle length	mean length of the lowermost tubercle from three fruits
LTUBW	fruit lowermost tubercle width	mean width of the lowermost tubercle from three fruits
LTUBH	fruit lowermost tubercle height	mean height of the lowermost tubercle from three fruits
NOAR	areole number	mean number of areoles as counted from one side of each of three fruits
FRTSPNO	fruit spine number	mean number of spines of an uppermost areole of three fruits (character deleted from multivariate analyses)
FRTSPL	fruit spine length	mean length of spines of an uppermost areole of three fruits (character deleted from multivariate analyses)

C. echinocarpa, and *C. multigeniculata* (Baker & Cloud-Hughes 2014). In an attempt to avoid age-dependent factors, only mature, fruit-bearing individuals were sampled. To further insure age-independency, stem characters were sampled only from previous year's growth. Fruits were measured only after they exhibited signs of maturation, such as loss of chlorophyll, but before they began to shrivel and dry.

Terminology for multivariate procedures follows that of Tabachnick and Fidell (2001). Principle factor analysis (PFA) was performed initially in order to determine, without *a priori* prejudice, which populations grouped together morphologically and which characters were most weighted in determining these groupings. The Grand Canyon population (site 9) was excluded from the final analysis because it included both diploid and tetraploid individuals, and the Black Gate Canyon population (site 8) was excluded because individuals were apparently morphologically intermediate between *C. multigeniculata* and *C. whipplei*. As far as is known, the individuals of Black Gate Canyon represent an isolated population of morphologically intermediates and not merely a small part of a widespread morphological continuum between the two species.

Once groups were determined, data were transformed as necessary to meet assumptions of homogeneity within groups, and discriminant function analysis (DFA) was used to test for multivariate outliers using the algorithm for determining the Mahalanobis distance-square of each individual from group means. Nineteen individuals (2.5%) were excluded as multivariate outliers ($p < 0.001$). Multivariate analysis of variance (MANOVA) was performed on transformed data in order to obtain homogeneous subsets of taxa, by character, which was useful in determining which characters differ significantly among taxa. Finally, DFA was used again, excluding multivariate outliers, to determine percent correct classification of individuals among taxa. SPSS® 22 software (SPSS IBM 2013) was used for the PFA, DFA and MANOVA.

RESULTS

Cytology.—Chromosome determinations were made for all populations (Table 1). For both diploid and tetraploid individuals, chromosome pairing was normal during prophase I of microgametogenesis. Individuals from all *a priori Cylindropuntia whipplei* var. *enodis* populations were tetraploid, while those from the Grand Canyon Caverns population, the type locality of *Opuntia hualpaensis*, were both diploid and tetraploid. Additional unpublished chromosome number determinations made during the course of this study for *C. whipplei* and not associated with study sites are listed in Table 3. These were included to better define the geographical distribution of chromosome races within *C. whipplei*.

Pollen stainability was fairly uniform throughout all populations of *Cylindropuntia multigeniculata* (Table 4). The mean pollen stainability for the western, spineless-fruited to somewhat spiny-fruited populations of *C. multigeniculata* was 89.0%; for the eastern, strictly spiny-fruited populations of *C. multigeniculata*, pollen stainability was 82.3%, and for the species as a whole, pollen stainability was 86.5%. The strictly spiny-fruited White Elephant Wash population of *C. multigeniculata* possessed the lowest value (77.8%). Pollen at this population was sampled primarily from older flowers in comparison to those of the other populations, and the ratio of fertile to sterile pollen may therefore have been reduced because of pollen aging or selective dispersal.

Morphological analysis.—The PFA analysis extracted three main factors, which explained a total of 61.9% of the variance: factor one 29.5%, factor two 18.8%, and factor three 13.6% (Table 5). When factors were plotted by populations, individuals were well-grouped within their expected taxa. Factors one and two show the greatest differences in fruit length, fruit uppermost tubercle width, stem length, fruit uppermost tubercle width, stem tubercle length, and fruit width, respectively. Factors two and three show the greatest differences in fruit areole number, fruit length, fruit uppermost tubercle width, radial spine length, fruit width, and central spine length. Factors one and three show the greatest differences in stem tubercle width, central spine sheath thickness, trunk internode length, central spine thickness, stem diameter, and stem tubercle length (Table 6).

Once populations were satisfactorily placed within their respective taxa, PFA scatter plots were made identifying individuals by taxon. The plot of factor one versus factor two indicated that individuals form more or less three groupings, one for each of the species, with only a weak segregation of individuals with respect to the two varieties of *Cylindropuntia whipplei* (Fig. 2). There was a better separation of *C. whipplei* individuals from those of the other two species with a strong weighting of fruit characters. A scatter plot of factor two versus factor three indicated that individuals form more or less four groupings, with the two varieties of *C. whipplei* having a much better segregation of individuals (Fig. 3), also with a strong weighting of fruit characters. Note that individuals of *C. multigeniculata* are shown as a morphological extreme rather than an intermediate. A scatter plot of factor one versus factor three showed the strongest separation of *C. echinocarpa* individuals from those of the other species, with stem characters being of primary importance (Fig. 4).

MANOVA was performed on populations of all taxa and included values for all characters, with the exception of plant height and width. Homogeneous subsets, by taxon, were created for each character, which provides character means and their significance at the $p < 0.01$ level (Table 6).

DFA was first run on all populations to test taxonomic affinities of individuals within the proposed taxa. Problematic populations were analyzed by leaving the individuals of those populations ungrouped, without *a priori* taxonomic classification. These ungrouped individuals were then classified by DFA into the taxon for which they possessed the closest morphological affinity.

TABLE 3. Additional unpublished chromosome determinations from localities not within study populations. NMT = Nichole M. Trushell (see N. Trushell 1985), all others are M. Baker.

