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SPECIES STATUS OF *SCLEROCACTUS BREVISPINUS*, *S. WETLANDICUS*, AND *S. GLAUCUS*: INFERENCES FROM MORPHOLOGY, CHLOROPLAST DNA SEQUENCES, AND AFLP MARKERS

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ABSTRACT

We examine patterns of variation in 12 continuous morphological traits, chloroplast DNA sequences from 10 intergenic spacer regions (*petA-psbJ*, *psbk-trnS*, *psbM-trnD*, *rpob-trnC*, *trnC-trnD*, *trn*GCU-*trn*G2S, *trn*FM-*trn*UGA, *atpF-atpH*, *trnT-trnD*, *trnQ-psbk*), *atp*F, and *rpl*16, and Amplified Fragment Length Polymorphism (AFLP) genetic markers in *Sclerocactus glaucus* sensu lato (=*S. brevispinus*, *S. glaucus*, and *S. wetlandicus*), a complex that historically has been considered conspecific and afforded protection under the Endangered Species Act. This complex is considered to represent three different species by some authors. We describe the expected patterns of morphological, DNA, and AFLP variation under the conditions that (a) the complex is a single species, and (b) that there are three antonymous species. We show that morphological evidence is consistent with the presence of three significantly different morphological species. Chloroplast DNA sequences provide evidence that the populations of *S. glaucus* (restricted to Colorado) are a lineage distinct from the populations of *S. brevispinus* and *S. wetlandicus* (restricted to Utah). AFLP genetic markers reveal significant genetic divergence among *S. brevispinus*, *S. glaucus*, and *S. wetlandicus*. Equally important, there is greater divergence among species than among populations within the species. The three sources of evidence all support the presence of three species and not a single species. These results indicate that protection of *S. glaucus* as a threatened species under the Endangered Species Act, as historically prescribed, includes populations of three species, two in Utah (*S. brevispinus* and *S. wetlandicus*) and one in Colorado (*S. glaucus*).

Key words: Cactaceae, conservation, molecular systematics, morphology, *Sclerocactus*, *Sclerocactus brevispinus*, *Sclerocactus glaucus*, *Sclerocactus wetlandicus*, species, systematics.

INTRODUCTION

Sclerocactus (Cactaceae: Cactoideae: Cacteae) has 22 species and eight additional heterotypic subspecies of the Colorado Plateau, Great Basin, Mojave Desert, Sonoran Desert, and Chihuahuan Desert (Porter and Prince 2011). Described early in the 20th century (Britton and Rose 1922), it originally included only *S. polyancistrus* (Engelm.) Brit. & Rose and *S. whipplei* (Engelm. & Bigelow) Brit. & Rose. Since that initial circumscription, many taxa have been described and assigned to *Sclerocactus* (Clover and Jotter 1941; Benson 1966, 1982; Woodruff and Benson 1976; Castetter et al. 1976; Heil 1979; Hochstätter 1989, 1990, 1992, 1993, 1995, 1996*a*,*b*, 1997), and recently the genera *Ancistrocactus* Brit. & Rose and *Echinomastus* Brit. & Rose were included (e.g., Barthlot and Hunt 1993; Hunt 1999, 2006). Although there is a growing consensus concerning generic circumscription, the merits of some of these species designations have been questioned (e.g., Welsh et al. 1987). One of the more controversial and confused species groups includes *S. brevispinus* Heil & J.M.Porter, *S. glaucus* (K.Schum.) L.D.Benson, and *S. wetlandicus* Hochstätter.

Near Myton, Utah, is found *S. brevispinus* (Fig. 1), possessing the most extreme morphological form of the three taxa. *Sclerocactus brevispinus* is depressed-globose, either lacking all central spines or if present they are solitary, very short (ca. 0.5–5.0 mm) and hooked, and have small, broad, pale pink to purple flowers (Heil and Porter 1994, 2003; see also Hochstätter 1990, 1992, 1993). It has been purported by Welsh (1987) that these individuals were the consequence of phenotypic plasticity, long remain in a juvenile stage, and providing the suggestion that these populations represent a pedomorphic form (a developmental mutation in which sexual maturity, in this case flowering, occurs when plants appear morphologically similar to juvenile individuals).

Sclerocactus glaucus was described from plants collected by C. A. Purpus on adobe clay hills in Delta County, Colorado, in 1892 (Fig. 2). The original description was very brief, only describing the flowers as pink; however, the name has consistently been applied to the *Sclerocactus* growing at the foot of Grand Mesa above the Gunnison River. These plants are moderately sized and have globose to sub-cylindrical stems (3–28 cm) with 1–4 straight or hooked central spines and narrow, red–purple flowers. This species has been particularly controversial both nomenclaturally and taxonomically (see below).

The third species in this group is *S. wetlandicus* (Fig. 3). This species has stems that are globose to cylindrical (3–15 cm) bearing 3–5 straight, unhooked or curved central spines. It was distinguished from *S. glaucus* based upon seed coat features. The testa of *S. wetlandicus* has cells that are clearly flattened, whereas those of *S. glaucus* are rounded and often referred to as papillate (Hochstätter 1989). In addition, *S. wetlandicus* is geographically isolated from *S. glaucus*, being restricted to the Uintah Basin of Utah, along the Green, White, and Strawberry rivers.

Historically, the distribution of *S. glaucus* was considered to incorporate two disjunct areas: (1) the Colorado and Gunnison

Fig. 1. *Sclerocactus brevispinus* Heil & J.M.Porter, growing at the Gilsonite Watertap (GW) population site, Duchesne County, Utah. The scale bar represents 1.0 cm.

River valleys of west-central Colorado and (2) Uintah Basin of northeastern Utah, on the Colorado Plateau (Atwood and Reveal 1975; Colorado Native Plant Society 1989; Weber 1987; Welsh et al. 1987, 1993). That is, all three species were treated as a single taxon. In fact, some treatments (e.g., Welsh et al. 1987) considered the entire collective to be unworthy of taxonomic recognition, treating them as conspecific with *S. whipplei*.

The segregation of *S. brevispinus* and *S. wetlandicus* from *S. glaucus* has found support from comparative *trnL-trnF* DNA sequencing (Porter et al. 2000). That study found *S.*

Fig. 2–3. *Sclerocactus glaucus* (Schum.) L.Benson (Fig. 2a,b) and *S. wetlandicus* Hochstätter (Fig. 3a,b).—2. *Sclerocactus glaucus* growing at the Reeder Mesa (RM) population site, Grand County, Colorado.—3. *Sclerocactus wetlandicus* growing at the Bonanza Power Plant (BPP) population site, Uintah County, Utah. Scale bars represent 1.0 cm.

glaucus to share more recent common ancestry with *S. whipplei* and *S. wrightiae* L.D.Benson than with *S. brevispinus* or *S. wetlandicus* that were sister taxa. At the same time it is important to recognize that a morphological cline has been suggested to exist along Pariette Wash, from the Myton populations of *S. brevispinus* to the type locality of *S. wetlandicus*. Across this cline, morphology has been suggested to shift from the typical *S. brevispinus* morphology to typical *S. wetlandicus* morphology. Whether this purported clinal variation represents secondary contact and hybridization between two formerly isolated species, or primary contact of a peripheral, diverging portion of a single species, is not known.

A comparative study of quantitative morphology in *Sclerocactus* (which did not include the seed traits discussed by Hochstätter 1989) revealed complex patterns of morphological similarity (Heil and Porter 1987). Although nearly all sampled populations showed some differences from one another, no significant differences in stem, spine, and floral features of *S. glaucus* and *S. wetlandicus* were found (Heil and Porter 1987). At the same time, there were significant differences in these same traits between *S. brevispinus* and both *S. glaucus* and *S. wetlandicus*. This evidence was used to support species status of *S. brevispinus* (Heil and Porter 1994).

Currently, those who work with the genus are left with a variety of alternative treatments provided by contemporary systematists. Heil and Porter (1994, 2003) believe that *S. glaucus* s.l. represents three different species. They suggest that *S. glaucus* s.s. is restricted to Colorado. In the Uintah Basin of Utah are two species: *S. brevispinus* and *S. wetlandicus* (Heil and Porter 2003). Hochstätter (1989, 1993) recognizes two species, *S. glaucus* (of Colorado) and *S. wetlandicus* (of Utah). The taxon that Heil and Porter treat as *S. brevispinus* is considered by Hochstätter to represent a different subspecies of *S. wetlandicus*, i.e., *S. wetlandicus* subsp. *ilseae* Hochstätter (Hochstätter 1995). Welsh et al. (2003) provide another alternative treatment in which there are two taxa, but both are varieties of *S. whipplei*. One taxon is *S. whipplei* var. *ilseae* (Hochstätter) S.Welsh, which corresponds to *S. brevispinus* and/or *S. wetlandicus* subsp. *ilseae*. The other is *S. whipplei* var. *glaucus* (K.Schum.) S.Welsh, which corresponds to *S. glaucus* and *S. wetlandicus* (subsp. *wetlandicus* sensu Hochstätter 1993). This conflict in species boundaries presents a further problem, given that these taxa all have protection under the Endangered Species Act.

Study Goals

During this recent period of taxonomic re-evaluation and change, the United States Fish and Wildlife Service (USFWS) has been charged with the protection and recovery of *S. glaucus*, a species protected under the Endangered Species Act as a threatened species (USFWS 1979, 1985). All of the recently named species, i.e., *S. brevispinus* and *S. wetlandicus*, have until recently been treated under the rubric of "*S. glaucus*," as has been the tradition of Utah botanists (Atwood and Reveal 1975; Welsh et al. 1987, 1993). This has afforded federal protection to all three named taxa, without the need of petitioning for federal listing of *S. brevispinus* and/or *S. wetlandicus*. The difficulty with this approach is that the numbers of populations of the three species combined may be high enough to question the need for protection; or, mitigations may impact one taxon more severely. In 2007 and 2009 (USFWS 2007, 2009*a*,*b*), *S. brevispinus* and *S. wetlandicus* were designated threatened species. However, the difference in opinion concerning taxonomy has left open to challenge the very existence of some of the taxa. Sound conservation and species management requires sound taxonomy.

It is frequently argued that taxonomy is largely the opinion of those who practice the naming of species. Both scientists and nonscientists alike have often suggested that whether a species is carved away from another (splitting) or two species are agglomerated together (lumping) is more an art than a science, being prone to subjectivity. However, speciation events result in characteristic patterns among populations, providing testable expectations for species. Species are evolutionarily independent, cohesive groups of populations, which are genetically differentiated from one another. Given this, we would expect that: (1) different species would be significantly different genetically and minimally possess diagnostic differences in allele frequencies, and (2) as a consequence we would usually observe significant differences in morphology, physiology, and/or reproductive features. Such properties of species can be tested (and potentially falsified) using comparative, population genetic, and phylogenetic methodologies.

