

Occurrence and phylogeny of the endangered Texas endemic *Echinocereus fitchii* subsp. *albertii*

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Summary: *Echinocereus fitchii* subsp. *albertii* (L.D.Benson) W.Blum & Mich.Lange, also known as *Echinocereus reichenbachii* var. *albertii* L.D.Benson, is a south Texas, USA endemic. The plant, commonly called black lace cactus, is listed as a federally endangered species in the United States. The objectives of this study are to 1 – survey populations of black lace cactus to determine current occurrence status, 2 – examine habitat and morphological features of black lace cactus across occurrences and 3 – examine the phylogeny of the *E. reichenbachii-fitchii* species complex to decipher the closest evolutionary relatives of black lace cactus. We photo-documented *E. fitchii* subsp. *albertii* individuals and confirmed extant populations in Kleberg, McMullen, and Refugio counties, Texas. Our study identified additional populations extending the sub-species' distributional range into Atascosa and La Salle counties, Texas. Flower colour of *E. fitchii* subsp. *albertii* is characteristic of the *E. fitchii* group. Central spines are absent to rare in *E. fitchii* subsp. *albertii* individuals in most populations, with

only plants growing in the Kleberg Co. population commonly exhibiting central spines. We used genome skimming and assembly of nuclear ribosomal regions and nearly complete chloroplast genomes to examine phylogenetic relationships of fifteen taxa of *Echinocereus*. The most significant finding emerging from this phylogenetic study is that two distinct chloroplast genome lineages can be distinguished in *E. fitchii* subsp. *albertii*. One lineage includes individuals from Refugio Co. and McMullen Co. populations which are most closely related to *E. reichenbachii* subsp. *caespitosus*. The other lineage includes individuals from Atascosa and Kleberg Cos., which are most closely related to *E. fitchii* subsp. *fitchii*. These two lineages are not defined by geographic proximity.

Zusammenfassung: *Echinocereus fitchii* subsp. *albertii* (L. D. Benson) W. Blum & Mich. Lange, auch bekannt als *Echinocereus reichenbachii* var. *albertii* L. D. Benson, ist in Südtexas, USA, endemisch. Die Pflanze, die gemeinhin als 'black lace cactus' bezeichnet wird, gilt in den Vereinigten Staaten als bundesweit gefährdet. Ziel der Studie war es, 1 – die Populationen dieser Unterart zu erfassen,

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Figure 1. *Echinocereus fitchii* subsp. *albertii* plant flowering in south Texas, USA.

um den aktuellen Status der Vorkommen zu bestimmen, 2 – die Lebensräume und die morphologischen Merkmale der Unterart in ihren Vorkommen zu untersuchen und 3 – die Phylogenie des *E. reichenbachii-fitchii*-Artenkomplexes zu untersuchen, um die evolutionär engsten Verwandten der Unterart zu ermitteln. Wir haben Individuen von *E. fitchii* subsp. *albertii* fotografisch dokumentiert und die Existenz von Populationen in den texanischen Bezirken (Countys) Kleberg, McMullen und Refugio bestätigt. Im Rahmen unserer Studie wurden weitere Populationen identifiziert, die das Verbreitungsgebiet der Unter-Art auf die Bezirke Atascosa und La Salle in Texas erweitern. Die Blütenfarbe von *E. fitchii* subsp. *albertii* ist charakteristisch für die *E. fitchii*-Gruppe. Mitteldornen sind bei Individuen von *E. fitchii* subsp. *albertii* in den meisten Populationen nicht vorhanden oder selten, wobei nur die Pflanzen in der Population des Bezirks Kleberg häufig Mitteldornen aufweisen. Mit ‘Genome Skimming’ und der Assemblierung ribosomaler Kernregionen sowie nahezu vollständiger Chloroplastengenome haben wir die

phylogenetischen Beziehungen von fünfzehn *Echinocereus*-Taxa untersucht. Das wichtigste Ergebnis dieser phylogenetischen Studie ist, dass bei *E. fitchii* subsp. *albertii* zwei verschiedene Chloroplastengenomlinien unterschieden werden können. Die Abstammungslinie umfasst Individuen aus den Populationen der Bezirke Refugio und McMullen, die am engsten mit *E. reichenbachii* subsp. *caespitosus* verwandt sind. Die andere Abstammungslinie umfasst Individuen der Bezirke Atascosa und Kleberg, die am engsten mit *E. fitchii* subsp. *fitchii* verwandt sind. Diese beiden Abstammungslinien sind nicht durch geografische Nähe definiert.

Introduction

Echinocereus fitchii subsp. *albertii* (L.D.Benson) W.Blum & Mich.Lange (Blum et al., 1998), also known as *Echinocereus reichenbachii* var. *albertii* L.D.Benson (Benson, 1969), is a south Texas, USA endemic (Poole et al., 2007). The plant (Figure 1), commonly called black lace cactus, is listed as a federally endangered species in the United States (USFWS, 1987, 2009). Poole et al. (2007) describes



Figure 2. Dense scrub habitat of the Kleberg Co. population with flowering *Echinocereus fitchii* subsp. *albertii* individuals in the foreground. Martin Terry and Anna Ermakova are shown at the field site.

the taxon's habitat as grasslands, thorn shrublands and mesquite woodlands, typically with saline soils. The U.S. Fish and Wildlife Service (1987) describes suitable habitat as openings in mesquite brush occurring along streams of the coastal plain at fifty metres or less in elevation. Figure 2 shows a typical habitat.

The greatest current threats to black lace cactus are considered to be clearing of its habitat for conversion to cropland and to improve pasture land, as well as competition from invasive, non-native grasses (USFWS, 1987, 2009). The species has also been impacted by pipeline construction (USFWS, 2009). Collection by cactus enthusiasts may also represent a considerable threat (USFWS, 1987, 2009).

Three populations were known when the species was listed as endangered in the United States in 1979: one population each in Jim Wells, Kleberg and Refugio counties (USFWS, 1987). The Jim Wells and Kleberg Co. populations were the first to be identified. As early as 1936, sightings of a small variety of what was initially thought to be *Echinocereus reichenbachii* var. *perbellus* were

reported from near Alice in Jim Wells Co. and in a limited area of adjoining Kleberg Co. (Lowry, 1936). This description was invalidly published without a Latin diagnosis or specific locality as *Echinocereus melanocentrus*, referring to the black colour of the central spine.

The first recorded herbarium specimen of the black lace cactus (Herbarium of the Rob and Bessie Welder Wildlife Foundation (WWF), record number 2965) was collected in 1959 by an amateur botanist, Fred B. Jones from the population in Kleberg Co. The plant's physical characteristics were well described by Jones on the specimen as 'stems usually single up to 8 inches high, sometimes caespitose with as many as 7 stems. Petals violet-purple, colour deeper near base; anther slightly orange-yellow; stigma green. Sloping silty sand. Plants usually in shade of chaparral', but only the name *Echinocereus* was provided by the collector. At some time after the publication of Benson's new name in 1969 in the first valid description, the herbarium added the words *E. reichenbachii* (Terschek) Haage f. var. *albertii* Benson to the type sheet. Fred B. Jones also

| Taxon | <i>E. fitchii</i> subsp. <i>albertii</i> | <i>E. fitchii</i> subsp. <i>fitchii</i> | <i>E. reichenbachii</i> subsp. <i>caespitosus</i> |
|----------------------|---|---|--|
| Features | | | |
| Rib number | 12–18 ¹ 10–13 ³ | 10–14 ³ | 14–21 ⁴ |
| Central spine colour | black ⁵ | brown-black, dark-tipped ³ | not present ³ |
| Radial spine colour | white to pink, tipped black ³ | white, more or less dark-tipped ³ | white to brown, tipped dark ³ |
| Central spine number | 0–1, straight, stiff ^{3,5} | 4–7, flexible ³ | 0 ³ |
| Radial spine number | 14–20 ^{3,5} 14–16 ^{1,2} | 16–20 ⁴ | 22–36 ⁴ |
| Flower colour | purple-pink, darker mid-stripe, throat crimson ³ | purple-pink, darker mid-stripe, throat crimson ³ | purple-pinkish-crimson, darker throat ³ |
| Altitude (meters) | 0 m ⁶ –100m ³ | 100–200m ⁴ | 100–700m ⁴ |
| Soil | sandy, somewhat saline soils on coastal prairie and along creeks ⁵ | gravelly limestone soils, calcareous sandy loam, or somewhat saline clay ⁵ | limestone/granite ⁴ |

Table 1. Distinguishing characteristics of *Echinocereus fitchii* subsp. *albertii*, *E. fitchii* subsp. *fitchii* and *E. reichenbachii* subsp. *caespitosus* according to various authorities, ¹USFWS (1987), ²Benson (1982), ³Blum et al. (1998), ⁴Blum et al., (2005), ⁵Poole et al. (2007), ⁶USFWS (2009).

identified a third population in 1974 located in Refugio Co. The Refugio Co. herbarium specimen is housed at the Ruth O'Brien Herbarium at Texas A&M University-Corpus Christi (TAMUCC, record number FB Jones 8043).