Chromosome number	Locality	Voucher specimens
	C. whipplei* var. *enodis	
n=22	Arizona, Mohave Co., 35.135°, -113.522°, Aquarius Mountains, 45 km ESE of Kingman, 2.7 km ENE of Mesquite Thicket Spring	16645.1 ASU, ASC; 16645.2 ASU, ASC
n=22	Arizona, Mohave Co., 36.3697°, -113.4959°, south base of Poverty Mountain, 82 km south of St. George, Utah	16713.3 ASU, US
n=22	Arizona, Mohave Co., 34.6378°, -113.5713°, just west of the Owen's Post Office site, along the Big Sandy River stems, mounted, no dup.	6804.2 ASU
n=22	Arizona, Yavapai Co., 34.2761°, -112.9053°, 17km WNW of Antelope Peak, 50 km SW of Prescott	*18268.2* ASU, DES; *18268.3* ASU, US; *18268.4* ASU, UCR
n=22	Arizona, Yavapai Co., 35.490°, -113.165°, 30 km WNW of Seligman, S of old Hwy 66	NMT 84.17 ASU
	C. whipplei* var. *whipplei	
n=11	Arizona, Apache Co., 35.4752°, -109.3970°, 11 km NE of Wide Ruins, 29 km SSE of Ganado	17600.2 ASU
n=11	Arizona, Mohave Co., 36.795°, -113.281°, 42 km SSE of St. George, Utah, along Navajo Trail, just W of Dutchman Draw	16654.1 ASU, ASC; 16654.2 ASU
n=11	Arizona, Navajo Co., 36.5875°, -110.4707°, Long House Valley, 25 km SW of Kayenta	17578.3 ASU; 17878.4 ASU, ASC; 17578.5 ASU, ASC
n=11	Arizona, Navajo Co. 35.4926°, -110.7050°, east of Tolani Lake, 90km ENE of Flagstaff	18264.1 ASU, ASC
n=11	Arizona, Yavapai Co., 34.896°, -112.469°, Big Chino Valley, 1 km N of Paulden	NMT84.11 ASU
n=11	Arizona, Yavapai Co., 34.990°, -112.588°, Big Chino Valley, 16 km NW of Paulden	NMT 84.12 ASU
n=11	Arizona, Yavapai Co., 35.453°, -113.098°, 15 mi E of Seligman on old Hwy 66	NMT 84.15 ASU
n=11	Arizona, Yavapai Co., 35.225°, -112.897°, Big Chino Valley, 11 km S of Seligman	NMT 84.22 ASU
n=11	Arizona, Yavapai Co., 35.080°, -112.925°, 20 mi S of Seligman at I-40 along Walnut Creek Road	NMT 84.23 ASU
n=11	Arizona, Yavapai Co., 34.689°, -112.540°, 17 km NNW of Prescott, 5.7 km NNE of Granite Mtn	16720 ASU
n=11	New Mexico, McKinley Co., 35.650°, -108.053°, ca. 2 km SW of Prewitt	11732.1 ASU
n=11	Nevada, Clark Co., 36.6866°, -115.0939°, Sheep Range, 9 km NE of Hayford Peak, 55 km N of Las Vegas	15925.5 ASU
n=11	Nevada, Lincoln Co., 37.898°, -114.415°, SE end of Pioche Hills, 4.5km SE of Pioche	17627.1 ASU
n=11	New Mexico, McKinley Co., 35.072°, -108.767°, Cheama Canyon, 12 km E of Zuni	16670.2 ASU; 16670.7 ASU, UNM
n=11	Utah, Iron Co., 37.8453°, -113.8961°, Escalante Desert, 5.6 km NNE of Modena	16957.2 ASU, UNLV
n=11	Utah, Millard Co., 38.5871°, -113.8046°, north slope of Warm Point, 72 km WNW of Milford	17788.2 ASU, NY

TABLE 4. Mean percent pollen stainability for *C. multigeniculata*, by population.

Population	N	Mean	SD
	Populations with spineless or nearly spineless fruits		
Blue Diamond	26	89.6	6.9
McCullough	30	90.1	5.5
La Madre	23	87.4	5.8
Population Average	79	89.0	4.0
	Populations with consistently spiny fruits		
Black Mountain	26	87.7	6.2
White Elephant Wash	23	77.8	10.7
Population Average	49	82.3	8.3
Overall Average	128	86.5	7.0

Table 5. Factor loadings for PFA (excluding populations 8 and 9). Values for the differences between factor 1 and factor 2, by character, are given in column F1-F2, and those for the differences between factor 1 and factor 3 are given in column F1-F3. The ten largest absolute values for each of these last two columns are in **bold**.

Character	**Factor 1**	**Factor 2**	**Factor 3**	**F2-F3**	**F1-F2**	**F1-F3**
branch angle	0.211	-0.181	-0.129	-0.052	0.392	0.34
trunk internode length	0.598	-0.185	-0.456	0.271	**0.783**	**1.054**
number of stems per whorl	-0.276	0.485	0.449	0.036	**-0.761**	**-0.725**
stem length	0.414	-0.584	-0.063	-0.521	**0.998**	0.477
stem diameter	0.725	0.301	-0.140	0.441	0.424	**0.865**
stem tubercle length	0.828	-0.144	-0.021	-0.123	**0.972**	**0.849**
stem tubercle width	0.627	-0.094	-0.482	0.388	0.721	**1.109**
stem tubercle height	0.683	0.291	0.034	0.257	0.392	**0.649**
central spine number	0.444	0.724	-0.066	0.79	-0.28	0.51
radial spine number	0.237	0.793	0.192	**0.601**	-0.556	0.045
central spine length	0.282	0.434	-0.325	**0.759**	-0.152	0.607
radial spine length	0.445	0.762	-0.070	**0.832**	-0.317	0.515
central spine thickness	0.430	-0.432	-0.624	0.192	**0.862**	**1.054**
central spine sheath thickness	0.556	-0.111	-0.553	0.442	0.667	**1.109**
fruit length	0.557	-0.537	0.331	**-0.868**	**1.094**	0.226
fruit width	0.605	-0.349	0.424	**-0.773**	**0.954**	0.181
fruit uppermost tubercle length	0.650	-0.398	0.336	**-0.734**	**1.048**	0.314
fruit uppermost tubercle width	0.290	-0.695	0.144	**-0.839**	**0.985**	0.146
fruit uppermost tubercle height	0.468	0.095	0.754	**-0.659**	0.373	-0.286
fruit lowermost tubercle length	0.832	0.049	0.200	-0.151	**0.783**	**0.632**
fruit lowermost tubercle width	0.603	-0.208	0.310	-0.518	**0.811**	0.293
fruit lowermost tubercle height	0.560	0.252	0.620	-0.368	0.308	-0.06
areole number	-0.341	-0.408	0.464	**-0.872**	0.067	**-0.805**
fruit spine number	0.661	0.442	-0.099	**0.541**	0.219	0.76
Percent of total variance explained	29.5	18.8	13.6			

Table 6. Selected homogeneous subsets from MANOVA performed on populations of *Cylindropuntia echinocarpa* (2), *C. whipplei* var. *whipplei* (3), *C. whipplei* var. *enodis* (4), and *C. multigeniculata* (5) where significance (Duncan's multiple range test) p <0 .01. Subset A has the smallest mean value for characters, and D has the largest. For all subsets containing a single taxon, $p < 0.01$. Means of character values for each taxon are in parentheses.