The purpose of this study is to examine the morphology, phylogenetic relationships, and patterns of genetic variation within and among populations of *S. brevispinus*, *S. glaucus*, and *S. wetlandicus*. If these three taxa represent a single, undifferentiated species, then we expect that chloroplast gene phylogenies will display all samples coalescing together, without respect to taxon names, rather than forming three different clades. If, on the other hand, they represent different species and have been reproductively isolated for a sufficiently long period of time, then we would expect populations of *S. glaucus* to coalesce (form a clade), those of *S. brevispinus* to coalesce, and those of *S. wetlandicus* to coalesce. We would further expect to find fixed mutations, unique to each of the species, provided sufficient time has occurred since speciation for mutations to become fixed in all populations. Similarly, if these three taxa represent a single, undifferentiated species, then we expect genetic variation to be uncorrelated with species assignment and be highly similar across all of the populations. If they represent different species, then we would expect genetic variation to be highly correlated with species assignment. In addition, we expect genetic divergence among the species. Here, we test these expectations.

METHODS

Floral buds from *S. glaucus* s.l. (including *S. brevispinus*, *S. glaucus*, and *S. wetlandicus*) were collected from eight wild populations located in Utah and Colorado (Table 1, Fig. 4). At the time of collection, latitude and longitude were recorded and a color digital photograph was made of each sampled plant. Floral tissues were dried in silica gel. Samples were stored in a -20°C freezer at Rancho Santa Ana Botanic Garden until DNA extraction.

Floral buds of *S. brevispinus* were collected from two small populations (N = 16–60 individuals; NF and GW, see Fig. 4) within oil fields near the Pariette Wash, southwest of Myton, Utah. Substrate of these sites was gravel pediment with sparse vegetation that included *Linanthus pungens* (Torr.) J.M.Porter & L.A.Johnson, *Oenothera caespitosa* Gilles ex Hook. & Arn., *Astragalus flavus* Nutt. ex Torr. & Gray, and *Aliciella triodon* Brand. Two individuals of *Sclerocactus wetlandicus* co-occurred with *S. brevispinus* at the GW site. Samples of *S. wetlandicus* were collected from three large populations ($N >$ 200 individuals) in Utah. The first population (PW) was located at the type location for *S. wetlandicus* on the slopes above Pariette Wetland, southwest of Myton, Utah. The second *S. wetlandicus* population (GR) was on an oil shale bench on the west bank of the Green River. The third population (BPP) of *S. wetlandicus* was outside Bonanza, Utah, southwest of the power plant. Samples of *S. glaucus*

Table 1. Location information for *Sclerocactus* spp. sample collections. Sample numbers beginning with SB represent *S. brevispinus*, SW prefixes represent *S. wetlandicus*, and SG prefixes represent *S. glaucus*.

Code	Location	Latitude/longitude	Elevation	Sample numbers
NF (16)	New Field Site, Myton, UT	40°06′N, 109°57′W	1518 m	SB001–SB016
GW (35)	Gilsonite Watertap, UT	40°04′N, 110°01′W	1518 m	SB017–SB052
PW (43)	Pariette Wetland, UT	40°01′N, 109°46′W	1450 m	SW003–SW046
GR (45)	Green River, UT	39°51′N, 109°54′W	1442 m	SW047–SW092
BPP (48)	Bonanza Power Plant, UT	40°05′N, 109°17′W	1550 m	SW093–SW141
GP (48)	Gravel pit near Grand Junction, CO	38°45′N, 108°15′W	1490 m	SG001–SG049
RM (50)	Reeder Mesa, CO	38°57′N, 108°21′W	1543 m	SG050–SG100
PR (49)	Pyramid Rock, CO	39°18′N, 108°16′W	1560 m	SG151–SG200

were collected from three large populations ($N > 600$ plants) in western Colorado. The first population (GP) was above a gravel pit along Gunnison River in the Escalante Canyon east of Grand Junction, Colorado. The second population (RM) was adjacent to a roadside and power line cut at Reeder Mesa. The third population (PR) grew on white sandy soil with a pediment of black volcanic rock at Pyramid Rock on the slopes above the Colorado River.

DNA was extracted from both ovary walls and perianth using a modified CTAB protocol. Extractions included three washes, the first with CTAB and then Nucleon PhytoPure Resin (Tepnel Life Sciences plc for Amersham Biosciences, Little Chalfont, UK) and chloroform, the second with CTAB and 1% w/v caylase (Cayla-InvivoGen, Toulouse, France) and then chloroform, and the third with 75% ethanol. DNAs from five individuals were used to screen 12 chloroplast markers for

Fig. 4. Map generated from Google Earth (Google Earth 2011), illustrating populations sampled for morphological, chloroplast DNA, and AFLP analyses. Red triangles denote populations sampled of *Sclerocactus brevispinus*; green circles represent populations sampled of *S. glaucus*; and blue squares identify sites of *S. wetlandicus*. Each site is denoted using the population codes described in Table 1.

genetic variation. The five individuals were: one sample of *S. brevispinus* from Pariette Wash, one sample of *S. wetlandicus* from GR, and three samples of *S. glaucus* representing populations from GP, RM, and PR. The 12 rapidly evolving chloroplast DNA regions surveyed for variation included 10 intergenic spacer regions [IGSRs] (*petA-psbJ*, *psbk-trnS*, *psbM-trnD*, *rpob-trnC*, *trnC-trnD*, *trn*GCU-*trn*G2S, *trn*FM-*trn*UGA, *atpF-atpH*, *trnT-trnD*, and *trnQ-psbk*), as well as *atp*F, and *rpl*16.

The chloroplast regions of 25 samples were amplified using the polymerase chain reaction (PCR) on a PTC-100 Thermal Controller (MJ Research, Inc., Watertown, MA): 94°C for 4 min, then 35 cycles of 94°C for 45 s, 56°C for 45 s, and 72°C for 2 min-30 s, concluding with 94°C for 45 s and 72°C for 5 min. A negative control excluding DNA was used in each set of reaction to detect contamination or false positives. The samples included four individuals from Pariette Wash, three individuals from GW, two from PW, four from BPP, four from GP, four from RM, and four from NF. The PCR reactions were cleaned using PEG precipitation, then subjected to the following Big-Dye™ (Applied Biosystems/Life Technologies, Foster City, CA) cycle sequence program for 35 cycles: 96°C for 30 s, 48°C for 15 s, 60°C for 4 min. The cycle sequencing product was placed on a 96-well plate and sequenced in a 3130xl sequencer (Applied Biosystems/Life Technologies). Sequences were aligned by eye in Se-Al vers. 2.0a11 (*http://tree.bio.ed.ac.uk/*).

Four samples representing each population of *Sclerocactus* were used to screen Amplified Fragment Length Polymorphism (AFLP) primers for population genetic analysis. Each DNA sample was subjected to restriction digestion using the AFLP Core Reagent Kit, Invitrogen, and *Eco*R1/*Mse*1 endonucleases. The restriction digestion was accomplished using the PTC-100 Thermal Controller following the suggested incubation period of 2 hrs at 37°C. Restriction digestion was inactivated by subjecting the mixture to 15 min at 70°C. Ligation of the adapters was accomplished using the restriction digest mixture subjected to 20°C for 2 hrs using the PTC-100 Thermal Controller. A 1:10 dilution of the ligation mixture was made and then subjected to a pre-amplification run using the PTC-100 Thermal Controller with the following reaction with 20 cycles: 94°C for 30 s, 56°C for 60 s, 72°C for 60 s. A 1:50 dilution of the pre-amplification mixture was made for the final selective AFLP amplification. The diluted mixture was then paired with a fluorescently labeled *Eco*R1-AAC primer and either *Mse*1-CAC or *Mse*1-CAG, in separate reaction. The AFLP amplification products were then run out on an ABI 3130xl sequencer and analyzed using GeneMapper software (Applied Biosystems/Life Technologies). Three replicons of each of the above reactions were completed and compared to ensure the alleles were present in each replicon, and that the results were reproducible. A 600 bp-standard (DG2611; Promega, Madison, WI) was used along with the AFLP amplifications for the sequencer run. Only fragments between 60 bp and 600 bp long were called as peaks by the GeneMapper software. The cutoff for allele calls was set at a peak height of 100. Sixteen individuals from each of the eight populations were surveyed using both *Mse*1-CAC and *Mse*1-CAG.

Allelic variation was analyzed using analysis of molecular variance (AMOVA), as implemented in GeneticStudio 2.0.1 (Dyer 2009) for Mac OS X. Allele frequencies were calculated using GeneticStudio 2.0.1. Frequencies were arcsine transformed and analyzed using multivariate analysis of variance (MANOVA), principal component analysis (PCA), and discriminant function analysis (DFA), as implemented in SPSS 11.0.2 (SPSS Inc., 2003, *http://www-01.ibm.com/software/analytics/spss/*) for Mac OS X.

Allelic variation was also investigated to estimate the likely number of ancestral populations giving rise to the standing genetic variation, using Bayesian model-based clustering for multilocus genotype data in Structure vers. 2.3.2 (Pritchard et al. 2000; Falush et al. 2003, 2007; Hubisz et al. 2009). Data were analyzed both as diploid recessive data and as haploid recessive data to contrast the results, given that the AFLP markers may represent both plastid and nuclear markers. Populations were analyzed with both naïve and population-informed clustering, $k = 1$–10, with 50,000 generations burn-in and posterior sampling of 50,000 generations, running 10 replicates of all analyses.

Twelve morphological characters (Table 2) were measured from 35 individuals at populations NF, PW, BBP, RM, and PR. Only continuous measurements (i.e., no meristic or qualitative traits) were used in analyses. Measurements were analyzed by PCA, DFA, and MANOVA using SPSS 11.0.2.

RESULTS

PCA analysis of 12 continuous morphological features finds six factors, each of which explain a significant proportion of variance (Table 2). The greatest variance proportion is associated with a factor that is characterized by a high correlation among stem length, stem width, spine length, and flower length (e.g., Factor 1, Table 2). However, this factor does not strongly aid in discriminating the three taxa. MANOVA of factor loadings demonstrates that there is a significant difference in the factors among the species (Table 3). Most of the variance associated with species differences is attributed to Factors 2 and 3. These differences are evident in Fig. 5, which shows the morphological isolation of *S. brevispinus*, based on Factors 2 and 3. Table 4 provides the results of a Bonferroni analysis of the factors used in the MANOVA. The Bonferroni analysis reveals which species are significantly different based on particular factors. For example, *S. brevispinus* and *S. glaucus* are significantly different only in Factor 2 (Table 4).

The stepwise DFA of *S. brevispinus*, *S. glaucus*, and *S. wetlandicus* required the addition of only seven continuous characters to discriminate among the three species (Table 5, Fig. 6). Even so, the separation among the three species is similar to the PCA, but with greater separation of *S. wetlandicus* and *S. glaucus*.