Within the past decade an additional population of black lace cactus was identified, extending the species' range into McMullen Co. (Lange & Sinclair, 2013). All known populations of black lace cactus occur on private property. Since endangered plants occurring exclusively on private lands receive little legal protection, landowner awareness and cooperation is important to the conservation of the species.

The type specimen was collected from the Jim Wells Co. population near Alice on 25 May 1965 by Benson and named after the discoverer, Richard O. Albert, with the field number 16550, as recorded in his field notes. Benson first designated the name *Echinocereus caespitosus* var. *albertii*, but changed this to *Echinocereus reichenbachii* var. *albertii* by the time the description was published (Benson, 1969). A reclassification to *E. fitchii*

subsp. *albertii* from *E. reichenbachii* var. *albertii* was made by Blum et al. (1998).

The Blum et al. (1998) *Echinocereus* taxonomy was based on morphological features examined from their field observations, photographic evidence and plants grown from commercial seed. Morphological features examined included shape and form of the flower, the seed, stem, roots, form of growth and spines. The presence or absence of central spines and the colour of any central spines is a characteristic defining the difference between the *E. reichenbachii* and *E. fitchii* groups (Blum et al., 1998).

Members of the *E. reichenbachii* group found in the United States either lack central spines, or when present they are the same colour as the radial spines, whereas a colour difference between central and radial spines automatically refers a plant to the *E. fitchii* group (Blum et al., 1998). Central spines from United States members of the *E. fitchii* group are either black, rusty brown, or dark-tipped, differing in colour from paler radial spines. *Echinocereus fitchii* subsp. *albertii* has

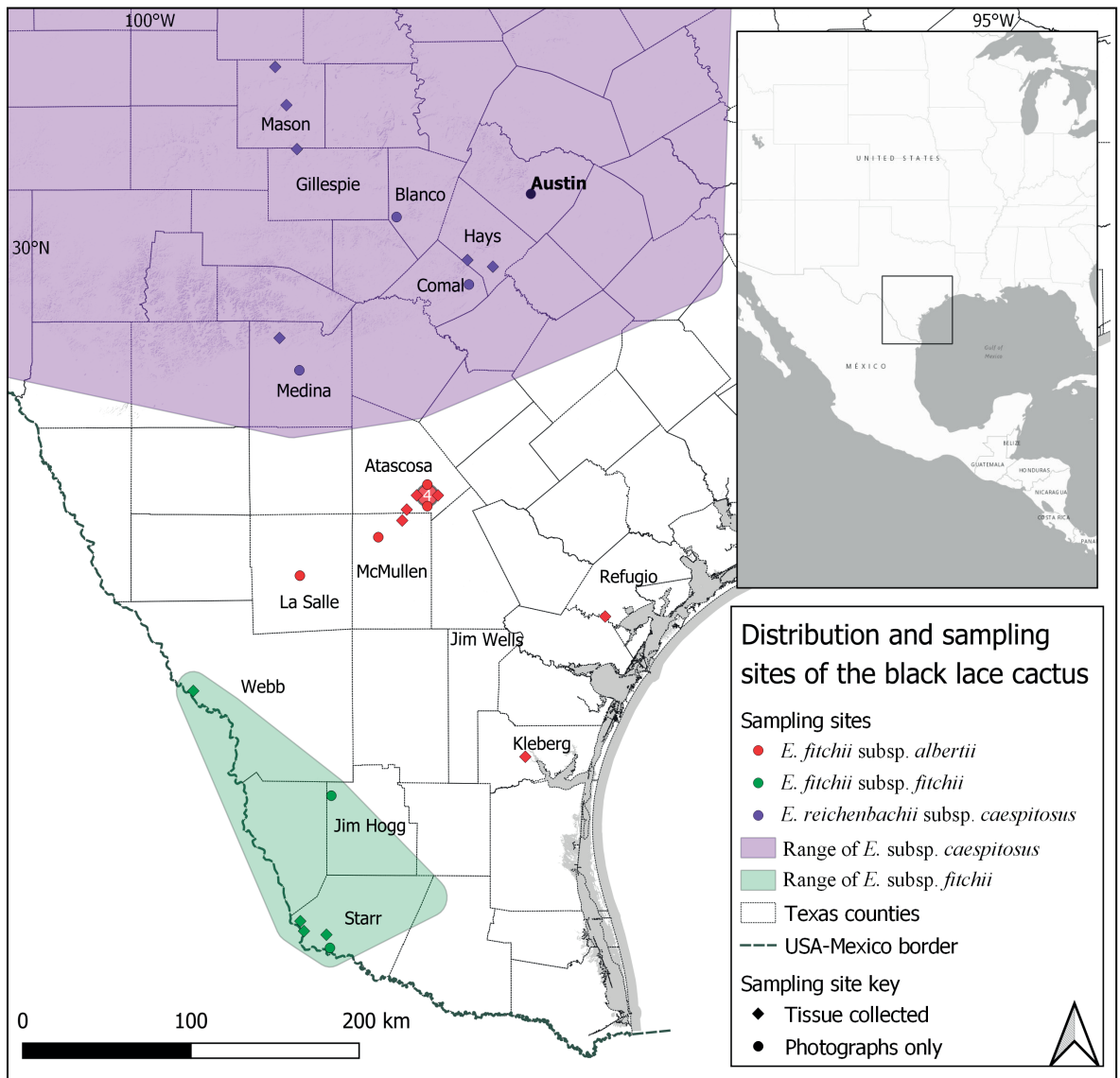


Figure 3. Map of south Texas, USA showing sites of *Echinocereus fitchii* subsp. *albertii* (red colour), *E. fitchii* subsp. *fitchii* (green colour), and *E. reichenbachii* subsp. *caespitosus* (purple colour). All sites shown (circle shape and rhombus shape) were photo-documented for the morphological study. Rhombus shape designates sites of tissue collection used in the phylogenetic study. Shaded areas show distributional range based on species occurrences obtained from GBIF of *E. fitchii* subsp. *fitchii* (green) and *E. reichenbachii* subsp. *caespitosus* (purple) within central and south Texas. Map created by Anna Ermakova.

0-1 black central spines and white radial spines, supporting placement of black lace cactus in the *E. fitchii* group by Blum et al. (1998), as opposed to the *E. reichenbachii* group.

Blum, Felix & Oldach (2005) studied the *E. reichenbachii-fitchii* complex and recognised eleven taxa native to the USA and Mexico. Taxa include: *E. reichenbachii* subsp. *reichenbachii*, *E. reichenbachii* subsp. *baileyi*, *E. reichenbachii* subsp. *burrensis*, *E. reichenbachii* subsp. *caespitosus*, *E.*

reichenbachii subsp. *perbellus*, *E. reichenbachii* subsp. *comanchensis*, *E. primolanatus*, *E. fitchii* subsp. *fitchii*, *E. fitchii* subsp. *albertii*, *E. fitchii* subsp. *bergmannii* and *E. armatus* (formerly *E. fitchii* subsp. *armatus*) (Appendix 1). Members of the complex that occur in closest geographic proximity to black lace cactus are *Echinocereus fitchii* Britton & Rose subsp. *fitchii* and *Echinocereus reichenbachii* subsp. *caespitosus* (Engelm.) W.Blum & Mich.Lange.