		Subset		
Character	**A**	**B**	**C**	**D**
Branch angle	5, 3 (49.21, 50.08)	4, 2 (57.34, 59.74)		
Branch internode length	5 (1.91)	4, 3 (3.80, 4.16)	2 (9.26)	
Branches per whorl	2 (2.81)	4 (3.15)	3 (4.16)	5 (5.79)
Stem length	5 (42.44)	2 (54.05)	4 (60.45)	3 (72.55)
Stem diameter	4 (14.07)	3, 5 (16.84, 17.12)	2 (22.41)	
Tubercle length	4, 5 (7.25, 7.40)	3 (9.70)	2 (11.27)	
Tubercle width	5 (3.75)	4 (4.11)	3 (4.39)	2 (5.84)
Tubercle height	4 (3.19)	3, 5 (4.24, 4.42)	2 (5.55)	
Number of central spines	4 (2.45)	3 (3.66)	5 (6.35)	2 (8.12)
Number of radial spines	4, 3 (3.23, 3.26)	2, 5 (6.42, 6.80)		
Central spine length	3, 4 (17.71, 18.20)	5 (20.14)	2 (23.14)	
Radial spine length	4 (3.89)	3 (4.96)	5 (8.65)	2 (10.90
Central spine thickness	5 (0.29)	3 (0.42)	4 (0.45)	2 (0.49)
Sheath thickness	5 (0.51)	4, 3 (0.61, 0.63)	2 (0.80)	
Fruit length	5 (16.32)	4, 2 (19.00, 19.04)	3 (20.43)	
Fruit width	5, 4, 2 (15.50, 15.71, 16.29)	3 (17.92)		
Upper fruit tubercle length	5 (4.72)	4 (5.39)	2, 3 (6.10, 6.25)	
Upper fruit tubercle width	5 (2.55)	2 (2.99)	3, 4 (3.56, 3.70)	
Upper fruit tubercle height	4 (1.59)	2 (2.46)	5, 3 (2.96, 2.99)	
Lower fruit tubercle length	4 (3.78)	5, 3 (5.47, 6.19)	2 (10.7)	
Lower fruit tubercle width	4, 5 (2.28, 2.47)	2 (3.16)	3 (3.35)	
Lower fruit height	4 (1.046)	2, 3, 5 (2.08, 2.09, 2.14)		
Number of areoles per fruit	2 (11.7)	5 (19.24)	4, 3 (20.53, 21.29)	
Number of spines per areole	3, 4 (0.00, 0.53)	5 (3.47)	2 (10.87)	

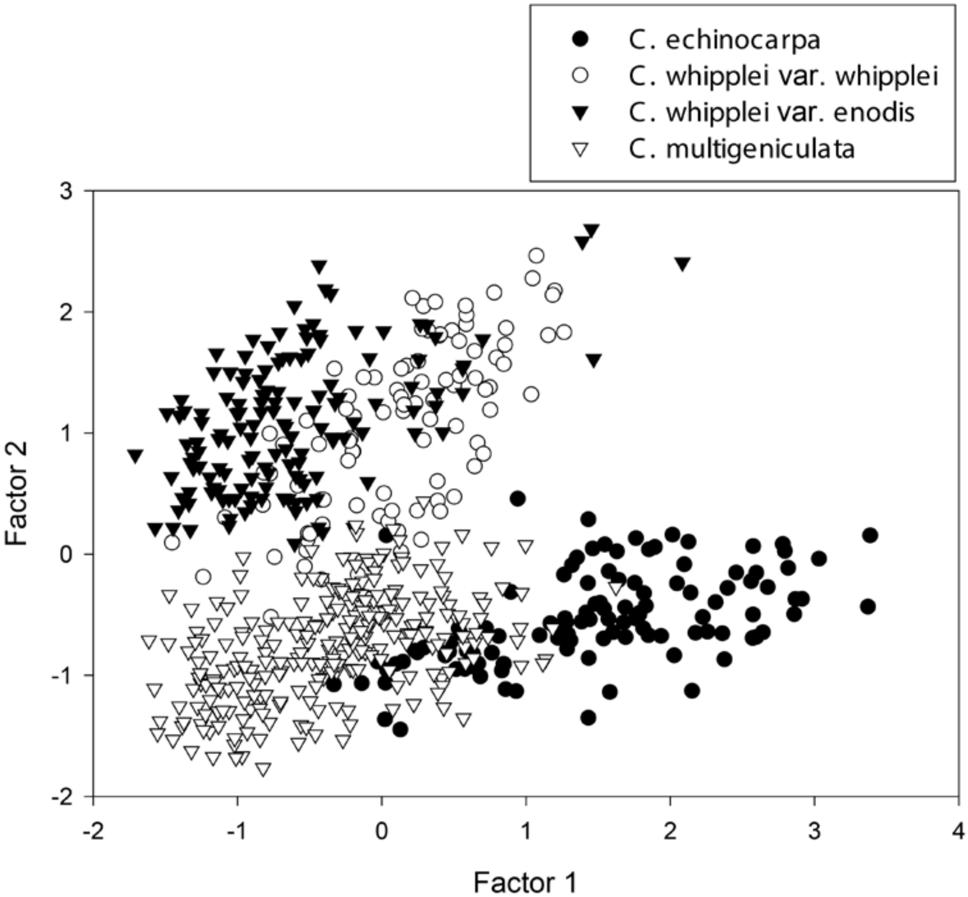

Fig. 2. Scatterplot of PFA factors one vs. two with individuals identified by taxon.

For individuals of the Black Gate Canyon population, which appeared to be morphologically intermediate between *Cylindropuntia multigeniculata* and *C. whipplei* var. *whipplei*, nearly half (41.2%) were classified by DFA as *C. whipplei,* and only slightly more than half (58.8%) as *C. multigeniculata*. With respect to the Grand Canyon Caverns population (*C. hualpaensis*), which is geographically and apparently morphologically intermediate between *C. whipplei* var. *whipplei* and *C. whipplei* var. *enodis*, 27 individuals (87.1%) were classified as *C. whipplei* var. *whipplei,* and only four (12.9%) were classified as *C. whipplei* var. *enodis*.

DFA was rerun after excluding the Grand Canyon Caverns population because of its mixture of chromosome races, and the Black Gate population because of its apparent morphological intermediacy. The between groups F-matrix is presented in Table 7, and correct classification results are presented in Table 8. Most misclassified individuals were between *Cylindropuntia whipplei* var. *whipplei* and *C. whipplei* var. *enodis*. Although a single individual each of *C. echinocarpa* and *C. whipplei* var. *whipplei* were misclassified as *C. multigeniculata*, all individuals within populations of *C. multigeniculata* were correctly classified. Descriptive statistics, by taxon, is presented in Table 9.