Of the 12 chloroplast regions examined, only one of the markers showed genetic variation. This was the *petA-psbJ* IGSR (Appendix 1). This region ranges between 570 and 598 nucleotides and includes a 29-base pair long indel (insertion-deletion feature). All of the individual samples of *S. glaucus* possess this 29-base pair segment of DNA; but in both *S. brevispinus* and *S. wetlandicus* it is absent. This indel feature was included in the phylogenetic analysis of *petA-psbJ* by adding a single binary character at the end of the DNA sequence matrix (see Appendix 1).

Parsimony analysis of the *petA-psbJ* region resulted in a single most-parsimonious tree (Fig. 7) of five steps, CI =

Table 2. Factor loadings from the orthogonal principal component analysis of 12 morphological characters measured for the samples of *Sclerocactus brevispinus*, *S. glaucus*, and *S. wetlandicus*. The bold values call attention to the traits that primarily contribute to each of the factors. Eigenvalues (γ) and proportion of variance (σ^2 Prop) contributed by each of the factors is provided for each factor. Morphological characters surveyed (measured in mm): stem length (stemL), stem diameter at 1/2 length (stemW), lower ("hooked") central spine length (cspineL), flower length (flrL), flower diameter at anthesis (flrdia), outer perianth lobe length (sepL), outer perianth lobe width (sepW), inner perianth lobe length (petL), fruit length (frtL), fruit diameter at 1/2 length (frtW), seed long axis length (seedL), and seed short axis length (seedW).

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
stemL	**0.939**	0.049	0.120	0.033	−0.039	0.040
stemW	**0.929**	0.144	0.001	−0.024	0.022	0.046
cspineL	**0.823**	−0.195	−0.072	0.191	0.071	0.078
seedL	0.020	**0.882**	0.046	−0.136	0.166	−0.121
seedW	0.002	**0.840**	0.047	0.196	−0.193	−0.092
flrL	**0.377**	−0.438	0.130	**0.601**	**0.206**	−0.017
flrdia	0.092	**0.504**	−0.161	**0.634**	0.035	0.024
sepL	0.001	0.037	0.397	**0.670**	−0.069	−0.078
sepW	0.112	−0.141	0.063	0.018	0.032	**0.957**
petL	0.032	−0.014	−0.057	0.026	**0.980**	0.031
frtL	0.029	0.017	**0.859**	**0.259**	−0.084	−0.035
frtW	0.025	0.023	**0.922**	−0.029	0.019	0.103
γ	2.890	2.108	1.892	1.127	0.960	0.934
σ^2 Prop	0.241	0.176	0.158	0.094	0.080	0.078

1.000, RI = 1.000. The consistency index (CI) and retention index (RI) indicate that there is no homoplasy in this data set. The tree unambiguously separates all *S. glaucus* samples from those of *S. brevispinus* and *S. wetlandicus*. This region does not differentiate *S. brevispinus* from *S. wetlandicus*; however, two *S. brevispinus* individuals from population GW share a unique mutation.

Five of the other markers (*psbk-trnS* IGSR, *atp*F, *psbM-trnD* IGSR, *rpob-trnC* IGSR, and *trnC-trnD* IGSR) were sequenced but displayed no genetic variation among the sampled individuals. Further, sequencing was attempted using the final six markers (*rpl*16, *trn*GCU-*trn*G2S IGSR, *trn*FM-*trn*UGA, *atpF-atpH* IGSR, *trnT-trnD* IGSR, and *trnQ-psbk* IGSR), but these regions could not be completely sequenced due to numerous poly-A/poly-T regions, producing *taq*-polymerase stutter. Completing sequencing of these regions would have required extensive primer design, manufacture, and trouble-shooting that were beyond the parameters of this study.

Three replicons (replicate runs) of fluorescently labeled *Eco*R1-AAC/*Mse*1-CAC primers and fluorescently labeled *Eco*R1-AAC/*Mse*1-CAG primers were completed and compared for the 10 individuals from each of the sampled populations (Table 1). The replication ensures that the alleles compared were consistently present, and the results are reproducible. We found 167 alleles. Analysis of molecular variance (AMOVA) of 167 AFLP markers, sampled from populations of *S. brevispinus*, *S. glaucus*, and *S. wetlandicus*, reveals that there is a significant degree of genetic divergence among the three species (Tables 5, 6). Although the greatest genetic diversity lies within species, there is greater divergence among species than among populations of the same species (Table 7).

The estimation of the number of populations using Structure vers. 2.3.2 produced different inferences depending upon the

Table 3. Multivariate Analysis of Variance (MANOVA) contrasting the principal component factor loadings of *Sclerocactus brevispinus*, *S. glaucus*, and *S. wetlandicus*. We provide a test and significance estimate of the model and a significance estimate for each of the dependent variables (factors) in species contrasts, including type III sums of squares (Type III SS), mean squares (MS), *f*-statistic values (*F*), and significance probability (*P*).

Effect[a]	Wilks' lambda value	*F*[b]	H_0 df	Error df	*P*
Intercept	0.557	14.073	6.000	106.000	0.000
Species	0.108	36.176	12.000	212.000	0.000

Source	Dependent variable	Type III SS	df	MS	*F*	*P*
Species	Factor 1	6.251[c]	2	3.126	3.250	0.042
	Factor 2	66.376[d]	2	33.188	79.013	0.000
	Factor 3	45.920[e]	2	22.960	37.993	0.000
	Factor 4	6.578[f]	2	3.289	3.430	0.036
	Factor 5	1.341	2	0.671	0.667	0.515
	Factor 6	24.456	2	12.228	15.329	0.000

[a] Design: Intercept + species; [b] Exact statistic; [c] R-squared = 0.055 (adjusted R-squared = 0.038); [d] R-squared = 0.587 (adjusted R-squared = 0.580); [e] R-squared = 0.406 (adjusted R-squared = 0.396); [f] R-squared = 0.058 (adjusted R-squared = 0.041)

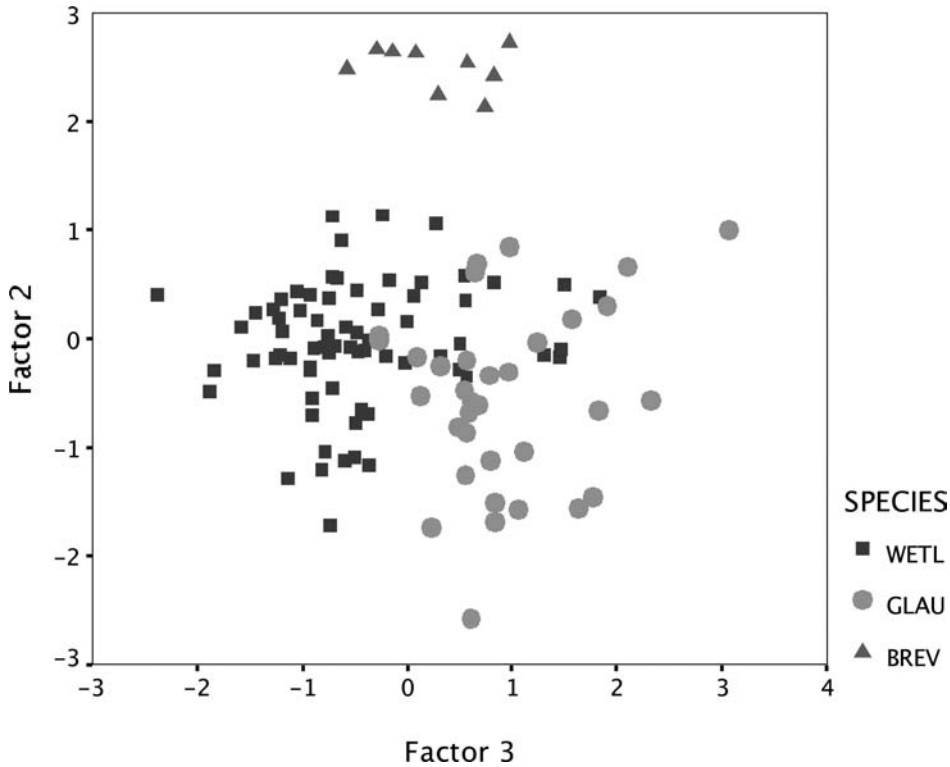

Fig. 5. Bivariate plot from principal component analysis Factors 2 and 3, based on 12 continuous morphological traits measured from *Sclerocactus brevispinus* (BREV), *S. glaucus* (GLAU), and *S. wetlandicus* (WETL).

assumptions associated with analyses. General patterns found in all estimations are: (1) most members of populations of *Sclerocactus brevispinus* cluster together in naïve clustering and most members of *S. wetlandicus* cluster together in a different cluster; (2) admixture is present in *Sclerocactus brevispinus*, involving *S. glaucus* and to a lesser extent *S. wetlandicus*. Admixture is also present in populations of *S. wetlandicus*, involving *S. glaucus*; however, the population at Bonanza, Utah, shows significant admixture involving *S. brevispinus*. The naïve estimation, assuming diploid populations, has a maximum likelihood at $k = 6$ (Fig. 8A) with a mean log-likelihood of −2940.55, averaged over 10 Markov Chain Monte Carlo (MCMC) runs. The mean log-likelihood value at $k = 6$ is significantly higher than other values of K; however, the likelihood values begin to plateau at $k = 4$ (lnL = −3018.2). The estimated number of diploid ancestral populations, informed by the hypothesized three-species membership similarly maximizes at $k = 6$ (Fig. 8B), with a mean log-likelihood of −3949.9, averaged over 10 MCMC runs. As was the case for the naïve clustering, the likelihood began to plateau at $k = 4$ (lnL = −3988.9), and the actual number of sampled populations, $k = 8$ (lnL = −3982.3), is not significantly different from $k = 6$. Fixation indices (ϕ_{ST}) for the three taxa based on Bayesian inference are relatively high (Table 8), leading to the deduction that the three species are reproductively isolated from one another.

Table 4. Bonferroni analysis of factor loadings, derived from continuous morphological characters, used in the Multivariate Analysis of Variance (MANOVA) of *Sclerocactus brevispinus* (brevi), *S. glaucus* (glau), and *S. wetlandicus* (wetl). The significance level (*) has been set a priori at $\alpha = 0.050$. Only the factors that demonstrate significant differences among species in the MANOVA are included.

	brevi vs. glau	brevi vs. wetl	glau vs. wetl
Factor 1	0.055	0.393	0.266
Factor 2	0.000*	0.000*	0.002*
Factor 3	0.083	0.022	0.000*
Factor 4	1.000	1.000	0.033
Factor 6	1.000	0.001*	0.000*

DISCUSSION

We have examined patterns of morphological variation, divergence in chloroplast sequences, and patterns of genetic variation within and among populations of *S. brevispinus*, *S. glaucus* and *S. wetlandicus*. The null hypothesis, i.e., these three

Table 5. Standardized canonical discriminant function coefficients, with percent of variance accounted for, canonical correlations and Eigenvalues for each function, from the discriminant function analysis of morphology of *Sclerocactus brevispinus*, *S. glaucus*, and *S. wetlandicus*.