| DNA Number | Taxon | County (population/site designation) |
|------------|---|---|
| 4120 | <i>E. fitchii</i> subsp. <i>albertii</i> | Refugio Co. TX |
| 4122 | <i>E. fitchii</i> subsp. <i>albertii</i> | Refugio Co. TX |
| 4130 | <i>E. fitchii</i> subsp. <i>albertii</i> | Refugio Co. TX |
| 4132 | <i>E. fitchii</i> subsp. <i>albertii</i> | Refugio Co. TX |
| 4183 | <i>E. fitchii</i> subsp. <i>albertii</i> | Refugio Co. TX |
| 4124 | <i>E. fitchii</i> subsp. <i>albertii</i> | Kleberg Co. TX |
| 4126 | <i>E. fitchii</i> subsp. <i>albertii</i> | Kleberg Co. TX |
| 4136 | <i>E. fitchii</i> subsp. <i>albertii</i> | Kleberg Co. TX |
| 4134 | <i>E. fitchii</i> subsp. <i>albertii</i> | Kleberg Co. TX |
| 4184 | <i>E. fitchii</i> subsp. <i>albertii</i> | Kleberg Co. TX |
| 4116 | <i>E. fitchii</i> subsp. <i>albertii</i> | McMullen Co. (B) TX |
| 4118 | <i>E. fitchii</i> subsp. <i>albertii</i> | McMullen Co. (B) TX |
| 4182 | <i>E. fitchii</i> subsp. <i>albertii</i> | McMullen Co. (B) TX |
| 4108 | <i>E. fitchii</i> subsp. <i>albertii</i> | Atascosa Co. (D) TX |
| 4195 | <i>E. fitchii</i> subsp. <i>albertii</i> | Atascosa Co. (D) TX |
| 4196 | <i>E. fitchii</i> subsp. <i>albertii</i> | Atascosa Co. (D) TX |
| 4106 | <i>E. fitchii</i> subsp. <i>albertii</i> | Atascosa Co. (B) TX |
| 4193 | <i>E. fitchii</i> subsp. <i>albertii</i> | Atascosa Co. (B) TX |
| 4194 | <i>E. fitchii</i> subsp. <i>albertii</i> | Atascosa Co. (B) TX |
| 4112 | <i>E. fitchii</i> subsp. <i>albertii</i> | Atascosa Co. (E) TX |
| 4114 | <i>E. fitchii</i> subsp. <i>albertii</i> | Atascosa Co. (E) TX |
| 4180 | <i>E. fitchii</i> subsp. <i>albertii</i> | Atascosa Co. (E) TX |
| 4181 | <i>E. fitchii</i> subsp. <i>albertii</i> | Atascosa Co. (E) TX |
| 4144 | <i>E. fitchii</i> subsp. <i>fitchii</i> | Starr Co. (D) TX |
| 4142 | <i>E. fitchii</i> subsp. <i>fitchii</i> | Starr Co. (C) TX |
| 4140 | <i>E. fitchii</i> subsp. <i>fitchii</i> | Starr Co. (B) TX |
| 4146 | <i>E. fitchii</i> subsp. <i>fitchii</i> | Webb Co. (A) TX |
| 4148 | <i>E. fitchii</i> subsp. <i>fitchii</i> | Webb Co. (A) TX |
| 4168 | <i>E. reichenbachii</i> subsp. <i>baileyi</i> | Comanche Co. OK |
| 4197 | <i>E. reichenbachii</i> subsp. <i>baileyi</i> | Comanche Co. OK |
| 4174 | <i>E. reichenbachii</i> subsp. <i>baileyi</i> | Greer Co. OK |
| 4188 | <i>E. reichenbachii</i> subsp. <i>caespitosus</i> | Gillespie Co. TX |
| 4100 | <i>E. reichenbachii</i> subsp. <i>caespitosus</i> | Hays Co. (B) TX |
| 4102 | <i>E. reichenbachii</i> subsp. <i>caespitosus</i> | Hays Co. (B) TX |
| 4191 | <i>E. reichenbachii</i> subsp. <i>caespitosus</i> | Hays Co. (A) TX |
| 4192 | <i>E. reichenbachii</i> subsp. <i>caespitosus</i> | Hays Co. (A) TX |
| 4160 | <i>E. reichenbachii</i> subsp. <i>caespitosus</i> | Mason Co. (A) TX |

Table 2. *Echinocereus* taxa sampled for this study with assigned DNA number and County of population location.

| | | |
|-------------------------------------|---|--------------------------------|
| 4189 | <i>E. reichenbachii</i> subsp. <i>caespitosus</i> | Mason Co. (B) TX |
| 4199 | <i>E. reichenbachii</i> subsp. <i>caespitosus</i> | Medina Co. TX |
| 4198 | <i>E. reichenbachii</i> subsp. <i>caespitosus</i> | Medina Co. TX |
| 4178 | <i>E. reichenbachii</i> subsp. <i>perbellus</i> | Childress Co. TX |
| 4176 | <i>E. reichenbachii</i> subsp. <i>perbellus</i> | Wheeler Co. TX |
| 4190 | <i>E. reichenbachii</i> var. <i>albispinus</i> | Johnston Co. OK |
| Outgroups DNA Number | Taxon | County |
| 4128 | <i>E. enneacanthus</i> subsp. <i>intermedius</i> | Starr Co. TX |
| 4138 | <i>E. poselgeri</i> | Starr Co. TX |
| 2843 | <i>E. arizonicus</i> subsp. <i>arizonicus</i> | Pinal Co. AZ |
| 3554 | <i>E. arizonicus</i> subsp. <i>nigrihorridispinus</i> | Hidalgo Co. NM |
| 3886 | <i>E. bonkerae</i> | Yavapai Co. AZ |
| 3923 | <i>E. bonkerae</i> | Pinal Co. AZ |
| 4201 | <i>E. chloranthus</i> | Dona Ana Co. NM |
| 4208 | <i>E. chloranthus</i> | Sierra Co. NM |
| 3890 | <i>E. fendleri</i> var. <i>fendleri</i> | Socorro Co. NM |
| 3894 | <i>E. fendleri</i> var. <i>fendleri</i> | Otero Co. NM |
| 4213 | <i>E. nicholii</i> | Pima Co. AZ |
| 3882 | <i>E. triglochidiatus</i> subsp. <i>triglochidiatus</i> | Lincoln Co. NM |
| Reference chloroplast genome | Taxon <i>E. reichenbachii</i> subsp. <i>caespitosus</i> | County Blanco Co. TX |

Table 2. (continued)

Morphological and habitat features of these three taxa described by several authors (Benson, 1982; Blum et al., 1998; Blum et al., 2005; Poole et al., 2007; USFWS, 1987) are provided in Table 1. *Echinocereus reichenbachii* subsp. *caespitosus* is described as lacking central spines. *Echinocereus fitchii* subsp. *fitchii* is described as having 4–7 flexible, brown to black, dark-tipped central spines and white radial spines. *Echinocereus fitchii* subsp. *albertii* is described as having 0–1 black, straight central spines and white radial spines. In addition to *E. fitchii* subsp. *albertii* and *E. fitchii* subsp. *fitchii* both possessing central spines that differ in colour from radial spines, the two taxa share identical flower colour and structure, supporting a close relationship between the two taxa (Berresford, 2011; Lange & Sinclair, 2013).

The three taxa are separated geographically and ecologically (Berresford, 2011; Blum et al., 1998; Blum et al., 2005; Lange, 2013; Poole et al., 2007; USFWS, 1987). *Echinocereus reichenbachii* subsp. *caespitosus* is widely distributed. Part of

the taxon's range is the Edwards Plateau in central Texas, where the plants typically grow in humus pockets on limestone cliffs or granite outcrops. *Echinocereus fitchii* subsp. *fitchii* occurs in thorn scrublands on gravelly soils, calcareous sandy loams, or somewhat saline clays in the western part of southernmost Texas in Dimmit, Jim Hogg, Starr, Zapata and Webb counties and adjacent Mexico. *Echinocereus fitchii* subsp. *albertii* is noted as growing on sandy, somewhat saline soils in the eastern part of South Texas.

While U.S. systematists continue to use the name *E. reichenbachii* var. *albertii* (Benson, 1969, 1982; Correll & Johnston, 1970), the newer rank *E. fitchii* subsp. *albertii* is widely accepted by most European authorities on the genus. Disagreement among systematists regarding the correct taxonomic treatment of black lace cactus underscores the need for genetic studies to decipher phylogenetic relationships within the *E. reichenbachii-fitchii* species complex.

The objectives of this study are to 1 – survey

populations of black lace cactus to determine current occurrence status, 2 – examine habitat and morphological features of black lace cactus across occurrences and 3 – examine the phylogeny of the *E. reichenbachii-fitchii* species complex to decipher the closest evolutionary relatives of black lace cactus.

Materials and methods

Site visits and photo-documentation: Populations of *E. fitchii* subsp. *albertii* were visited with landowner permission between 2015 and 2020 to assess current occurrence status. The altitude and geographical coordinates at each site were determined using a handheld GPS device. Photographs were taken of the habitat and individual plants for photo-vouchers.

We attempted to confirm presence of the *E. fitchii* subsp. *albertii* population at the type locality near Alice, Jim Wells Co., TX. The Black Lace Recovery Plan (USFWS, 1987) indicates the original population reported by Dr. R. Albert in 1965 was bulldozed and ploughed, but that a second population discovered by Dr. R. Albert in an area of approximately 1.5 hectares within dense mesquite brush was undisturbed as of 1985. The population was reportedly large with hundreds if not thousands of individuals (USFWS, 1987). We examined Google Earth images of the area, which revealed that the eastern end of the range had been bulldozed but that the remainder was dense thornscrub with breaks in the canopy that looked similar to known habitats in Kleberg and Refugio Cos. We conducted an extensive search of potential habitat at the type location in Jim Wells County over an area of approximately 125,000 square metres.

Morphological examination: We examined morphological features of *E. fitchii* subsp. *albertii* and the two taxa of the *E. reichenbachii-fitchii* species complex that occur in closest geographic proximity to black lace cactus, *E. fitchii* subsp. *fitchii* and *E. reichenbachii* subsp. *caespitosus*. Photographs were taken of plants in Texas populations of *E. fitchii* subsp. *albertii*, *E. fitchii* subsp. *fitchii*, and *E. reichenbachii* subsp. *caespitosus* between 2015 and 2019. Figure 3 shows populations visited for photographic evidence. The photographs were analysed to determine rib counts, presence/absence and number of central spines, colour of central spines, number of radial spines, colour of radials, and flower colour. Means and standard deviations were calculated for the number of ribs and number of radial spines.