DISCUSSION

These analyses indicate that both *Cylindropuntia multigeniculata* and *C. whipplei* var. *enodis* represent taxa that are defined by characteristics with mean values that are significantly different than those of their closest rela-

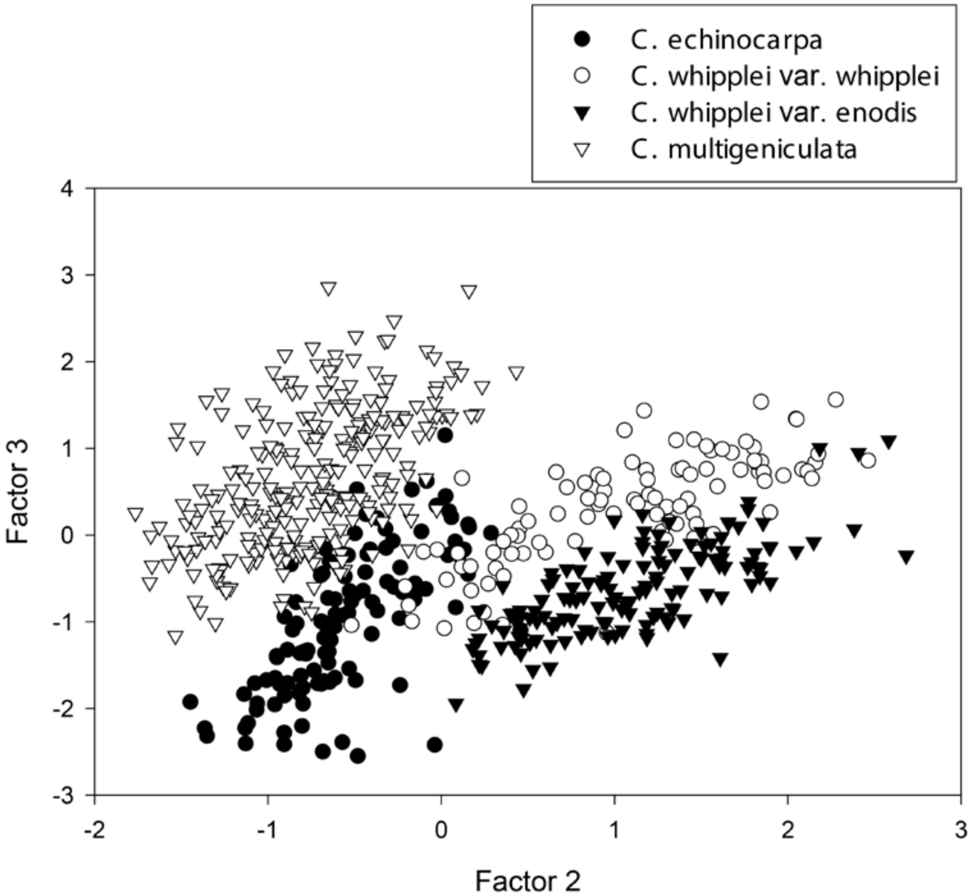

Fig. 3. Scatterplot of PFA factors two vs. three with individuals identified by taxon.

tives. The high percentage of correct classification for individuals of *C. multigeniculata* suggests that it should be maintained at the species level, and the lower percentage of correct classification for individuals of *C. whipplei* var. *enodis* suggests that it is worthy of recognition at the subspecific level. In both cases, data are in close agreement with similar studies within the Cactaceae (Baker 2006a, 2006b; Baker & Butterworth 2013; Baker & Cloud-Hughes 2014; Baker & Porter 2016).

Stebbins (1950) holds that a species must consist of a system of populations that are separated from those of other species by a complete or at least a sharp discontinuity with a genetic basis. This genetic basis may be reflected by one or more isolating mechanisms that prevent or greatly hinder the transfer of genes between two or more species. In the present study, both polyploidy and geography, especially the deeper gorges of the Grand Canyon, play major roles in genetic isolation. Davis and Heywood (1973) further assert that most plant taxonomists insist that species must be delimited by morphological characters. It is primarily this morphological-geographical species concept that is employed here, augmented by cytological/genetic data.

Subspecies, like species, are defined as occupying unique geographic distributions (Davis & Heywood 1973) but with weaker morphological boundaries than those between species. With respect to studies employing multivariate analyses of morphological characters, species tend to be comprised of individuals that are correctly classified by DFA at the 98–100% range, and subspecific taxa in the 80–97% range (Baker 2006a, 2006b; Baker & Butterworth 2013; Baker & Cloud-Hughes 2014; Baker & Porter 2016). Taxonomic decisions

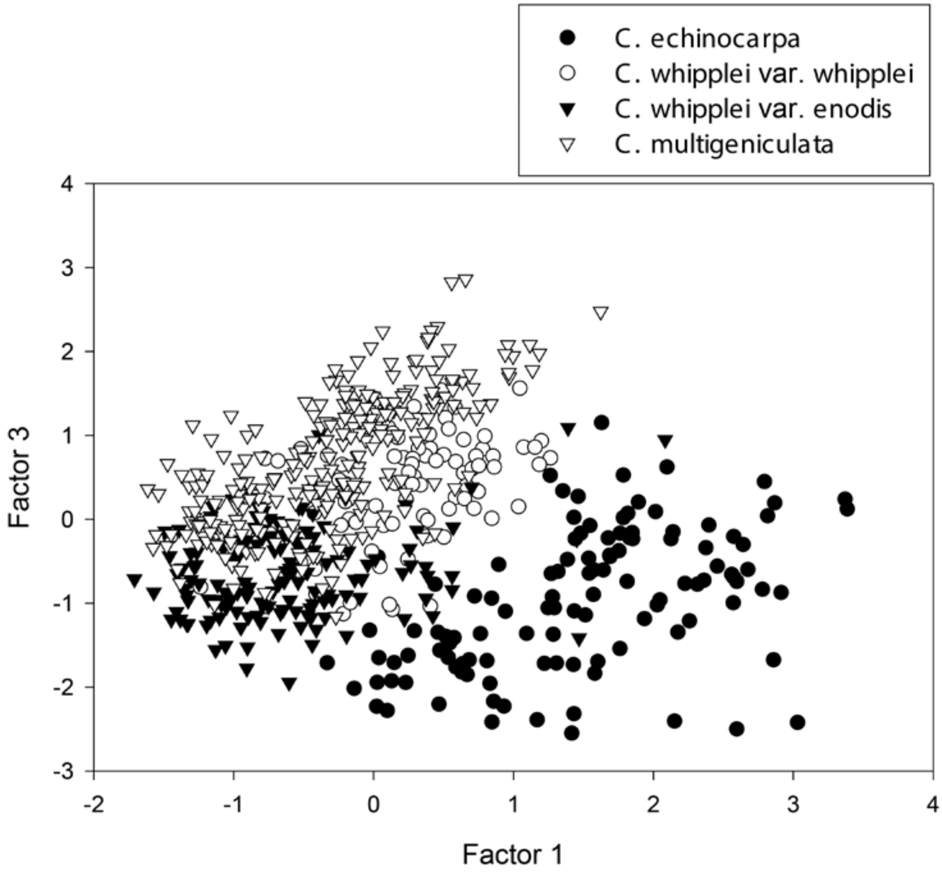

Fig. 4. Scatterplot of PFA factors one vs. three with individuals identified by taxon.