	Function 1	Function 2
cspineL	0.240	−0.503
seedL	−0.526	0.435
seedW	0.136	0.403
flrdia	0.222	0.482
sepL	0.353	0.247
petL	−0.711	−0.421
frtL	0.454	−0.012
% of variance	63.5	36.5
Canonical correlation	0.857	0.783
Eigenvalue	2.760	1.586

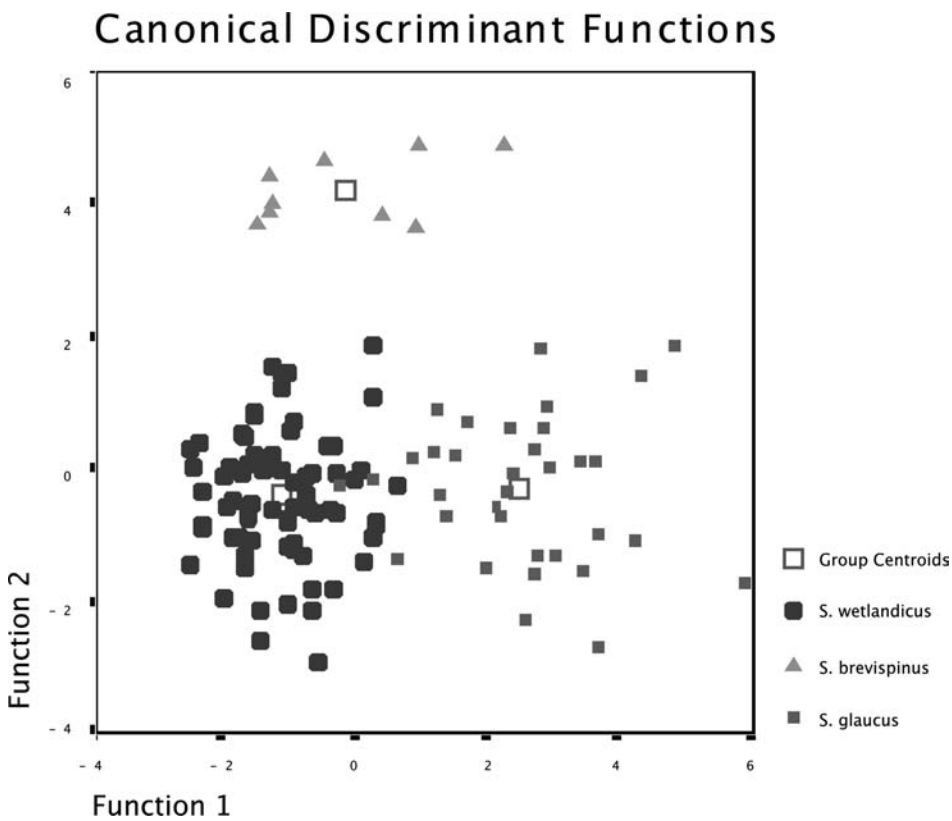

Fig. 6. Bivariate plot from discriminant function analysis Functions 1 and 2, based on 12 continuous morphological traits measured from *Sclerocactus brevispinus*, *S. glaucus*, and *S. wetlandicus*.

taxa represent a single, undifferentiated species, leads to three expectations: (1) the three named taxa should be morphologically cohesive, or represent a continuum of morphological variation; (2) chloroplast gene phylogenies should show that all samples coalesce together, without respect to taxon naming, or show a branching pattern independent of taxon naming; (3) genetic variation should be uncorrelated with species assignment and be highly similar across all of the populations or, at least, there should be greater divergence among populations of the same taxon than among the assigned species. By contrast, our alternative hypothesis, i.e., *S. brevispinus*, *S. glaucus*, and *S. wetlandicus* represent differentiated species, leads to three contrary expectations: (1) the three named taxa should be morphologically distinct and thus can be discriminated on the basis of morphological variation; (2) chloroplast gene phylogenies should show populations of *S. glaucus* coalescing, *S. brevispinus* coalescing, and *S. wetlandicus* coalescing, or show a branching pattern that is in some way consistent with taxon naming; (3) genetic variation should be correlated with species assignment, and species should show significant genetic divergence, i.e., there should be greater divergence among species than among populations of the same species. If the alternative hypotheses—and thus the three expectations—are true, then by any criterion used for recognizing species (morphological, phylogenetic, genetic isolation), *S. brevispinus*, *S. glaucus*, and *S. wetlandicus* would be considered different species.

The different markers used in this study possess the potential to provide different information or aspects of information concerning the species status of our three study taxa. Markers such as chloroplast DNA sequences are known to evolve slowly, providing information about more ancient events, but may provide little or no information concerning more recent speciation events, because often there is insufficient or no variation in the DNA sequences. By contrast, rapidly evolving molecular markers such as allozymes, microsatellites, or AFLPs are variable enough to provide information about populations and closely related species, but are often too variable to be useful for understanding relationships beyond closely related species. Morphological data can represent a powerful inference tool for discrimination of taxa; however, failure to discriminate taxa may not necessarily reflect that taxa cannot be discriminated: any morphological analysis is limited by the morphological traits included in the analysis. If the set of included traits fails to separate taxa it may be either because the two taxa do not differ in the particular traits, or that the two taxa are in fact morphologically identical. Even given this reality, our data provide a very consistent picture of phylogenetic, morphological, and genetic relatedness. These patterns are consistent with our alternative hypothesis, that our sample represents three species.

Morphological data (Fig. 5, 6) provide evidence that *S. brevispinus*, *S. glaucus*, and *S. wetlandicus* are morphologically different ($F = 15.771$, $P = 0.000$) and distinct from one another. In fact, *S. brevispinus* is the most distinctive of the three, significantly differing from *S. glaucus* and *S. wetlandicus* in five of the 12 traits examined: central spine length, flower length, flower diameter, seed length, and seed width. Although

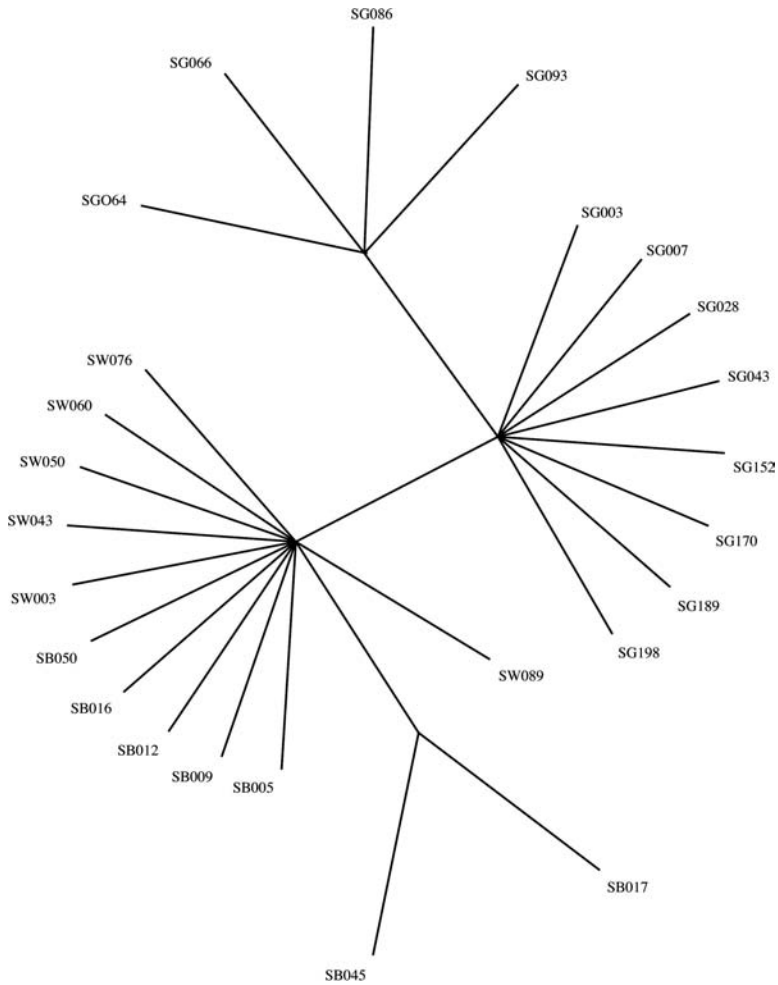

Fig. 7. Unrooted tree depicting mutational differences among *Sclerocactus brevispinus* (SB), *S. glaucus* (SG), and *S. wetlandicus* (SW), based on parsimony analysis of the *petA-psbJ* intergenic spacer region. In this diagram the length of the branches does not have meaning.

there has been a longstanding debate concerning the recognition of *S. brevispinus* as a species, it is one of the most distinctive taxa in the genus in terms of morphology. In addition, we find significant differences between *S. glaucus* and *S. wetlandicus* that are morphologically very similar. *Sclerocactus glaucus* and *S. wetlandicus* differ from one another in flower length, outer perianth segment length, inner perianth length, fruit length, seed length, and seed width. The patterns of morphological variation are consistent with the hypothesis that the three are different species.

Since the chloroplast genome is maternally inherited and nonrecombining, sequence data can be compared and interpreted to assess the phylogenetic and phylogeographic relationships among *S. glaucus*, *S. wetlandicus*, and *S. brevispinus*. These data reveal a 29-base difference in length between *S. glaucus* that has the 29-base span and *S. brevispinus* and *S. wetlandicus* that lack it. Similarly, mean evolutionary distances, based upon Tamura and Nei (1993) distance of chloroplast DNA sequences, are greatest in comparisons involving *S. glaucus*, i.e., *S. glaucus*–*S. wetlandicus* = 0.00266; *S. glaucus*–*S. brevispinus* = 0.00318. This is considerably larger than the mean evolutionary distance between *S. brevispinus* and *S. wetlandicus* of 0.00051 (see also Fig. 7). The chloroplast DNA sequences unequivocally support the evolutionary separation of the Colorado populations of *S. glaucus* from the Utah populations of *S. brevispinus* and *S. wetlandicus*. The *S. glaucus* lineage has been reproductively isolated for a sufficiently long period of time that length differences and point mutations could evolve and become fixed in all of the sampled individuals, but remain absent from *S. brevispinus* and *S. wetlandicus*. While this is consistent with species status for *S. glaucus*, the sequence data lack sufficient variation to make any inference concerning species status of *S. brevispinus* and *S. wetlandicus*. The chloroplast *petA-psbJ* IGSR data (Fig. 7) provide nearly identical inference as does the *trnL-trnF* region (Porter et al. 2000). These new data differ in that two members of *S. brevispinus* (from population GW) share a unique chloroplast type, derived from the common type in other *S. brevispinus* and *S. wetlandicus*. In addition, different chloroplast variants are found in different populations of *S. glaucus*, suggesting population differentiation in that species.