Phylogenetic study: Taxa examined: We included six taxa of the *E. reichenbachii-fitchii* species complex native to the United States in the

phylogenetic study at the same time collecting photo vouchers of each specimen sampled (Table 2). All populations of *E. fitchii* subsp. *albertii* included in the morphological examination were also included in the phylogenetic study, with the exception of the McMullen Co. population A and the La Salle Co. population (Figure 3). Populations of the taxa are well-documented in publicly accessible herbarium records and summarised in the literature (Blum et al. 2005), which proved a successful source for selecting sampling locations. We collected specimens of *E. reichenbachii* subsp. *comanchensis*, however DNA extraction from this taxon was unsuccessful. We were not able to collect specimens from outside of the United States, thus did not include members of the complex native to Mexico (*E. reichenbachii* subsp. *reichenbachii*, *E. reichenbachii* subsp. *burrensis*, *E. fitchii* subsp. *bergmanii*, *E. primolanatus*, *E. armatus*). Taxa used as outgroups are shown in Table 2.

Genome skimming: Genome skimming can be defined as using next-generation DNA sequencing technology (e.g. the Illumina sequencing platform) to obtain hundreds of thousands of short DNA sequence ‘reads’ from the high copy fraction of the genome. Using bioinformatics tools and/or other available genomes as references, genome skimming sequence reads can be assembled into nearly complete sequences for the nuclear ribosomal region and the chloroplast genome (Straub et al., 2012). Aligned nuclear ribosomal sequences and chloroplast genome sequences from across the species of interest can be used to build a phylogenetic tree and infer evolutionary relationships.

Sample preparation: To acquire genome skimming data, DNA was extracted from tepal tissue or epidermal tissue of one to five individuals from each of six populations of *E. fitchii* subsp. *albertii*, four populations of *E. fitchii* subsp. *fitchii*, three of *E. reichenbachii* subsp. *baileyi*, seven of *E. reichenbachii* subsp. *caespitosus*, two of *E. reichenbachii* subsp. *perbellus*, and one of *E. reichenbachii* var. *albispinus* (Table 2). Data were also acquired for one to two individuals from each of nine outgroup or sister species (Table 2), as part of the current project or as part of other projects in Dr. Fehlberg’s lab, including *E. arizonicus* subsp. *arizonicus*, *E. arizonicus* subsp. *nigrihorridispinus*, *E. bonkeriae*, *E. chloranthus*, *E. enneacanthus* subsp. *intermedius*, *E. fendleri* var. *fendleri*, *E. nicholii*, *E. poselgeri* and *E. triglochidiatus* subsp. *triglochidiatus*.

DNA was extracted using a modified CTAB Direct Column Cleaning procedure (Doyle and Doyle, 1987; Cullings, 1992). For each individual

sample, 0.030–0.060g of dried tissue was weighed and placed in a 2mL plastic, screw top tube with two 5mm steel balls. The screw top tubes were placed in a five-tube adapter for the Retsch MM200 mixer mill (Retsch GmbH, Haan, Germany), and the mixer mill was run at 25Hz for two minutes. Once samples were ground, 800 μ L of CTAB, which included approximately 0.032g of PVP-10 and 4 μ L of RNase A (Qiagen, Hilden, Germany), was added to each tube. Tubes were inverted to mix the contents and placed in a heat block at 55 $^{\circ}$ C overnight for lysis. Contents were mixed by inverting the tubes at least twice during the incubation period.

Following incubation and lysis, 500 μ L of 24:1 chloroform/isoamyl alcohol solution was added to each tube, and tubes were vortexed vigorously and centrifuged at 14,000 rpm for ten minutes. While tubes were centrifuging, two DNA silica membrane binding columns (EconoSpin #1920, Epoch Life Sciences, Missouri City, TX, USA) for each sample were prepared for use by placing each column in a 2mL collection tube and adding 20 μ L of 3M sodium acetate. Following centrifugation, 600 μ L of the supernatant was transferred to a clean 2mL tube and gently mixed by pipetting with 900 μ L of PB binding buffer (Qiagen). Half of the mixture (750 μ L) was transferred to each of the two columns prepared for each sample. Columns and collection tubes were immediately centrifuged at 14,000rpm for one minute and the filtrate was discarded. Columns were washed by adding 750 μ L of PE wash buffer (Qiagen) and centrifuging at 14,000rpm for one minute. The filtrate was discarded, and columns and collection tubes were centrifuged at 14,000rpm for two minutes to dry. Columns were transferred to a clean 1.5mL tube and 100 μ L of TE buffer (10mM TRIS, 1mM EDTA, pH 8.0), pre-heated to 55 $^{\circ}$ C, was added to each column. The tubes and columns were incubated at 55 $^{\circ}$ C for 15 min and centrifuged at 4,000 rpm for 1 minute. These steps were repeated with an additional 100 μ L of TE buffer for a total of 200 μ L elute per column, 400 μ L elute per extraction. To obtain enough DNA for genome skimming, multiple extractions were performed from the same individual sample, combined, and concentrated using a vacufuge (Eppendorf, Hauppauge, NY, USA). DNA concentrations for combined/concentrated extractions were determined using a Qubit 2.0 fluorometer (Life Technologies, Waltham, MA, USA) to verify the presence of enough DNA (average 1337ng total, between 230ng and 3623ng) and submitted to RAPiD Genomics (<http://rapid-genomics.com/home/>), a next-generation sequencing service provider.

RAPiD Genomics uses established protocols to acquire genome skimming sequence data (150 base pair (bp), paired-end reads) on an Illumina HiSeq platform. Briefly, this protocol includes shearing the DNA into 150bp fragments, adding Illumina-specific ‘adapter’ sequences to these sheared fragments, and adding unique ‘barcode’ sequences individually to fragments from each sample, prior to pooling and running all samples together in a single lane or across several lanes of the Illumina HiSeq platform. Once samples were run at RAPiD Genomics, raw data were parsed into separate data files for each individual sample using the unique barcodes and returned to us via FTP.

Genome skimming data analyses: To assemble nuclear ribosomal sequences and nearly complete chloroplast sequences for each *Echinocereus* sample submitted for genome skimming, raw sequence data in fastq format (called ‘reads’) were first imported into the program GENEIOUS (Biomatters, Auckland, NZ). Once in GENEIOUS, forward and reverse reads from each sample were linked as ‘paired-ends’, and linked reads were trimmed and filtered using the ‘BBduk’ plugin (<https://sourceforge.net/projects/bbmap/>) to remove adapter sequences, low quality sequences (<20 Q score), and short sequences (<36bp).

A nearly complete sequence of the 26S-18S nuclear ribosomal region, including partial non-transcribed spacer region, external transcribed spacer region [ETS], 18S rRNA gene, internal transcribed spacer region 1 (ITS1), 5.8S rRNA gene, internal transcribed spacer region 2 (ITS2) and partial 26S rRNA gene, for *Echinocereus arizonicus* (generated by our lab through a combination of traditional DNA sequencing and genome skimming) was used as a reference for mapping all trimmed, filtered reads from each sample separately using the built-in GENEIOUS ‘map to reference’ function with the following settings: medium-low sensitivity fast, iterate up to twenty-five times, save contigs, and generate consensus based on a majority threshold. This resulted in complete nuclear ribosomal region assemblies for each *Echinocereus* sample submitted for genome skimming.

A de novo assembly of the chloroplast genome for *E. reichenbachii* subsp. *caespitosus* (available from Lucas C. Majure, Assistant Curator of the Herbarium, Florida Museum of Natural History, University of Florida, Gainesville, FL; Majure 5679) was used as a reference for mapping all trimmed, filtered reads from each sample as described above. This resulted in nearly complete chloroplast genome assemblies for each *Echinocereus* sample submitted for genome skimming.

| Population locality by County and site (if applicable) | Atascosa A | Atascosa B | Atascosa C | Atascosa D |
|--|--------------|--------------|-------------|--------------|
| Sample size | 10 | 15 | 5 | 12 |
| Morphological features | | | | |
| Mean rib number (SD) | 13.25 (0.54) | 12.97 (0.9) | 13 (0) | 13 (0) |
| Rib number (min–max) | 13–15 | 10–14 | 13–13 | 13–13 |
| Number of mature individuals with a central spine | 0 | 0 | 0 | 0 |
| Central spine number (min–max) | 0 | 0 | 0 | 0 |
| Mean radial spine number (SD) | 22.1 (1.43) | 20.73 (1.75) | 20.1 (0.96) | 19.33 (1.07) |
| Radial spine number (min–max) | 18–24 | 16–24 | 18–22 | 16–22 |
| Number of individuals with dark-tipped radial spines | 10 | 15 | 5 | 12 |
| Number of individuals lacking | 0 | 0 | 0 | 0 |
| Number of individuals with radial tip inconsistent in colour | 0 | 0 | 0 | 0 |

Table 3. Morphological characteristics of *Echinocereus fitchii* subsp. *albertii* populations.