are further based on the degree of morphological intergradation between the taxa in question and the ability to identify the taxa in a practical sense. Although a strong case can be made to include many of the varieties within *Cylindropuntia* under subspecies, a conservative approach is maintained for present purposes.

Although this study indicates some degree of morphological intermediacy between *Cylindropuntia multigeniculata* and *C. whipplei* var. *whipplei*, populations of the two taxa are almost entirely allopatric and occur within separate habitats. The occurrence of a morphologically intermediate population at Black Gate Canyon suggests a close evolutionary relationship between *C. multigeniculata* and *C. whipplei* var. *whipplei*. However, it does little to weaken the case for their separate taxonomic recognition, especially in comparison to most other species within the genus. The number of morphologically intermediate or putative interspecific hybrids within the genus probably exceeds the number of recognized non-hybrid species (Peebles 1936; Grant & Grant 1971; Arp 1973; Pinkava et al. 1985, 1992; Baker & Pinkava 1987, 1999; Baker & Rebman 2012). In the greater Southwest, for example, *C. acanthocarpa* (Engelm. & Bigelow) F.M. Knuth is thought to hybridize with *C. abyssi* (Hester) Backeb., *C. bigelovii* (Engelmann) F.M. Knuth, *C. echinocarpa*, *C. leptocaulis* (DC.) F.M. Knuth, *C. spinosior* (Engelm.) F.M. Knuth, *C. versicolor* (Engelm. ex J.M. Coulter) F.M. Knuth, and *C. whipplei* var. *whipplei*. At least some interspecific hybrids in *Cylindropuntia* are abundant and geographically widespread.

The evidence provided here does not support the hypothesis that *Cylindropuntia multigeniculata* is of hybrid origin between *C. whipplei* and *C. echinocarpa*. For at least two of the three main factors, PFA analysis does not place individuals of *C. multigeniculata* intermediate to those of the other two putative parental species

Table 7. Between groups F-matrix for DFA excluding *Cylindropuntia* populations in Grand Canyon Caverns and Black Gate Canyon populations. df = 21, 735.

	C. echinocarpa	*C. whipplei* var. *enodis*	*C. multigeniculata*
C. echinocarpa	0.0		
C. whipplei var. *enodis*	224.5	0.0	
C. multigeniculata	188.6	156.1	0.0
C. whipplei var. *whipplei*	207.9	33.7	118.8

Wilk's lambda = 0.0005, df = 21, 5, 755
Approx. F= 125.8020, df = 105, 3598, p = 0.0000

Table 8. DFA correct classification matrix. Individuals of the Grand Canyon Caverns and Black Gate Canyon populations were excluded from the analysis.

Taxon	*C. echinocarpa*	*C. whipplei* var. *whipplei*	*C. whipplei* var. *enodis*	*C. multigeniculata*	% correct
C. echinocarpa	114	0	0	0	100
C. whipplei var. *whipplei*	0	95	1	1	98
C. whipplei var. *enodis*	0	11	133	0	92
C. multigeniculata	0	0	0	264	100
Totals	114	106	144	265	

98% of original groups correctly classified

(Adams 1982; Leveille-Bourret et al. 2014). For several non-intermediate characters, values for *C. multigeniculata* possess means that are significantly different from those of either of the putative parents (Table 8). With the possible exception of the Gold Butte area of Clark County, Nevada (M. Baker, pers. obs.), no individuals of intermediate morphology between *C. multigeniculata* and *C. echinocarpa* have been documented. In contrast, several individuals have been recorded with morphology intermediate between *C. multigeniculata* and *C. acanthocarpa* (Clokey 1943; Baker & Cloud-Hughes 2014), and putative morphological intermediates between *C. echinocarpa* and *C. acanthocarpa* (*C.* ×*deserta* [Griffiths] Pinkava) are fairly common wherever the two taxa are sympatric (Pinkava 1999; M. Baker, pers. obs.). In addition, mean pollen stainability (89.0%) in *C. multigeniculata* matches that reported for non-hybrid diploids of *Cylindropuntia* (Baker 1985; Pinkava et al. 1985, 1992). In comparison, mean pollen stainability for published putative diploid hybrids is 59.0%.

The spiny condition of the fruits in some populations of *Cylindropuntia multigeniculata* may or may not be an introgressive trait that resulted from past hybridization between *C. multigeniculata* and *C. echinocarpa*. Although the spiny condition of the fruits in *C. multigeniculata* may also have originated from introgression from hybridization with *C. acanthocarpa*, no other morphological traits of *C. multigeniculata* resemble those of *C. acanthocarpa*. The development of spiny fruits in *C. multigeniculata* may also be a case of homoplasy, perhaps as an adaptive response to drier habitats. Individuals of most other *Cylindropuntia* species that occur near populations of *C. multigeniculata,* including *C. acanthocarpa, C. echinocarpa*, and *C. ramosissima*, have spiny fruits, with *C. whipplei* var. *enodis* being the exception. Finally, spiny fruits could be ancestral among these species and spineless fruits the derived state.

Although speculative, there is at least some evidence that *Cylindropuntia echinocarpa* may have originated through hybridization between *C. multigeniculata* and *C. acanthocarpa*. Putative hybrids between *C. multigeniculata* and *C. acanthocarpa* are morphologically similar to individuals of *C. echinocarpa* (Baker & Cloud-Hughes 2014).

PFA and DFA did not support the recognition of two morphological (spineless vs. spiny-fruited) groups within *Cylindropuntia multigeniculata*. The apparent lack of additional morphological characteristics to satisfactorily separate the spiny-fruited form of *C. multigeniculata* from the typical form is notable in the sense that the spiny-fruited form was historically placed within *C. echinocarpa*.

Table 9. Descriptive statistics based on final taxonomic groupings of populations.