Our examination of genetic variation using AFLP markers reveals that there is significant genetic divergence among population samples of *S. glaucus*, *S. wetlandicus*, and *S. brevispinus* (Table 6, 7), based on direct measures. Unlike the chloroplast DNA sequence data, AFLP markers show significant ($P = 0.010$) evolutionary divergence ($\phi_{GT} =$

Table 6. Analysis of molecular variance (AMOVA) of 167 AFLP markers, sampled from populations of *Sclerocactus brevispinus*, *S. glaucus*, and *S. wetlandicus*.

Source	df	SS	MS	σ^2 Prop
Among species	2	111.6250	55.8125	0.1640
Among populations within species	5	33.1861	6.6372	−0.0647
Within populations	52	721.0556	13.8665	0.9007
Total	59	865.8667		

0.3018) between *S. brevispinus* and *S. wetlandicus*. Further, there is three times the divergence between *S. brevispinus* and *S. wetlandicus* as there is among populations within each species. This points to a significant period of isolation between *S. brevispinus* and *S. wetlandicus*. It is difficult to imagine such a degree of divergence developing if there were long-term gene flow between the two, given that they are parapatric in distribution and *S. brevispinus* is represented by a single metapopulation along a 10-mile stretch of Pariette Draw (species census numbers are estimated at 8000–12,000; USFWS 2007). Similar divergences between species are also revealed in the Bayesian estimates of F_{ST} (Table 8). This bolsters the hypothesis that *S. glaucus*, *S. wetlandicus*, and *S. brevispinus* are different, genetically differentiated species.

Konnert (2005) concluded that *S. glaucus* and *S. parviflorus* were the most similar, while the differences between *S. wetlandicus* subsp. *ilseae* (=*S. brevispinus*) and *S. wetlandicus* subsp. *wetlandicus* were so slight that they could be attributed to different individuals of the same population. However, this was based on examination of a single individual from each of 24 species or subspecies in *Sclerocactus*, using nine enzyme systems. In contrast, we found greater allele frequency divergence between *S. brevispinus* and *S. wetlandicus* (0.0760) than between *S. brevispinus* and *S. glaucus* (0.0283) when examining diploid populations ($k = 3$), using Structure vers. 2.3.2.

While the AFLP data are supportive of the hypothesis that there are three species, there is also strong evidence for admixture. Perplexingly, the source populations for admixture are not those that are geographically proximal; rather, they are the most distant. For example, populations of *S. brevispinus* show admixture involving the Colorado populations of *S. glaucus*. Similarly, the population of *S. wetlandicus* at Bonanza is characterized by admixture involving *S. brevispinus*, but other populations of *S. wetlandicus* do not show admixture. This may be due to the maintenance of ancestral genetic polymorphism rather than recent gene flow. The pollinators of both *S. brevispinus* and *S. wetlandicus* are ground-dwelling bees of the family Halictidae (Tepedino et al. 2010). These insects do not have large home ranges. In addition, the fruits of both species are dry (not dispersed by birds), and the seeds fall to the base of the plants (not dispersed by ants) to be moved only by rainfall and wind. As a result, seed dispersal is limited.

While we have argued that the AFLP data are consistent with the three-species hypothesis, it is important to recognize that the number of populations estimated with Structure vers. 2.3.2 was six rather than three (the number of hypothesized species) or eight (the actual number of populations sampled). The six populations identified by an informed population prior (Fig. 8B) discriminates the two populations (NF and GW) of *S. brevispinus* with evident admixture between these two populations. Similarly, the three populations of *S. glaucus* (GP, RM, and PR) are found to have significant differentiation. The admixture detected in these populations seems to represent markers from *S. brevispinus* and *S. wetlandicus* rather than from other populations of *S. glaucus*. As noted above, this seems more likely to be the result of the persistence of genetic markers from common ancestors than from recent gene flow between these two species, given the geographic isolation of the populations.

One caveat regarding the AFLP data is that it suffers from limited sampling of individuals at the populations investigated. While reasonable population samples were acquired in the field, funding restrictions reduced that number of individuals analyzed significantly. Moreover, the AFLP markers show great variation, with 167 variable loci. This results in a high degree of noise in the data, more so than would be desirable. Another possible contributing factor to noise in the data is the presence of an unsampled species, *S. parviflorus*, which may be playing a genetic role.

We have examined patterns of variation in morphology, chloroplast DNA sequences, and AFLP markers in *S. brevispinus*, *S. glaucus*, and *S. wetlandicus*, a group of species that historically have been considered conspecific, under the name *S. glaucus*. By considering two sets of expected patterns of variation under the conditions that this group represents a

Table 7. Phi (ϕ) statistics derived from analysis of molecular variance (AMOVA) of *Sclerocactus brevispinus*, *S. glaucus*, and *S. wetlandicus*. We provide estimates of genetic differentiation among species (ϕ_{GT}), among populations within species (ϕ_{SG}), and among populations (ϕ_{ST}).

Statistic	Value	*P*
ϕ_{GT}	0.1640	0.002
ϕ_{SG}	−0.0774	0.001
ϕ_{ST}	0.0993	0.001

Table 8. F_{ST} statistics derived from Bayesian estimation (Structure vers. 2.3.2) for *Sclerocactus brevispinus*, *S. glaucus*, and *S. wetlandicus*. We provide among-species estimates of genetic differentiation from AFLP markers assumed to represent haploid and diploid data, $k = 3$, informed by species membership. Estimates of expected heterozygosity (H_E) are provided parenthetically.

Taxon	Haploid F_{ST} (H_E)	Diploid F_{ST} (H_E)
S. brevispinus	0.4496 (0.1299)	0.4082 (0.0732)
S. glaucus	0.0527 (0.2831)	0.0717 (0.2151)
S. wetlandicus	0.2546 (0.2408)	0.1851 (0.2249)

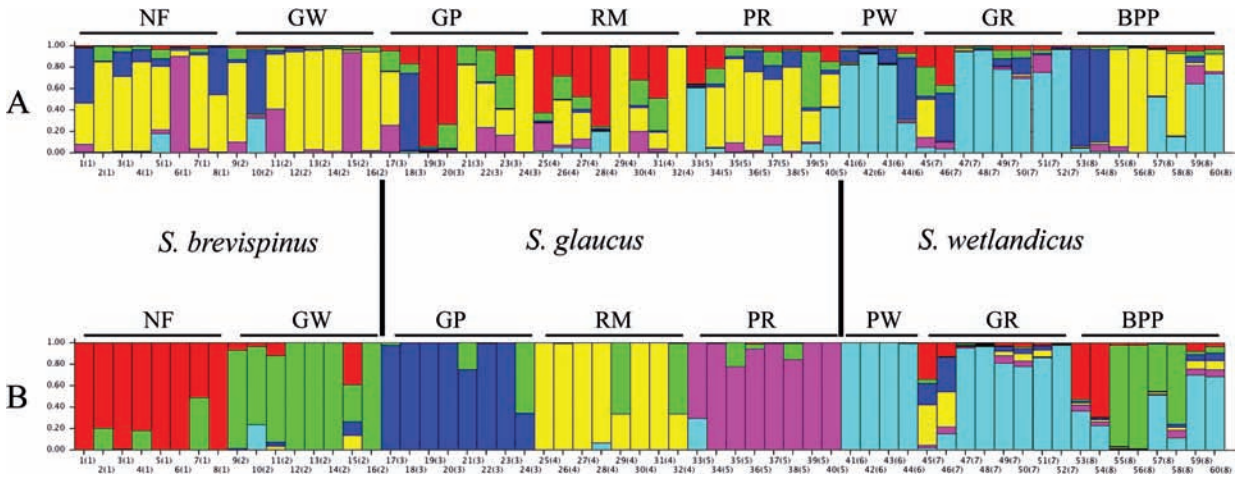

Fig. 8. Population structure of sampled individuals of *Sclerocactus brevispinus*, *S. glaucus*, and *S. wetlandicus* as inferred using Structure 2.3.2 at the maximum likelihood cluster setting $k = 6$ (color-coded by hypothesized ancestral population), based on naïve clustering (A) and population-informed clustering (B). Populations and individuals are identified using the code and numbering provided in Table 1.

single species, or three genetically independent species, we show that morphological evidence is consistent with the presence of three species that differ significantly in morphology. Chloroplast DNA sequences provide evidence that the Colorado populations of *S. glaucus* have a long history of reproductive isolation from the Utah populations of *S. brevispinus* and *S. wetlandicus*. Similar to the morphological data, AFLP markers reveal significant genetic divergence among *S. brevispinus*, *S. glaucus*, and *S. wetlandicus*. Equally important, there is greater divergence among species than among populations within the species. The three sources of evidence all support the presence of three species and not a single panmictic species.

The three species can be distinguished morphologically, using the following key:

2. Seed coat composed of flattened cells; UT
   3. Lower central spines usually not hooked; flowers red-purple, 2.0–3.5 cm long, 2.5–5.0 cm in diam., funnelform . . . . . *S. wetlandicus*
   3. Lower central spines absent, if present then curved or hooked; flowers often pink, 2.0–3.0 cm long, 1.1–3.0 cm in diam., campanulate . . . . . *S. brevispinus*
2. Seed coat composed of rounded cells; CO . . . *S. glaucus*