All nuclear ribosomal region assemblies were aligned using the ‘MAFFT’ plug-in (Katoh et al., 2002; Katoh and Standley, 2013) in GENEIOUS and visually inspected for DNA sequence quality and proper alignment. When low coverage regions or regions with poor alignment were observed (indicated by few mapped reads and low-quality sequence information), these regions were deleted from the alignment. All chloroplast genome assemblies from *Echinocereus* were also aligned using the ‘MAFFT’ plug-in and inspected and edited as described above.

Phylogenetic trees were estimated from edited alignments for nuclear ribosomal region or chloroplast genome assemblies in two ways 1 using the built-in GENEIOUS tree builder based on Jukes-Cantor genetic distances and neighbour-joining and 2 using the ‘PhyML’ plug-in (Guindon et al., 2010) in GENEIOUS based on maximum likelihood with a GTR+gamma model of substitution and NNI branch swapping. Strengths

of inferred phylogenetic relationships from either tree building method were evaluated with 1000 bootstrap replicates.

Results and discussion

Site visits: We photo-documented *E. fitchii* subsp. *albertii* individuals and confirmed extant populations in Atascosa, Kleberg, La Salle, McMullen, and Refugio counties (Figure 3). Despite an extensive search, we were not able to relocate the Jim Wells Co. population.

Kleberg Co. and Refugio Co. each have one known population. The Kleberg Co. population occurs in small clearings among dense thorn scrub on sandy loam soil in close proximity to tidal creeks of the Jaboncillos Creek drainage system. The Refugio Co. population occurs in saline silty soil in pockets of open land in Tamaulipan thorn scrub. The Refugio Co. site lies within the Aransas River drainage system.

Jeremiah McKinney of our research team has

| Atascosa E | Kleberg | La Salle | McMullen A | McMullen B | Refugio | All |
|--------------|--------------|--------------|--------------|--------------|-----------------|-------|
| 10 | 25 | 8 | 16 | 12 | 17 | 130 |
| | | | | | | |
| 13 (0) | 11.98 (0.78) | 12.82 (0.37) | 13.72 (0.86) | 12.92 (0.29) | 12.06 (1.14) | 12.76 |
| 13–13 | 11–13 | 12–13 | 12–16 | 12–13 | 10–13 | 10–16 |
| 0 | 23 | 1 | 1 | 1 | 0 | 26 |
| 0 | 0–1 | 0–1 | 0–1 | 0–1 | 0 | 0–1 |
| 21.56 (1.55) | 16.92 (1.5) | 17.94 (2.72) | 21.5 (2.13) | 18.67 (1.53) | 16.92 (2.35) | 19.14 |
| 19–24 | 12–20 | 14–22 | 16–26 | 14–22 | 12–21 | 12–26 |
| 10 | 25 | 7 | 8 | 10 | 17 | 119 |
| 0 | 0 | 0 | 3 | 1 | 0 | 4 |
| 0 | 0 | 1 | 5 | 1 | 0 | 7 |

Table 3. (continued)

identified populations on lands leased for surface mining by the San Miguel Electric Cooperative, Inc. extending the species' range into Atascosa Co. At least fourteen distinct small clusters of *E. fitchii* subsp. *albertii* plants in Atascosa Co., broadly split into a northern group and a southern one, have been found. The Atascosa Co. sites occur in proximity to Metate Creek within the Nueces River drainage system. The clusters occur in clearings in mesquite scrub. Impacts from mining may have contributed to fragmentation of what was previously a more contiguous population into the clusters of plants that exist today. We examined five of the clusters in this study, which we designate as Atascosa sites A–E. Distances between the sampled locations, starting with the most northern and working south, are as follows: Atascosa site B–C is 3.3km; Atascosa site C–D is 0.44km; Atascosa site D–A is 0.96km. Two clusters we did not sample occur between sites B and C. This northern group (Atascosa sites A–D) is likely

best treated as one population.

The distance between Atascosa site A and E is 13.84km. It is likely that the southern-most Atascosa site E should be considered a second population distinct from the more northern population due to its distance from site A with no known clusters between sites A and E. However, it is uncertain whether the northern and southern groups may have previously been connected prior to establishment of the mine.

Populations occurring on land used by a mining company in McMullen Co. were identified by Jeremiah McKinney of our research team. We know of two populations in McMullen Co., which we have designated as populations A and B in this study. The McMullen Co. population A site occurs within the Nueces River drainage system. *Echinocereus fitchii* subsp. *albertii* plants in McMullen Co. population A grow on the very low banks of a creek and in clearings slightly inland. Plants in McMullen Co. population B grow in

| Population locality by County and site (if applicable) | Jim Hogg | Starr A | Starr B | Starr C | Starr D | Webb A | Webb B | All |
|--|----------------|----------------|----------------|----------------|----------------|----------------|----------------|-------|
| Sample size | 2 | 4 | 6 | 2 | 3 | 3 | 5 | 25 |
| Morphological features | | | | | | | | |
| Mean rib number (SD) | 13 (1.41) | 13.3 (0.5) | 13.7 (1.21) | 13.5 (0.71) | 13.3 (0.58) | 13 (0) | 12.8 (0.45) | 13.2 |
| Rib number (min-max) | 12-14 | 13-14 | 12-15 | 13-14 | 13-14 | 13-13 | 12-13 | 12-15 |
| Number of mature individuals with a central spine | 2 | 4 | 6 | 2 | 3 | 3 | 5 | 25 |
| Central spine number (min-max) | 3-5 | 4-5 | 1-6 | 3-5 | 2-4 | 3-5 | 1-7 | 1-7 |
| Mean radial spine number (SD) | 20.5 (2.12) | 19.7 (1.32) | 20.1 (2.08) | 19.8 (1.06) | 19.0 (3.12) | 18.3 (2.31) | 18.7 (1.60) | 19.4 |
| Radial spine number (min-max) | 18-22 | 18-22 | 16-22 | 18-21 | 15-23 | 16-22 | 16-21 | 15-23 |
| Number of individuals with dark-tipped radial spines on new growth | 2 | 4 | 6 | 2 | 3 | 3 | 5 | 25 |
| Number of individuals with dark-tipped radial spines | 2 | 4 | 6 | 2 | 3 | 3 | 5 | 25 |
| Number of individuals lacking dark-tipped radial spines | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Number of individuals with radial tip inconsistent in colour | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 4. Morphological characteristics of *Echinocereus fitchii* subsp. *fitchii* populations.

| Population locality by County and site (if applicable) | Blanco | Comal | Gillespie | Hays A | Hays B | Mason A | Mason B | Medina A | Medina B | All |
|--|--------------|--------------|--------------|--------------|--------------|--------------|-------------|-------------|--------------|-------|
| Sample size | 3 | 3 | 6 | 8 | 7 | 6 | 9 | 5 | 7 | 54 |
| Morphological features | | | | | | | | | | |
| Mean rib number (SD) | 15.33 (1.53) | 13.17 (0.29) | 13.67 (1.51) | 15.07 (1.92) | 15.07 (1.74) | 14.33 (0.82) | 17 (2.45) | 14.7 (0.91) | 14.71 (1.58) | 14.98 |
| Rib number (min–max) | 14–17 | 13–14 | 11–15 | 13–20 | 13–21 | 13–15 | 15–21 | 13–16 | 13–18 | 11–21 |
| Number of mature individuals with a central spine | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mean radial spine number (SD) | 26 (0.5) | 21.83 (2.57) | 24.9 (1.87) | 22.5 (2.62) | 24.0 (1.66) | 24.5 (4.38) | 19.75 (2.8) | 25.2 (1.79) | 22.64 (2.61) | 22.32 |
| Radial spine number (min–max) | 25–28 | 18–25 | 22–29 | 18–28 | 22–28 | 15–31 | 15–23 | 22–28 | 20–29 | 15–31 |
| Number of individuals with dark-tipped radial spines on new growth | 1 | 3 | 4 | 1 | 5 | 3 | 7 | 4 | 3 | 31 |
| Number of individuals with dark-tipped radial spines | 1 | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 5 |

Table 5. Morphological characteristics of *Echinocereus reichenbachii* subsp. *caespitosus* populations.

clearings in the Tamaulipan thorn scrub west of Choke Canyon Reservoir, within the Frio River drainage system. McMullen Co. population B occurs 16.57km north-east of population A. The McMullen Co. population B is approximately 6.69 km from the Atascosa Co. site E.

Most recently a population has been confirmed extending the species' range into La Salle County. The landowner of the site contracted an environmental consultant regarding a proposed pipeline across the ranch. At the consultant's request one of our research team members, Chris Best, visited the site on the 18 June 2020 and verified the presence of black lace cactus. The La Salle Co. population occurs in gravelly saline soils containing gypsum on a low ridge just above the flood plain of a tributary of the Nueces River.