		Cylindropunta echinocarpa					***Cylindropunta whipplei* var. *whipplei***			
	N	**Min**	**Max**	**Mean**	**SD**	**N**	**Min**	**Max**	**Mean**	**SD**
PLANT H	114	23.00	198.00	74.38	27.38	97	11.00	130.00	45.65	25.84
PLANT W	114	15.00	136.00	67.93	27.39	97	6.00	218.00	77.38	41.38
BRANG	114	28.33	90.00	59.74	15.21	97	21.67	88.33	50.08	15.89
INTL	114	3.33	21.67	9.26	3.64	97	1.00	10.67	4.16	1.93
WHORL	114	1.00	5.67	2.81	0.93	97	1.67	6.33	4.16	1.12
STEML	114	31.00	82.00	54.05	11.86	97	36.00	129.00	72.55	18.64
STEMDIA	114	15.00	31.00	22.41	2.86	97	11.30	24.00	16.84	2.71
TUBL	114	7.00	17.50	11.27	2.25	97	4.70	15.30	9.70	2.41
TUBW	114	3.70	8.70	5.84	1.02	97	3.00	6.70	4.39	0.82
TUBH	114	3.30	8.00	5.55	1.22	97	2.30	6.00	4.24	0.78
NOCSP	114	3.00	13.70	8.12	2.15	97	1.00	8.00	3.66	1.72
NORSP	114	2.70	12.00	6.42	1.96	97	1.30	5.70	3.26	1.02
CSPL	114	11.50	35.00	23.14	4.87	97	7.30	31.00	17.71	5.39
RSPL	114	5.30	17.30	10.90	2.54	97	2.00	10.30	4.96	1.61
CSPTH	114	0.20	0.78	0.49	0.11	97	0.24	0.60	0.42	0.08
SHTH	114	0.43	1.20	0.80	0.17	97	0.33	0.88	0.63	0.11
FRTL	114	10.00	29.00	19.04	3.88	97	10.70	30.70	20.43	5.25
FRTW	114	10.00	23.50	16.29	2.85	97	12.00	25.00	17.92	2.74
UTUBL	114	3.00	11.00	6.10	1.67	97	2.70	11.30	6.25	1.75
UTUBW	114	1.30	5.00	2.99	0.79	97	2.00	5.00	3.56	0.61
UTUBH	114	1.00	4.70	2.46	1.08	97	1.30	4.30	2.99	0.68
LTUBL	114	3.70	19.30	10.71	4.04	97	2.50	13.30	6.19	1.92
LTUBW	114	1.00	6.30	3.16	1.28	97	1.70	5.70	3.35	0.68
LTUBH	114	1.00	4.00	2.08	0.93	97	1.00	3.70	2.09	0.59
NOAR	114	6.00	22.30	11.68	2.71	97	14.30	34.00	21.29	3.40
FRTSPNO	114	5.00	18.30	10.87	2.79	97	0.00	0.00	0.00	0.00
FRTSPL	114	7.00	22.00	14.62	3.22	0	n/a	n/a	n/a	n/a
		Cylindropunta whipplei* var. *enodis					***Cylindropunta multigeniculata***			
PLANT H	144	9.00	72.00	27.60	10.22	264	15.00	127.00	42.88	15.52
PLANT W	144	22.00	149.00	57.05	20.52	264	19.00	402.00	74.91	49.88
BRANG	144	18.33	88.33	57.34	15.37	264	8.33	80.00	49.21	14.05
INTL	144	1.00	11.00	3.80	1.75	264	0.50	5.67	1.91	0.92
WHORL	144	1.00	5.67	3.15	0.96	264	2.33	8.00	5.79	1.04
STEML	144	29.50	127.00	60.45	19.67	264	18.50	71.30	42.44	9.47
STEMDIA	144	9.70	25.70	14.07	3.32	264	10.60	28.70	17.12	3.46
TUBL	144	3.50	12.30	7.25	1.89	264	3.70	11.70	7.40	1.82
TUBW	144	2.70	7.30	4.11	0.70	264	2.30	6.60	3.75	0.82
TUBH	144	1.30	6.70	3.19	1.03	264	2.00	7.60	4.41	1.19
NOCSP	144	0.70	4.30	2.45	1.12	264	2.70	13.00	6.35	1.89
NORSP	144	0.30	5.30	3.23	0.80	264	2.00	11.30	6.80	1.63
CSPL	144	5.70	31.00	18.20	5.36	264	10.00	38.50	20.14	4.52
RSPL	144	1.00	9.00	3.89	1.54	264	5.00	14.70	8.65	1.83
CSPTH	144	0.27	0.67	0.45	0.08	264	0.16	0.52	0.29	0.06
SHTH	144	0.31	0.94	0.61	0.12	264	0.25	0.87	0.51	0.11
FRTL	144	12.70	36.30	18.99	3.83	264	7.30	28.00	16.33	4.09
FRTW	144	11.00	27.00	15.71	3.21	264	9.50	24.70	15.50	2.67
UTUBL	144	3.00	10.30	5.39	1.50	264	1.60	10.30	4.72	2.04
UTUBW	144	1.30	6.00	3.70	1.13	264	1.00	4.00	2.55	0.57
UTUBH	144	1.00	4.70	1.59	0.70	264	1.00	6.70	2.96	1.11
LTUBL	144	2.00	8.70	3.78	1.22	264	1.00	11.70	5.47	2.60
LTUBW	144	1.00	5.00	2.28	0.91	264	1.00	5.00	2.47	0.73
LTUBH	144	0.50	2.30	1.05	0.20	264	1.00	4.00	2.14	0.80
NOAR	144	12.30	30.70	20.53	3.55	264	12.00	31.70	19.24	3.68
FRTSPNO	144	0.00	2.70	0.05	0.29	264	0.00	15.70	3.47	4.26
FRTSPL	7	4.50	7.00	5.93	0.79	149	2.00	24.30	12.89	3.98

The recognition of *Cylindropuntia whipplei* var. *enodis* as a separate taxon from *C. whipplei* var. *whipplei* is based on morphological, ecological, and geographical factors that correlate with genetic isolation. Benson (1982) attributed the morphology of the fruit as a reaction of the fruits to parasitic insects. Although he may have observed parasitic insects associated with the fruits of *C. whipplei* var. *enodis*, common garden observations by the author have shown that parasitic insects have little, if any, effect on fruit morphology for individuals of either variety. Unfortunately, the thickness of the pericarpel wall was not included in our morphological analysis. The diagnostic importance of the character was not realized until after the morphological sites were sampled. Individuals of *C. whipplei* var. *whipplei* nearly always have a thin (< 5 mm) pericarpel wall at the middle of the fruit, while those of *C. whipplei* var. *enodis* nearly always have a thick (> 7 mm) pericarpel wall. In addition, the fruits of tetraploid individuals often form chains, with fruits emerging from the areoles of older fruits, and are sterile or contain few seeds, whereas the fruits of diploids rarely form chains and are generally fertile with many seeds. Based on morphology and geographic distribution, *C. whipplei* var. *enodis* most likely originated as an autotetraploid at the eastern end of their present-day distribution near the type locality of *C. hualpaensis* at Grand Canyon Caverns and evolved as genetic isolates from their diploid counterparts, invading new niches (Fowler and Levin 1984), and finally lower, drier regions to the west. The somewhat isolated occurrence of *C. whipplei* var. *enodis* on the Arizona strip north of the Grand Canyon probably resulted from its migration across the Grand Canyon rather than as an independent polyploid origin. This is evidenced by morphology and by the rarity of tetraploidy in *C. whipplei* and its nearest relatives.