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APPENDIX 1

Aligned DNA sequence file of the *petA-psbJ* chloroplast intergenic spacer region. Sample acronyms correspond to those in Table 1. The length positions of each nucleotide are displayed in brackets above the sequences. Within the sequences, dashes indicate an insertion/deletion, where one or more nucleotides are absent. Following each line of sequence data is the cumulative number of nucleotides, in brackets.

```
       [         10        20        30        40        50        60        70        80        90       100]
       [         .         .         .         .         .         .         .         .         .         .]
SB005  CGTGGATTTATCAACATCAAGTTCGTAACAAGAACAAAATTTTTTTTTATAATTCTTTTACAATTTTGGACGATCAAGAAATAAAAAAATTATAAGAAGG  [100]
SB009  CGTGGATTTATCAACATCAAGTTCGTAACAAGAACAAAATTTTTTTTTATAATTCTTTTACAATTTTGGACGATCAAGAAATAAAAAAATTATAAGAAGG  [100]
SB012  CGTGGATTTATCAACATCAAGTTCGTAACAAGAACAAAATTTTTTTTTATAATTCTTTTACAATTTTGGACGATCAAGAAATAAAAAAATTATAAGAAGG  [100]
SB016  CGTGGATTTATCAACATCAAGTTCGTAACAAGAACAAAATTTTTTTTTATAATTCTTTTACAATTTTGGACGATCAAGAAATAAAAAAATTATAAGAAGG  [100]
SB017  CGTGGATTTATCAACATCAAGTTCGTAACAAGAACAAAATTTTTTTTTATAATTCTTTTACAATTTTGGACGATCAAGAAATAAAAAAATTATAAGAAGG  [100]
SB045  CGTGGATTTATCAACATCAAGTTCGTAACAAGAACAAAATTTTTTTTTATAATTCTTTTACAATTTTGGACGATCAAGAAATAAAAAAATTATAAGAAGG  [100]
SB050  CGTGGATTTATCAACATCAAGTTCGTAACAAGAACAAAATTTTTTTTTATAATTCTTTTACAATTTTGGACGATCAAGAAATAAAAAAATTATAAGAAGG  [100]
SW003  CGTGGATTTATCAACATCAAGTTCGTAACAAGAACAAAATTTTTTTTTATAATTCTTTTACAATTTTGGACGATCAAGAAATAAAAAAATTATAAGAAGG  [100]
SW043  CGTGGATTTATCAACATCAAGTTCGTAACAAGAACAAAATTTTTTTTTATAATTCTTTTACAATTTTGGACGATCAAGAAATAAAAAAATTATAAGAAGG  [100]
SW050  CGTGGATTTATCAACATCAAGTTCGTAACAAGAACAAAATTTTTTTTTATAATTCTTTTACAATTTTGGACGATCAAGAAATAAAAAAATTATAAGAAGG  [100]
SW060  CGTGGATTTATCAACATCAAGTTCGTAACAAGAACAAAATTTTTTTTTATAATTCTTTTACAATTTTGGACGATCAAGAAATAAAAAAATTATAAGAAGG  [100]
SW076  CGTGGATTTATCAACATCAAGTTCGTAACAAGAACAAAATTTTTTTTTATAATTCTTTTACAATTTTGGACGATCAAGAAATAAAAAAATTATAAGAAGG  [100]
SW089  CGTGGATTTATCAACATCAAGTTCGTAACAAGAACAAAATTTTTTTTTATAATTCTTTTACAATTTTGGACGATCAAGAAATAAAAAAATTATAAGAAGG  [100]
SG003  CGTGGATTTATCAACATCAAGTTCGTAACAAGAACAAAATTTTTTTTTATAATTCTTTTACAATTTTGGACGATCAAGAAATAAAAAAATTATAAGAAGG  [100]
SG007  CGTGGATTTATCAACATCAAGTTCGTAACAAGAACAAAATTTTTTTTTATAATTCTTTTACAATTTTGGACGATCAAGAAATAAAAAAATTATAAGAAGG  [100]
SG028  CGTGGATTTATCAACATCAAGTTCGTAACAAGAACAAAATTTTTTTTTATAATTCTTTTACAATTTTGGACGATCAAGAAATAAAAAAATTATAAGAAGG  [100]
SG043  CGTGGATTTATCAACATCAAGTTCGTAACAAGAACAAAATTTTTTTTTATAATTCTTTTACAATTTTGGACGATCAAGAAATAAAAAAATTATAAGAAGG  [100]
SG066  CG-GGATTT-TCA-CATCAAGTTCGTAACAAGAACAAAATTTTTTTTTATAATTCTTTTACAATTTTGGACGATCAAGAAATAAAAAAATTATAAGAAGG  [97]
SG086  CGTGGATTTATCAACATCAAGTTCGTAACAAGAACAAAATTTTTTTTTATAATTCTTTTACAATTTTGGACGATCAAGAAATAAAAAAATTATAAGAAGG  [100]
SG093  CGTGGATTTATCAACATCAAGTTCGTAACAAGAACAAAATTTTTTTTTATAATTCTTTTACAATTTTGGACGATCAAGAAATAAAAAAATTATAAGAAGG  [100]
SG152  CGTGGATTTATCAACATCAAGTTCGTAACAAGAACAAAATTTTTTTTTATAATTCTTTTACAATTTTGGACGATCAAGAAATAAAAAAATTATAAGAAGG  [100]
SG170  CGTGGATTTATCAACATCAAGTTCGTAACAAGAACAAAATTTTTTTTTATAATTCTTTTACAATTTTGGACGATCAAGAAATAAAAAAATTATAAGAAGG  [100]
SG189  CGTGGATTTATCAACATCAAGTTCGTAACAAGAACAAAATTTTTTTTTATAATTCTTTTACAATTTTGGACGATCAAGAAATAAAAAAATTATAAGAAGG  [100]
SG198  CGTGGATTTATCAACATCAAGTTCGTAACAAGAACAAAATTTTTTTTTATAATTCTTTTACAATTTTGGACGATCAAGAAATAAAAAAATTATAAGAAGG  [100]
SGO64  CGTGGATTTATCAACATCAAGTTCGTAACAAGAACAAAATTTTTTTTTATAATTCTTTTACAATTTTGGACGATCAAGAAATAAAAAAATTATAAGAAGG  [100]

       [         110       120       130       140       150       160       170       180       190       200]
       [         .         .         .         .         .         .         .         .         .         .]
SB005  CTCTTTTTTTGTTAGATTTAGATTTATTATAATAAAATATTATTATTACACATATAATTCTAATAGACTTTACTTTCTAATATACTACTTTATAATATAC  [200]
SB009  CTCTTTTTTTGTTAGATTTAGATTTATTATAATAAAATATTATTATTACACATATAATTCTAATAGACTTTACTTTCTAATATACTACTTTATAATATAC  [200]
SB012  CTCTTTTTTTGTTAGATTTAGATTTATTATAATAAAATATTATTATTACACATATAATTCTAATAGACTTTACTTTCTAATATACTACTTTATAATATAC  [200]
SB016  CTCTTTTTTTGTTAGATTTAGATTTATTATAATAAAATATTATTATTACACATATAATTCTAATAGACTTTACTTTCTAATATACTACTTTATAATATAC  [200]
SB017  CTCTTTTTTTGTTAGATTTAGATTTATTATAATAAAATATTATTATTACACATATAATTCTAATAGACTTTACTTTCTAATATACTACTTTATAATATAC  [200]
SB045  CTCTTTTTTTGTTAGATTTAGATTTATTATAATAAAATATTATTATTACACATATAATTCTAATAGACTTTACTTTCTAATATACTACTTTATAATATAC  [200]
SB050  CTCTTTTTTTGTTAGATTTAGATTTATTATAATAAAATATTATTATTACACATATAATTCTAATAGACTTTACTTTCTAATATACTACTTTATAATATAC  [200]
SW003  CTCTTTTTTTGTTAGATTTAGATTTATTATAATAAAATATTATTATTACACATATAATTCTAATAGACTTTACTTTCTAATATACTACTTTATAATATAC  [200]
SW043  CTCTTTTTTTGTTAGATTTAGATTTATTATAATAAAATATTATTATTACACATATAATTCTAATAGACTTTACTTTCTAATATACTACTTTATAATATAC  [200]
SW050  CTCTTTTTTTGTTAGATTTAGATTTATTATAATAAAATATTATTATTACACATATAATTCTAATAGACTTTACTTTCTAATATACTACTTTATAATATAC  [200]
SW060  CTCTTTTTTTGTTAGATTTAGATTTATTATAATAAAATATTATTATTACACATATAATTCTAATAGACTTTACTTTCTAATATACTACTTTATAATATAC  [200]
SW076  CTCTTTTTTTGTTAGATTTAGATTTATTATAATAAAATATTATTATTACACATATAATTCTAATAGACTTTACTTTCTAATATACTACTTTATAATATAC  [200]
SW089  CTCTTTTTTTGTTAGATTTAGATTTATTATAATAAAATATTATTATTACACATATAATTCTAATAGACTTTACTTTCTAATATACTACTTTATAATATAC  [200]
SG003  CTCTTTTTTTGTTAGATTTAGATTTATTATAATAAAATATTATTATTACACATATAATTCTAATAGACTTTACTTTCTAATATACTACTTTATAATATAC  [200]
SG007  CTCTTTTTTTGTTAGATTTAGATTTATTATAATAAAATATTATTATTACACATATAATTCTAATAGACTTTACTTTCTAATATACTACTTTATAATATAC  [200]
SG028  CTCTTTTTTTGTTAGATTTAGATTTATTATAATAAAATATTATTATTACACATATAATTCTAATAGACTTTACTTTCTAATATACTACTTTATAATATAC  [200]
SG043  CTCTTTTTTTGTTAGATTTAGATTTATTATAATAAAATATTATTATTACACATATAATTCTAATAGACTTTACTTTCTAATATACTACTTTATAATATAC  [200]
SG066  CTCTTTTTTTGTTAGATTTAGATTTATTATAATAAAATATTATTATTACACATATAATTCTAATAGACTTTACTTTCTAATATACTACTTTATAATATAC  [197]
SG086  CTCTTTTTTTGTTAGATTTAGATTTATTATAATAAAATATTATTATTACACATATAATTCTAATAGACTTTACTTTCTAATATACTACTTTATAATATAC  [200]
SG093  CTCTTTTTTTGTTAGATTTAGATTTATTATAATAAAATATTATTATTACACATATAATTCTAATAGACTTTACTTTCTAATATACTACTTTATAATATAC  [200]
SG152  CTCTTTTTTTGTTAGATTTAGATTTATTATAATAAAATATTATTATTACACATATAATTCTAATAGACTTTACTTTCTAATATACTACTTTATAATATAC  [200]
SG170  CTCTTTTTTTGTTAGATTTAGATTTATTATAATAAAATATTATTATTACACATATAATTCTAATAGACTTTACTTTCTAATATACTACTTTATAATATAC  [200]