While previous descriptions of *E. fitchii* subsp. *albertii* habitat in Jim Wells, Refugio and Kleberg

counties indicated an altitude of less than 50m (Blum et al., 1998; Blum et al., 2005; USFWS, 2009), our observations reveal that the taxon occurs at altitudes up to 116m. At the lowest elevations, the Kleberg Co. population occurs at 2–5m and the Refugio Co. population at 1–6m. The clusters of plants in Atascosa Co. occur at altitudes ranging from 67–103m. The McMullen Co. population A occurs at 96m. The McMullen Co. population B occurs at 85–87m. The recently discovered La Salle population occurs at altitudes ranging from 112–116m.

Morphological study: Examination of the morphological characteristics reveals that *E. fitchii* subsp. *albertii*, *E. fitchii* subsp. *fitchii* and *E. reichenbachii* subsp. *caespitosus* are similar in rib number (Tables 3, 4, 5). We have observed plants of *E. fitchii* subsp. *albertii* in flower at all sites visited in this study, with the exception of



Figure 4. Flower of *Echinocereus fitchii* subsp. *albertii* growing in Refugio Co. population. Photograph Peter Berresford. **Figure 5.** Flower of *Echinocereus fitchii* subsp. *fitchii* plant from Starr Co. population growing in cultivation. Photograph Martin Terry. **Figure 6.** Flower of *Echinocereus reichenbachii* subsp. *caespitosus* growing in Hays Co. B population. Photograph Paula S. Williamson. **Figure 7a.** *Echinocereus fitchii* subsp. *albertii* plants growing in McMullen Co. A population showing mature plant lacking central spines and juveniles at base. **Figure 7b.** Shows closer view of central spine exhibited by juvenile.

the McMullen Co. population A that was visited at a time plants were not in flower. The flowers are pink-purple with darker centres in the flower throat (Figure 4). *Echinocereus fitchii* subsp. *fitchii* flowers are pink with dark centres (Figure 5). *Echinocereus reichenbachii* subsp. *caespitosus* flowers are pink with a white-green zone in the centre (Figure 6). Flower colour of *E. fitchii* subsp. *albertii* is characteristic of the *E. fitchii* group rather than the *E. reichenbachii* group that have a whitish zone in the flower centre, which is not present in flowers of the *E. fitchii* group.

Central spines are absent to rare in *E. fitchii* subsp. *albertii* individuals in most populations (Table 3). Central spines are rare in plants growing



Figure 8a. *Echinocereus fitchii* subsp. *albertii* plant growing in Kleberg Co. population showing mature plant with central spines.



Figure 8b. *Echinocereus fitchii* subsp. *albertii* plant growing in Kleberg Co. population. Closeup of central spine.

in the McMullen Co. populations. One mature plant examined growing in the McMullen Co. population A exhibited a single central spine, and we also noted that a juvenile present around the base of a mature plant of *E. fitchii* subsp. *albertii* in this population exhibited central spines (Figure 7). One plant growing in the McMullen Co. population B was also found to possess single central spines. Only plants growing in the Kleberg Co. population commonly exhibit central spines (Table 3). A single, large ($\leq 1\text{cm}$), black central spine that is straight and stiff is present in the areoles of most individuals (Figure 8).

All *E. fitchii* subsp. *fitchii* individuals examined exhibit central spines, with central spine number ranging from 1–7 (Table 4). Central spines were not present on either juvenile or mature *E. reichenbachii* subsp. *caespitosus* plants in any population examined in this study (Table 5). Although most *E. fitchii* subsp. *albertii* individuals do not exhibit central spines, the presence of

central spines in any population aligns with Blum et al.'s (1998) placement of black lace cactus in the *E. fitchii* group as opposed to the *E. reichenbachii* group.

Echinocereus fitchii subsp. *albertii* radial spines number between 12 and 26 (Table 3). *Echinocereus fitchii* subsp. *fitchii* individuals have 15–23 radial spines (Table 4). Both of these taxa have light-coloured radial spines with black or dark-tips (Tables 3, 4). Individuals growing in the Refugio Co. population have lighter coloured radial spine tips than individuals from other populations. *Echinocereus reichenbachii* subsp. *caespitosus* plants have white radial spines ranging in number from 15–31, and tend to only have dark-tipped radial spines on new growth (Table 5).

Genetic study: Genome skimming and assembly of nuclear ribosomal regions and nearly complete chloroplast genomes was successful for all 55 samples representing 15 taxa of *Echinocereus*. Nuclear ribosomal region assemblies for the 43

| nr nucleotide differences | <i>Echinocereus reichenbachii</i> | | | | <i>Echinocereus fitchii</i> subsp. | | |
|---|-----------------------------------|-----------------------|------------------------|-------------------------|------------------------------------|----------------------------|----------------|
| | subsp. <i>caespitosus</i> | subsp. <i>baileyi</i> | var. <i>albispinus</i> | subsp. <i>perbellus</i> | <i>albertii</i> Refugio/Tilden | <i>albertii</i> all others | <i>fitchii</i> |
| <i>E. reichenbachii caespitosus</i> | 6 | | | | | | |
| <i>E. reichenbachii baileyi</i> | 5 | 5 | | | | | |
| <i>E. reichenbachii albispinus</i> | 10 | 10 | 0 | | | | |
| <i>E. reichenbachii perbellus</i> | 8 | 8 | 4 | 1 | | | |
| <i>E. fitchii albertii</i> Refugio/Tilden | 10 | 8 | 15 | 12 | 12 | | |
| <i>E. fitchii albertii</i> all others | 18 | 14 | 19 | 18 | 16 | 14 | |
| <i>E. fitchii fitchii</i> | 17 | 15 | 14 | 13 | 16 | 14 | 11 |

A

| cp nucleotide differences | <i>Echinocereus reichenbachii</i> | | | | <i>Echinocereus fitchii</i> subsp. | | |
|---|-----------------------------------|-----------------------|------------------------|-------------------------|------------------------------------|----------------------------|----------------|
| | subsp. <i>caespitosus</i> | subsp. <i>baileyi</i> | var. <i>albispinus</i> | subsp. <i>perbellus</i> | <i>albertii</i> Refugio/Tilden | <i>albertii</i> all others | <i>fitchii</i> |
| <i>E. reichenbachii caespitosus</i> | 57 | | | | | | |
| <i>E. reichenbachii baileyi</i> | 74 | 16 | | | | | |
| <i>E. reichenbachii albispinus</i> | 107 | 94 | 0 | | | | |
| <i>E. reichenbachii perbellus</i> | 160 | 160 | 135 | 14 | | | |
| <i>E. fitchii albertii</i> Refugio/Tilden | 79 | 56 | 94 | 161 | 24 | | |
| <i>E. fitchii albertii</i> all others | 1730 | 1727 | 1728 | 1738 | 1716 | 68 | |
| <i>E. fitchii fitchii</i> | 1725 | 1723 | 1721 | 1731 | 1711 | 84 | 60 |

B

Table 6. Mean number of differences found between aligned sequences for 43 ingroup samples representing six populations of *Echinocereus fitchii* subsp. *albertii*, four of *E. fitchii* subsp. *fitchii*, three of *E. reichenbachii* subsp. *baileyi*, seven of *E. reichenbachii* subsp. *caespitosus*, two of *E. reichenbachii* subsp. *perbellus*, and one of *E. reichenbachii* var. *albispinus* for a) nuclear ribosomal region assemblies (6696 bp) and b) chloroplast genome assemblies (108,275 bp).