The apparent evolutionary and geographical migration of *Cylindropuntia whipplei* and *C. multigeniculata*, as evidenced by morphological and cytogenetic analyses is indicative of a ring species (Irwin et al. 2001), a phenomenon that has only been recently documented in plants (Cacho & Baum 2012). However, in the present scenario, populations are morphologically distinct enough to place within two species. Typical *C. whipplei* occurs in the highlands from northern New Mexico, west into Mohave County, Arizona, as far north as central Utah and east-central Nevada. Spinier forms of *C. whipplei*, with some combination of a greater number of spines per stem areole, larger spines, and/or larger spine sheaths, occur sporadically from the Little Colorado Gorge, westward north of the Grand Canyon. The Hurricane Cliffs population of *C. whipplei* represents one of these spiny forms, and the DFA classified one individual from this population as *C. multigeniculata*. The Black Gate Canyon population, which is morphologically and geographically intermediate between *C. whipplei* and *C. multigeniculata*, occurs near the westernmost distribution of *C. whipplei* in the Sheep Range of Clark County, Nevada. Populations of *C. multigeniculata* with fruits nearly identical to those of *C. whipplei* occur immediately to the southwest of the Black Gate Canyon population at the southern end of the Las Vegas Range and to the southeast at La Madre Mountain. The average number of spines per fruit increases toward the south at Blue Diamond, then southeast to the McCullough Range, and to the east in Arizona. The spiniest forms are found in Arizona at the east end of the morphological cline and to the north across the Colorado River in Nevada at Bonelli Peak (Fig. 5). There is evidence that populations of *C. multigeniculata* occurred much farther south in the past. Van Devender (1987) reported finding spine clusters and seeds of *C. whipplei* dated 14,120yr BP from fossilized packrat middens in the Puerto Blanco Mountains of southwestern Arizona. However, upon further examination of the material, the number of spines per areole matched that for the average number of spines in *C. multigeniculata* (Baker pers. observ.) Van Devender also reported the habitat as one similar to that of present-day *C. multigeniculata* populations: a woodland dominated by *Juniperus californica* Carriere and *Yucca brevifolia* Engelm. with *Hesperoyucca torreyi* (Torr.) Baker ex Trel., *Monardella arizonica* Epling, *Opuntia chlorotica* Engelm. & J.M. Bigelow, *Salvia mohavensis* E. Greene, and *Yucca whipplei* Engelm.

The apparent western migration of *Cylindropuntia whipplei*, south of the deeper gorges of the Grand Canyon, begins as a morphological cline with the easternmost tetraploid populations near Grand Canyon Caverns and continues toward lower and drier regions. Populations of *C. whipplei* var. *enodis* and *C. multigeniculata* are sympatric at the southern end of the geographic ring, where individuals of the two species bear little morphological resemblance to one another.

Although our data strongly suggest that populations of *Cylindropuntia whipplei* var. *enodis* migrated west-

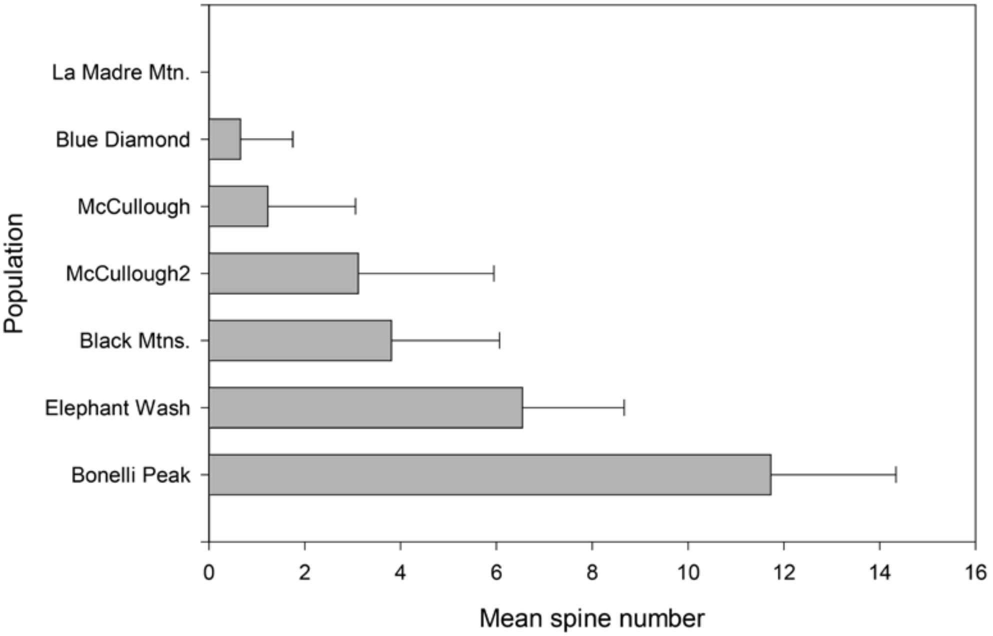

Fig. 5. Mean spine number per upper fruit areole in *Cylindropuntia multigeniculata*, by population.

ward from those of *C. whipplei* var. *whipplei*, the morphological analysis herein does not address whether *C. multigeniculata* evolved from an ancestor of *C. whipplei* or vice versa. Furthermore, the study herein only reflects a present day scenario, and greater insights with respect to evolutionary trends among *C. echinocarpa*, *C. multigeniculata*, and *C. whipplei* would be gained through rigorous DNA analyses.

KEY TO THE TAXA

The following key is intended for live material in the field and may not function for dried herbarium specimens, especially those that are poorly preserved. Because of overlapping character states, it is best to sample a number of individuals within a population.