SG189  CTCTTTTTTTGTTAGATTTAGATTTATTATAATAAAATATTATTATTACACATATAATTCTAATAGACTTTACTTTCTAATATACTACTTTATAATATAC  [200]
SG198  CTCTTTTTTTGTTAGATTTAGATTTATTATAATAAAATATTATTATTACACATATAATTCTAATAGACTTTACTTTCTAATATACTACTTTATAATATAC  [200]
SGO64  CTCTTTTTTTGTTAGATTTAGATTTATTATAATAAAATATTATTATTACACATATAATTCTAATAGACTTTACTTTCTAATATACTACTTTATAATATAC  [200]

       [         210       220       230       240       250       260       270       280       290       300]
       [         .         .         .         .         .         .         .         .         .         .]
SB005  T----------------------AAAGTAGTGGACAAACTGAAAAAAAGATAGAGGAAAATTGATTGAAAAAATGGAACTTCTTGTACGATAT  [271]
SB009  T----------------------AAAGTAGTGGACAAACTGAAAAAAAGATAGAGGAAAATTGATTGAAAAAATGGAACTTCTTGTACGATAT  [271]
SB012  T----------------------AAAGTAGTGGACAAACTGAAAAAAAGATAGAGGAAAATTGATTGAAAAAATGGAACTTCTTGTACGATAT  [271]
SB016  T----------------------AAAGTAGTGGACAAACTGAAAAAAAGATAGAGGAAAATTGATTGAAAAAATGGAACTTCTTGTACGATAT  [271]
SB017  T----------------------AAAGTAGTGGACAAACTGAAAAAAAGATAGAGGAAAATTGATTGAAAAAATGGAACTTCTTGTACGATAT  [271]
SB045  T----------------------AAAGTAGTGGACAAACTGAAAAAAAGATAGAGGAAAATTGATTGAAAAAATGGAACTTCTTGTACGATAT  [271]
SB050  T----------------------AAAGTAGTGGACAAACTGAAAAAAAGATAGAGGAAAATTGATTGAAAAAATGGAACTTCTTGTACGATAT  [271]
SW003  T----------------------AAAGTAGTGGACAAACTGAAAAAAAGATAGAGGAAAATTGATTGAAAAAATGGAACTTCTTGTACGATAT  [271]
SW043  T----------------------AAAGTAGTGGACAAACTGAAAAAAAGATAGAGGAAAATTGATTGAAAAAATGGAACTTCTTGTACGATAT  [271]
SW050  T----------------------AAAGTAGTGGACAAACTGAAAAAAAGATAGAGGAAAATTGATTGAAAAAATGGAACTTCTTGTACGATAT  [271]
SW060  T----------------------AAAGTAGTGGACAAACTGAAAAAAAGATAGAGGAAAATTGATTGAAAAAATGGAACTTCTTGTACGATAT  [271]
SW076  T----------------------AAAGTAGTGGACAAACTGAAAAAAAGATAGAGGAAAATTGATTGAAAAAATGGAACTTCTTGTACGATAT  [271]
SW089  T----------------------AAAGTAGTGGACAAACTGAAAAAAAGATAGAGGAAAATTGATTGAAAAAATGGAACTTCTTGTACGATAT  [271]
SG003  TCTTTCTAATATACTACTTTATAATATACTAAAGTAGTGGACAAACTGAAAAAAAGATAGAGGAAAATTGATTGAAAAAATGGAACTTCTTGTACGATAT  [300]
SG007  TCTTTCTAATATACTACTTTATAATATACTAAAGTAGTGGACAAACTGAAAAAAAGATAGAGGAAAATTGATTGAAAAAATGGAACTTCTTGTACGATAT  [300]
SG028  TCTTTCTAATATACTACTTTATAATATACTAAAGTAGTGGACAAACTGAAAAAAAGATAGAGGAAAATTGATTGAAAAAATGGAACTTCTTGTACGATAT  [300]
SG043  TCTTTCTAATATACTACTTTATAATATACTAAAGTAGTGGACAAACTGAAAAAAAGATAGAGGAAAATTGATTGAAAAAATGGAACTTCTTGTACGATAT  [300]
SG066  TCTTTCTAATATACTACTTTATAATATACTAAAGTAGTGGACAAACTGAAAAAAAGATAGAGGAAAATTGATTGAAAAAATGGAACTTCTTGTACGATAT  [297]
SG086  TCTTTCTAATATACTACTTTATAATATACTAAAGTAGTGGACAAACTGAAAAAAAGATAGAGGAAAATTGATTGAAAAAATGGAACTTCTTGTACGATAT  [300]
SG093  TCTTTCTAATATACTACTTTATAATATACTAAAGTAGTGGACAAACTGAAAAAAAGATAGAGGAAAATTGATTGAAAAAATGGAACTTCTTGTACGATAT  [300]
SG152  TCTTTCTAATATACTACTTTATAATATACTAAAGTAGTGGACAAACTGAAAAAAAGATAGAGGAAAATTGATTGAAAAAATGGAACTTCTTGTACGATAT  [300]
SG170  TCTTTCTAATATACTACTTTATAATATACTAAAGTAGTGGACAAACTGAAAAAAAGATAGAGGAAAATTGATTGAAAAAATGGAACTTCTTGTACGATAT  [300]
SG189  TCTTTCTAATATACTACTTTATAATATACTAAAGTAGTGGACAAACTGAAAAAAAGATAGAGGAAAATTGATTGAAAAAATGGAACTTCTTGTACGATAT  [300]
SG198  TCTTTCTAATATACTACTTTATAATATACTAAAGTAGTGGACAAACTGAAAAAAAGATAGAGGAAAATTGATTGAAAAAATGGAACTTCTTGTACGATAT  [300]
SGO64  TCTTTCTAATATACTACTTTATAATATACTAAAGTAGTGGACAAACTGAAAAAAAGATAGAGGAAAATTGATTGAAAAAATGGAACTTCTTGTACGATAT  [300]
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      [         310        320        330        340        350        360        370        380        390        400]
      [         .          .          .          .          .          .          .          .          .          .]
SB005 TTGATCTATAAAAATATAGATTCTATTGTGTGATTCAATAAATCAAGTGATTCCTTCTTTCTTTTAGGAAGGGTCAATTAAAGAAGACTATCGAGAAATA [371]
SB009 TTGATCTATAAAAATATAGATTCTATTGTGTGATTCAATAAATCAAGTGATTCCTTCTTTCTTTTAGGAAGGGTCAATTAAAGAAGACTATCGAGAAATA [371]
SB012 TTGATCTATAAAAATATAGATTCTATTGTGTGATTCAATAAATCAAGTGATTCCTTCTTTCTTTTAGGAAGGGTCAATTAAAGAAGACTATCGAGAAATA [371]
SB016 TTGATCTATAAAAATATAGATTCTATTGTGTGATTCAATAAATCAAGTGATTCCTTCTTTCTTTTAGGAAGGGTCAATTAAAGAAGACTATCGAGAAATA [371]
SB017 TTGATCTATAAAAATATAGATTCTATTGTGCGATTCAATAAATCAAGTGATTCCTTCTTTCTTTTAGGAAGGGTCAATTAAAGAAGACTATCGAGAAATA [371]
SB045 TTGATCTATAAAAATATAGATTCTATTGTGCGATTCAATAAATCAAGTGATTCCTTCTTTCTTTTAGGAAGGGTCAATTAAAGAAGACTATCGAGAAATA [371]
SB050 TTGATCTATAAAAATATAGATTCTATTGTGTGATTCAATAAATCAAGTGATTCCTTCTTTCTTTTAGGAAGGGTCAATTAAAGAAGACTATCGAGAAATA [371]
SW003 TTGATCTATAAAAATATAGATTCTATTGTGTGATTCAATAAATCAAGTGATTCCTTCTTTCTTTTAGGAAGGGTCAATTAAAGAAGACTATCGAGAAATA [371]
SW043 TTGATCTATAAAAATATAGATTCTATTGTGTGATTCAATAAATCAAGTGATTCCTTCTTTCTTTTAGGAAGGGTCAATTAAAGAAGACTATCGAGAAATA [371]
SW050 TTGATCTATAAAAATATAGATTCTATTGTGTGATTCAATAAATCAAGTGATTCCTTCTTTCTTTTAGGAAGGGTCAATTAAAGAAGACTATCGAGAAATA [371]
SW060 TTGATCTATAAAAATATAGATTCTATTGTGTGATTCAATAAATCAAGTGATTCCTTCTTTCTTTTAGGAAGGGTCAATTAAAGAAGACTATCGAGAAATA [371]
SW076 TTGATCTATAAAAATATAGATTCTATTGTGTGATTCAATAAATCAAGTGATTCCTTCTTTCTTTTAGGAAGGGTCAATTAAAGAAGACTATCGAGAAATA [371]
SW089 TTGATCTATAAAAATATAGATTCTATTGTGTGATTCAATAAATCAAGTGATTCCTTCTTTCTTTTAGGAAGGGTCAATTAAAGAAGACTATCGAGAAATA [371]
SG003 TTGATCTATAAAAATATAGATTCTATTGTGTGATTCAATAAATCAAGTGATTCCTTCTTTCTTTTAGGAAGGGTCAATTAAAGAAGACTATCGAGAAATA [400]
SG007 TTGATCTATAAAAATATAGATTCTATTGTGTGATTCAATAAATCAAGTGATTCCTTCTTTCTTTTAGGAAGGGTCAATTAAAGAAGACTATCGAGAAATA [400]
SG028 TTGATCTATAAAAATATAGATTCTATTGTGTGATTCAATAAATCAAGTGATTCCTTCTTTCTTTTAGGAAGGGTCAATTAAAGAAGACTATCGAGAAATA [400]
SG043 TTGATCTATAAAAATATAGATTCTATTGTGTGATTCAATAAATCAAGTGATTCCTTCTTTCTTTTAGGAAGGGTCAATTAAAGAAGACTATCGAGAAATA [400]
SG066 TTGATCTATAAAAATATAGATTCTATTGTGTGATTCAATAAATCAAGTGATTCCTTCTTTCTTTTAGGAAGGGTCAATTAAAGAAGACTATCGAGAAATA [397]
SG086 TTGATCTATAAAAATATAGATTCTATTGTGTGATTCAATAAATCAAGTGATTCCTTCTTTCTTTTAGGAAGGGTCAATTAAAGAAGACTATCGAGAAATA [400]
SG093 TTGATCTATAAAAATATAGATTCTATTGTGTGATTCAATAAATCAAGTGATTCCTTCTTTCTTTTAGGAAGGGTCAATTAAAGAAGACTATCGAGAAATA [400]
SG152 TTGATCTATAAAAATATAGATTCTATTGTGTGATTCAATAAATCAAGTGATTCCTTCTTTCTTTTAGGAAGGGTCAATTAAAGAAGACTATCGAGAAATA [400]
SG170 TTGATCTATAAAAATATAGATTCTATTGTGTGATTCAATAAATCAAGTGATTCCTTCTTTCTTTTAGGAAGGGTCAATTAAAGAAGACTATCGAGAAATA [400]
SG189 TTGATCTATAAAAATATAGATTCTATTGTGTGATTCAATAAATCAAGTGATTCCTTCTTTCTTTTAGGAAGGGTCAATTAAAGAAGACTATCGAGAAATA [400]
SG198 TTGATCTATAAAAATATAGATTCTATTGTGTGATTCAATAAATCAAGTGATTCCTTCTTTCTTTTAGGAAGGGTCAATTAAAGAAGACTATCGAGAAATA [400]