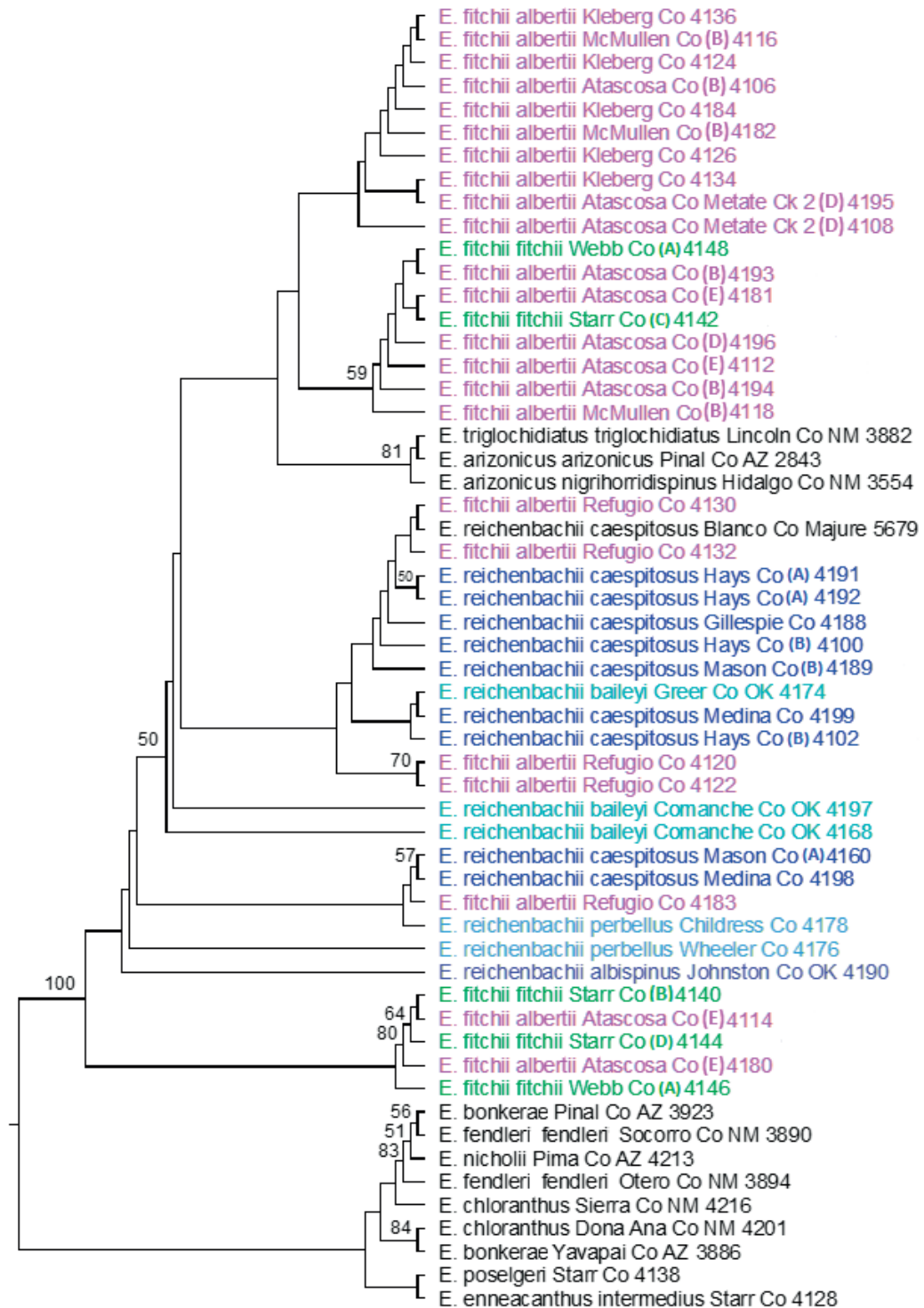


Figure 9. Phylogenetic relationships among 36 populations of *Echinocereus* sampled for this study based on maximum likelihood analysis with a GTR+gamma model of evolution for complete nuclear ribosomal sequences. Number above branches represent bootstrap values from 1,000 replicates. Counties are from Texas unless otherwise noted.

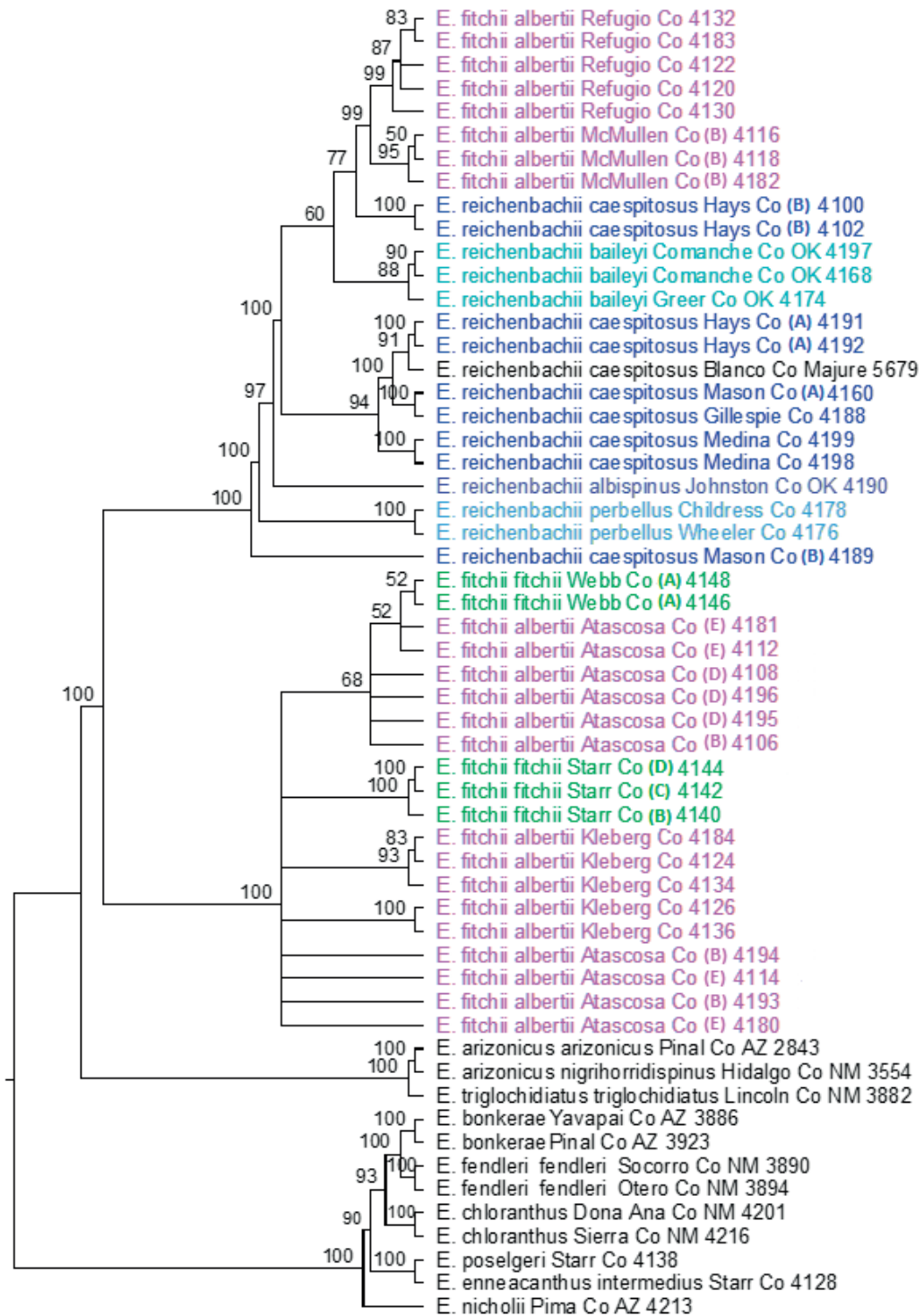


Figure 10. Phylogenetic relationships among 36 populations of *Echinocereus* sampled for this study based on neighbour-joining analyses of Jukes-Cantor genetic distances of nearly complete chloroplast genome sequences. Number above branches represent bootstrap values from 1,000 replicates. Counties are from Texas unless otherwise noted.

ingroup samples averaged 12,056bp each with 4282× coverage (depth of assembled reads), and the edited alignment of 55 samples plus the reference *E. reichenbachii* was 6696bp long. Reported coverage is artificially high due to the stacking of thousands of repetitive reads just outside the analysed nuclear ribosomal region. Chloroplast genome assemblies for the 43 ingroup samples averaged 111,201bp each with 819× coverage, and the edited alignment of 55 samples plus the reference *E. reichenbachii* was 108,275bp long. Based on comparisons of edited, aligned nuclear ribosomal region assemblies, the mean number of raw sequence differences (including base substitutions and insertion/deletions) among ingroup taxa was low, ranging from 4bp between *E. reichenbachii* subsp. *perbellus* and *E. reichenbachii* var. *albispinus* to 19bp between *E. reichenbachii* var. *albispinus* and some populations of *E. fitchii* subsp. *albertii* (Table 6a). Comparisons of edited, aligned chloroplast genome assemblies showed greater differences among ingroup taxa, with the mean number of sequence differences ranging from 56bp between *E. reichenbachii* subsp. *baileyi* and some populations of *E. fitchii* subsp. *albertii* to 1738bp between *E. reichenbachii* subsp. *perbellus* and some populations of *E. fitchii* subsp. *albertii* (Table 6b).

Results of nuclear ribosomal and chloroplast genomes are presented in Figures 9 and 10. The number above branches represent bootstrap values from 1,000 replicates. Bootstrap values indicate the robustness of the phylogenetic relationships shown in the tree as well as how many characters/base pairs support that relationship. Bootstrap values from 90–100 are considered highly supported (high confidence), values from 70–80 are moderately supported, and values 50–60 are poorly supported.

Phylogenetic analyses based on the nuclear ribosomal region resulted in poorly resolved genetic relationships due to the low number of sequence differences (Figure 9). Both the neighbour joining analysis of genetic distances (results not shown) and the maximum likelihood analysis similarly resolved populations of *E. fitchii* subsp. *albertii* across several unresolved clades. Most individuals of *E. fitchii* subsp. *albertii* were found in a single clade containing only *E. fitchii* subsp. *albertii*, with the exception of two individuals of *E. fitchii* subsp. *fitchii* from Webb Co. and Starr Co. Individuals of *E. fitchii* subsp. *albertii* from Refugio Co. were found in a clade with individuals of *E. reichenbachii* subsp. *caespitosus*. Two individuals of *E. fitchii* subsp. *albertii* from the southernmost Atascosa Co. site E were found

in a clade containing all other individuals of *E. fitchii* subsp. *fitchii*.