1. Trunks with few or no old persistent stem segments, ultimate stem segments averaging 22mm in diameter, tubercle height averaging >5 mm, and central spines averaging 0.5 mm thick; most areoles of fruit with >10 permanent spines ____ **C. echinocarpa**
1. Trunks generally bearing several to numerous persistent stem segments, ultimate stem segments averaging <17 mm in diameter, tubercle height averaging <4 mm; fruit areoles without spines, occasionally with a few deciduous spines, or, if fruits spiny, then central spines of stems thin, averaging 0.3 mm.
    2. Central spines generally 6 or 7 and averaging 0.3 mm in thickness at mid-spine; radial spines ca. 7 ____ **C. multigeniculata** (Fig. 6)
    2. Central spines few in number, generally 2–4, averaging 0.4 mm in thickness, radial spines 5 or 6 in number.
        3. Fruits generally with 20 or more seeds, definitely tuberculate, generally bright yellow, rarely chaining; lateral walls of the pericarpel <5 mm thick, much thinner than the width of the seed cavity; mean values include: stems 67 mm long and 18.2 mm in diameter, tubercles 9.3 mm long and 4.7 mm high; central spines 4.2 in number; radial spine length 6 mm ____ **C. whipplei** var. **whipplei**
        3. Fruits generally sterile or with fewer than 10 seeds, smooth to obscurely tuberculate; green to dull yellow, often tinged purple-brown, often chaining; wall of the pericarpel >7 mm thick, approximately equal to the width of the seed cavity; mean values include: stems 57 mm long and 13.4 mm in diameter; tubercles 7.0 mm long and 3.1 mm high; central spines 2.5 in number; radial spine length 3.4 mm ____ **C. whipplei** var. **enodis**

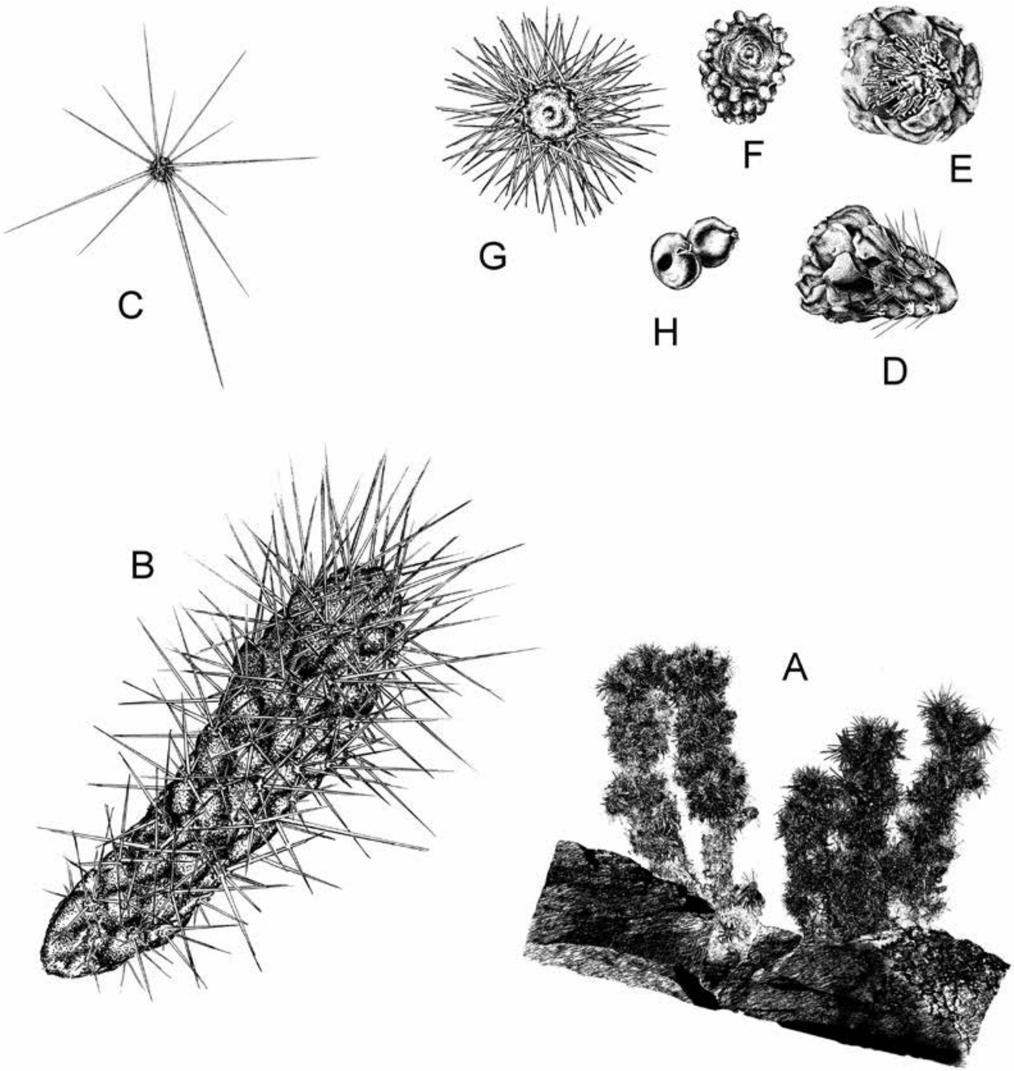

Fig. 6. ***Cylindropuntia multigeniculata*****. A.** **habit; B. stem; C. spine cluster; D. flower, side view; E. flower, face view; F. fruit of *C. multigeniculata* (type locality); G. fruit of *C. multigeniculata* (spiny-fruited); H. seeds. Drawn by Mara Trushell.**

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For over fifty years after its discovery, *Cylindropuntia multigeniculata* was thought to occur only at the type locality. Fortunately, additional populations were discovered by Gina Glenne of the U.S. Fish & Wildlife Service, Las Vegas, Nevada. Wesley Niles of the Wesley E. Niles Herbarium, University of Nevada, Las Vegas, discovered the population of *C. multigeniculata* near Bonelli Peak. Jody Fraser of the U.S. Fish & Wildlife Service, Reno, Nevada, aided in funding, editing, and fieldwork. James Rohlf, Department of Ecology and Evolution, State University of New York at Stony Brook and Robert Johnson, from Arizona State University, Tempe, Arizona aided in the statistical analysis. Mara Trushell of Northern Arizona University, Flagstaff, Arizona, produced the line drawing of *C. multigeniculata*. Michelle Cloud-Hughes repeatedly reviewed the manuscript and assisted with the fieldwork. Lucas Majure of the Desert Botanical Garden, Phoenix, Arizona also graciously

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