SGO64 TTGATCTATAAAAATATAGATTCTATTGTGTGATTCAATAAATCAAGTGATTCCTTCTTTCTTTTAGGAAGGGTCAATTAAAGAAGACTATCGAGAAATA [400]

      [         410        420        430        440        450        460        470        480        490        500]
      [         .          .          .          .          .          .          .          .          .          .]
SB005 GATGTTTTGAATGACCAAATAAAAGAATTTCCAAAAAGCATCATTGCATGGATCCTTTTTTCATTTAGACGAAAAAGGATTATGATAATTAAATAAAATA [471]
SB009 GATGTTTTGAATGACCAAATAAAAGAATTTCCAAAAAGCATCATTGCATGGATCCTTTTTTCATTTAGACGAAAAAGGATTATGATAATTAAATAAAATA [471]
SB012 GATGTTTTGAATGACCAAATAAAAGAATTTCCAAAAAGCATCATTGCATGGATCCTTTTTTCATTTAGACGAAAAAGGATTATGATAATTAAATAAAATA [471]
SB016 GATGTTTTGAATGACCAAATAAAAGAATTTCCAAAAAGCATCATTGCATGGATCCTTTTTTCATTTAGACGAAAAAGGATTATGATAATTAAATAAAATA [471]
SB017 GATGTTTTGAATGACCAAATAAAAGAATTTCCAAAAAGCATCATTGCATGGATCCTTTTTTCATTTAGACGAAAAAGGATTATGATAATTAAATAAAATA [471]
SB045 GATGTTTTGAATGACCAAATAAAAGAATTTCCAAAAAGCATCATTGCATGGATCCTTTTTTCATTTAGACGAAAAAGGATTATGATAATTAAATAAAATA [471]
SB050 GATGTTTTGAATGACCAAATAAAAGAATTTCCAAAAAGCATCATTGCATGGATCCTTTTTTCATTTAGACGAAAAAGGATTATGATAATTAAATAAAATA [471]
SW003 GATGTTTTGAATGACCAAATAAAAGAATTTCCAAAAAGCATCATTGCATGGATCCTTTTTTCATTTAGACGAAAAAGGATTATGATAATTAAATAAAATA [471]
SW043 GATGTTTTGAATGACCAAATAAAAGAATTTCCAAAAAGCATCATTGCATGGATCCTTTTTTCATTTAGACGAAAAAGGATTATGATAATTAAATAAAATA [471]
SW050 GATGTTTTGAATGACCAAATAAAAGAATTTCCAAAAAGCATCATTGCATGGATCCTTTTTTCATTTAGACGAAAAAGGATTATGATAATTAAATAAAATA [471]
SW060 GATGTTTTGAATGACCAAATAAAAGAATTTCCAAAAAGCATCATTGCATGGATCCTTTTTTCATTTAGACGAAAAAGGATTATGATAATTAAATAAAATA [471]
SW076 GATGTTTTGAATGACCAAATAAAAGAATTTCCAAAAAGCATCATTGCATGGATCCTTTTTTCATTTAGACGAAAAAGGATTATGATAATTAAATAAAATA [471]
SW089 GATGTTTTGAATGACCAAATAAAAGAATTTCCAAAAAGCATCATTGCATGGATCCTTTTTTCATTTAGACGAAAAAGGATTATGATAATTAAATAAAATA [471]
SG003 GATGTTTTGAATGACCAAATAAAAGAATTTCCAAAAAGCATCATTGCATGGATCCTTTTTTCATTTAGACGAAAAAGGATTATGATAATTAAATAAAATA [500]
SG007 GATGTTTTGAATGACCAAATAAAAGAATTTCCAAAAAGCATCATTGCATGGATCCTTTTTTCATTTAGACGAAAAAGGATTATGATAATTAAATAAAATA [500]
SG028 GATGTTTTGAATGACCAAATAAAAGAATTTCCAAAAAGCATCATTGCATGGATCCTTTTTTCATTTAGACGAAAAAGGATTATGATAATTAAATAAAATA [500]
SG043 GATGTTTTGAATGACCAAATAAAAGAATTTCCAAAAAGCATCATTGCATGGATCCTTTTTTCATTTAGACGAAAAAGGATTATGATAATTAAATAAAATA [500]
SG066 GATGTTTTGAATGACCAAATAAAAGAATTTCCAAAAAGCATCATTGCATGGATCCTTTTTTCATTTAGACGAAAAAGGATTATGATAATTAAATAAAATA [497]
SG086 GATGTTTTGAATGACCAAATAAAAGAATTTCCAAAAAGCATCATTGCATGGATCCTTTTTTCATTTAGACGAAAAAGGATTATGATAATTAAATAAAATA [500]
SG093 GATGTTTTGAATGACCAAATAAAAGAATTTCCAAAAAGCATCATTGCATGGATCCTTTTTTCATTTAGACGAAAAAGGATTATGATAATTAAATAAAATA [500]
SG152 GATGTTTTGAATGACCAAATAAAAGAATTTCCAAAAAGCATCATTGCATGGATCCTTTTTTCATTTAGACGAAAAAGGATTATGATAATTAAATAAAATA [500]
SG170 GATGTTTTGAATGACCAAATAAAAGAATTTCCAAAAAGCATCATTGCATGGATCCTTTTTTCATTTAGACGAAAAAGGATTATGATAATTAAATAAAATA [500]
SG189 GATGTTTTGAATGACCAAATAAAAGAATTTCCAAAAAGCATCATTGCATGGATCCTTTTTTCATTTAGACGAAAAAGGATTATGATAATTAAATAAAATA [500]
SG198 GATGTTTTGAATGACCAAATAAAAGAATTTCCAAAAAGCATCATTGCATGGATCCTTTTTTCATTTAGACGAAAAAGGATTATGATAATTAAATAAAATA [500]
SGO64 GATGTTTTGAATGACCAAATAAAAGAATTTCCAAAAAGCATCATTGCATGGATCCTTTTTTCATTTAGACGAAAAAGGATTATGATAATTAAATAAAATA [500]

      [         510        520        530        540        550        560        570        580        590        ]
      [         .          .          .          .          .          .          .          .          .         .]
SB005 AAGAAATTAACGGGACCTCCCCCTTCTTTGTTTGTCTAATTCAAGGGAAAAGGAAGGTCCCGTCGAGTTCTTATACTTTCATATCGATACCTCGGTTCa [570]
SB009 AAGAAATTAACGGGACCTCCCCCTTCTTTGTTTGTCTAATTCAAGGGAAAAGGAAGGTCCCGTCGAGTTCTTATACTTTCATATCGATACCTCGGTTCa [570]
SB012 AAGAAATTAACGGGACCTCCCCCTTCTTTGTTTGTCTAATTCAAGGGAAAAGGAAGGTCCCGTCGAGTTCTTATACTTTCATATCGATACCTCGGTTCa [570]
SB016 AAGAAATTAACGGGACCTCCCCCTTCTTTGTTTGTCTAATTCAAGGGAAAAGGAAGGTCCCGTCGAGTTCTTATACTTTCATATCGATACCTCGGTTCa [570]
SB017 AAGAAATTAACGGGACCTCCCCCTTCTTTGTTTGTCTAATTCAAGGGAAAAGGAAGGTCCCGTCGAGTTCTTATACTTTCATATCGATACCTCGGTTCa [570]
SB045 AAGAAATTAACGGGACCTCCCCCTTCTTTGTTTGTCTAATTCAAGGGAAAAGGAAGGTCCCGTCGAGTTCTTATACTTTCATATCGATACCTCGGTTCa [570]
SB050 AAGAAATTAACGGGACCTCCCCCTTCTTTGTTTGTCTAATTCAAGGGAAAAGGAAGGTCCCGTCGAGTTCTTATACTTTCATATCGATACCTCGGTTCa [570]
SW003 AAGAAATTAACGGGACCTCCCCCTTCTTTGTTTGTCTAATTCAAGGGAAAAGGAAGGTCCCGTCGAGTTCTTATACTTTCATATCGATACCTCGGTTCa [570]
SW043 AAGAAATTAACGGGACCTCCCCCTTCTTTGTTTGTCTAATTCAAGGGAAAAGGAAGGTCCCGTCGAGTTCTTATACTTTCATATCGATACCTCGGTTCa [570]
SW050 AAGAAATTAACGGGACCTCCCCCTTCTTTGTTTGTCTAATTCAAGGGAAAAGGAAGGTCCCGTCGAGTTCTTATACTTTCATATCGATACCTCGGTTCa [570]
SW060 AAGAAATTAACGGGACCTCCCCCTTCTTTGTTTGTCTAATTCAAGGGAAAAGGAAGGTCCCGTCGAGTTCTTATACTTTCATATCGATACCTCGGTTCa [570]
SW076 AAGAAATTAACGGGACCTCCCCCTTCTTTGTTTGTCTAATTCAAGGGAAAAGGAAGGTCCCGTCGAGTTCTTATACTT-CATATCGA-ACCTCGGTTCa [568]
SW089 AAGAAATTAACGGGACCTCCCCCTTCTTTGTTTGTCTAATTCAAGGGAAAAGGAAGGTCCCGTCGAGTTCTTATACTTTCATATCGATACCTCGGTTCa [570]
SG003 AAGAAATTAACGGGACCTCCCCCTTCTTTGTTTGTCTAATTCAAGGGAAAAGGAAGGTCCCGTCGAGTTCTTATACTTTCATATCGATACCTCGGTTCc [599]
SG007 AAGAAATTAACGGGACCTCCCCCTTCTTTGTTTGTCTAATTCAAGGGAAAAGGAAGGTCCCGTCGAGTTCTTATACTTTCATATCGATACCTCGGTTCc [599]
SG028 AAGAAATTAACGGGACCTCCCCCTTCTTTGTTTGTCTAATTCAAGGGAAAAGGAAGGTCCCGTCGAGTTCTTATACTTTCATATCGATACCTCGGTTCc [599]
SG043 AAGAAATTAACGGGACCTCCCCCTTCTTTGTTTGTCTAATTCAAGGGAAAAGGAAGGTCCCGTCGAGTTCTTATACTTTCATATCGATACCTCGGTTCc [599]
SG066 AAGAAATTAACGGGACCTCCCCCTTCTTTGTTTGTCTAATTCAAGGGAAAAGGAAGGTCCCGTCGAGTTCTTATGCTTTCATATCGA-ACCTCCGTTCc [595]
SG086 AAGAAATTAACGGGACCTCCCCCTTCTTTGTTTGTCTAATTCAAGGGAAAAGGAAGGTCCCGTCGAGTTCTTATGCTTTCATATCGATACCTCGGTTCc [599]
SG093 AAGAAATTAACGGGACCTCCCCCTTCTTTGTTTGTCTAATTCAAGGGAAAAGGAAGGTCCCGTCGAGTTCTTATGCTTTCATATCGATACCTCGGTTCc [599]
SG152 AAGAAATTAACGGGACCTCCCCCTTCTTTGTTTGTCTAATTCAAGGGAAAAGGAAGGTCCCGTCGAGTTCTTATACTTTCATATCGATACCTCGGTTCc [599]
SG170 AAGAAATTAACGGGACCTCCCCCTTCTTTGTTTGTCTAATTCAAGGGAAAAGGAAGGTCCCGTCGAGTTCTTATACTTTCATATCGATACCTCGGTTCc [599]
SG189 AAGAAATTAACGGGACCTCCCCCTTCTTTGTTTGTCTAATTCAAGGGAAAAGGAAGGTCCCGTCGAGTTCTTATACTTTCATATCGATACCTCGGTTCc [599]
SG198 AAGAAATTAACGGGACCTCCCCCTTCTTTGTTTGTCTAATTCAAGGGAAAAGGAAGGTCCCGTCGAGTTCTTATACTTTCATATCGATACCTCGGTTCc [599]
SGO64 AAGAAATTAACGGGACCTCCCCCTTCTTTGTTTGTCTAATTCAAGGGAAAAGGAAGGTCCCGTCGAGTTCTTATGCTTTCATATCGATCCCTCGGTTCc [599]
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