Phylogenetic analyses based on the chloroplast genome resulted in much better resolution of genetic relationships, including two well-supported, genetically distinct clades for the ingroup taxa (Figure 10). Results from neighbour joining analysis of genetic distances are shown as results from maximum likelihood analysis were less resolved, possibly because the model of evolution used was a poor fit for the chloroplast data. The first large clade included separate subclades for each subspecies or variety of *E. reichenbachii*, with the exception of individuals of *E. reichenbachii* subsp. *caespitosus* from Hays Co. population B. These individuals were resolved as sister to a clade containing individuals of *E. fitchii* subsp. *albertii* from Refugio Co. and McMullen Co. population B. One individual of *E. reichenbachii* subsp. *caespitosus* from Mason Co. population B displayed a large number of substitutions, possibly due to a lab error or due to hybridisation at this site. The second large clade included all individuals of *E. fitchii* subsp. *albertii* from sites B, D and E in Atascosa Co. and the Kleberg Co. population. Three individuals of *E. fitchii* subsp. *fitchii*, excluding those individuals from Webb Co. were resolved as a separate subclade, but the relationship of that subclade to other individuals in the larger clade was unresolved. Genetic differences between *E. fitchii* subsp. *albertii* individuals found in the two separate clades were large, with a mean of 1716bp differences (Table 6b). One difference observed between the nuclear ribosomal and chloroplast genome phylogenies was the placement of *E. fitchii* subsp. *albertii* individuals from the McMullen Co. population B.

Echinocereus fitchii subsp. *albertii* has been considered most closely related to *E. fitchii* subsp. *fitchii* (Blum et al., 1998). Our phylogenetic study, however, indicates two distinct clades. *Echinocereus fitchii* subsp. *albertii* individuals from Refugio Co. and McMullen Co. population B occur in a large clade with *E. reichenbachii* subsp. *caespitosus*, *baileyi*, *perbellus* and *albispinus*. A second large clade includes all individuals of *E. fitchii* subsp. *albertii* from Atascosa Co. and Kleberg Co. with individuals of *E. fitchii* subsp. *fitchii* from Webb Co. and Starr Co. Additionally, our morphological study revealed that *E. fitchii* subsp. *albertii* shows similarities to both the *E. fitchii* group and the *E. reichenbachii* group. Flower colour of *E. fitchii* subsp. *albertii* is characteristic of the *E. fitchii* group, while the absence of central spines in *E. fitchii* subsp. *albertii* individuals in most populations aligns with the *E. reichenbachii* group.

The most significant finding emerging from

this phylogenetic study is that two distinct chloroplast genome lineages can be distinguished in *E. fitchii* subsp. *albertii*. One lineage includes individuals from Refugio Co. and McMullen Co. population B, which are most closely related to *E. reichenbachii* subsp. *caespitosus*. The other lineage includes individuals from Atascosa and Kleberg Cos., which are most closely related to *E. fitchii* subsp. *fitchii*. These two lineages are not defined by geographic proximity. Such large chloroplast sequence differences observed within taxa can sometimes be explained by contemporary hybridisation and chloroplast capture (for example, acquisition of the chloroplast genome from *E. reichenbachii* subsp. *caespitosus* by *E. fitchii* subsp. *albertii* during a hybridisation event). However, contemporary hybridisation seems unlikely given the current disparate geographic distribution of the populations and taxa involved. It is possible that an ancestral hybridisation event(s) played a role in the formation of the taxon represented by individuals for *E. fitchii* subsp. *albertii* from Refugio and McMullen Cos., which would explain their close genetic relationship with *E. reichenbachii* subsp. *caespitosus* in the phylogenetic tree. While the distinction of individuals of *E. fitchii* subsp. *albertii* from Refugio Co. is also evident in the nuclear ribosomal phylogeny, individuals of *E. fitchii* subsp. *albertii* from McMullen Co. are found in a clade with all other *E. fitchii* subsp. *albertii*. Perhaps this phylogenetic pattern can best be explained by contemporary gene flow among individuals of *E. fitchii* subsp. *albertii* from Atascosa and McMullen Cos., as nuclear ribosomal genes are more likely to reflect contemporary genetic interactions and gene flow.

Beyond this significant finding, the phylogenetic study also provides some information about the relationship between *E. fitchii* subsp. *albertii* from Atascosa and Kleberg Cos. and *E. fitchii* subsp. *fitchii*. Phylogenetic analyses of both the nuclear ribosomal region and chloroplast genome indicate a close genetic relationship between these taxa, as would be expected for subspecific taxa. However, their recognition as separate entities is compatible with the current phylogenetic results. Both analyses support a genetically distinct *E. fitchii* subsp. *fitchii*, even though there is some shared genetic variation with *E. fitchii* subsp. *albertii*, specifically from Atascosa Co. The exact relationship between these two taxa will need to be further explored with additional sampling and a population genetic approach that provides greater resolution between closely related taxa.

While our understanding of relationships

among these taxa has been improved by phylogenetic analyses of the nuclear ribosomal region and chloroplast genome, additional genetic information from the nuclear genome of these taxa is needed. Because of the close relationship among these taxa, such genetic information will need to be based on genomic approaches that are able to gather large amounts of data across the nuclear genome. Understanding relationships among these taxa may require both a phylogenomic approach, such as targeted enrichment and sequencing of nuclear genes with a breadth of taxonomic and population sampling (Johnson et al., 2019), and a population genomic approach such as RADseq (Andrews et al., 2016) with deeper sampling of individuals and populations among a subset of taxa. Both types of data could provide greater resolution of relationships and could also be used for sophisticated analyses that jointly consider phylogenetic history and population genetic history, including gene exchange.

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Appendix 1. Overview of taxonomic relationships of examined materials in ingroups and outgroups.

| Genus: <i>Echinocereus</i> | | | | Author |
|-------------------------------|--|--|--|--------------------------|
| Subgenus: <i>Echinocereus</i> | | | | |
| INGROUPS | | | | |
| Section: <i>Reichenbachia</i> | | | | |
| Series: <i>Reichenbachia</i> | | | | |
| Species: | | | | |
| Subspecies: | | | | |
| <i>armatus</i> | | | | (Poselger) A.Berger |
| <i>chisoensis</i> * | | | | W.T.Marshall |
| <i>fitchii</i> | | | | subsp. <i>fitchii</i> * |
| | | | | subsp. <i>albertii</i> * |
| | | | | subsp. <i>bergmannii</i> |

Appendix 1. (continued)

| | | | | | |
|---|----------------------------------|------------------------------|-------------------------------|---|--------------------------------------|
| | | | <i>fobeanus</i> | subsp. <i>fobeanus</i> | Oehme |
| | | | | subsp. <i>metornii</i> | (G.Frank) W.Blum |
| | | | <i>primolanatus</i> | | Fritz Schwarz ex N.P.Taylor |
| | | | <i>reichenbachii</i> | subsp. <i>reichenbachii</i> | (Terschek ex Walpers) Britton & Rose |
| | | | | subsp. <i>baileyi</i>* | (Rose) N.P.Taylor |
| | | | | var. <i>albispinus</i>* | (L.D.Benson) |
| | | | | subsp. <i>burrensis</i> | G.Frank, Metorn et E.Scherer |
| | | | | subsp. <i>caespitosus</i>* | (Engelmann) W.Blum & Mich.Lange |
| | | | | subsp. <i>comanchensis</i> * | D.Felix, Oldach & J.Oldach |
| | | | | subsp. <i>perbellus</i>* | Britton & Rose) N.P.Taylor |
| | | | <i>rigidissimus</i> | subsp. <i>rigidissimus</i> | Engelmann) Rose |
| | | | | subsp. <i>rubispinus</i> | (G.Frank) N.P.Taylor |
| | | | <i>scheneri</i> | | G.Frank |
| OUTGROUPS | | | | | |
| | | Section: <i>Costati</i> | | | |
| | | Series: <i>Costati</i> | | | |
| | | | <i>enneacanthus</i> | subsp. <i>intermedius</i>* | (Moore) W.Blum & Mich.Lange |
| | | Section: <i>Echinocereus</i> | | | |
| | | Series: <i>Echinocereus</i> | | | |
| | | | <i>chloranthus</i> | subsp. <i>chloranthus</i>* | |
| | | Section: <i>Erecti</i> | | | |
| | | Series: <i>Erecti</i> | | | |
| | | | <i>bonkerae</i> | subsp. <i>bonkerae</i>* | Thornber & Bonker |
| | | | <i>fendleri</i> | subsp. <i>fendleri</i>* | (Engelmann) Sencke ex J.N.Haage |
| | | Series: <i>Fasciculati</i> | | | |
| | | | <i>nicholii</i>* | | L.D.Benson) B.D.Parfitt |
| | | Section: <i>Wilcoxia</i> | | | |
| | | | <i>poselgeri</i> | subsp. <i>poselgeri</i>* | Lemaire |
| | Subgenus: <i>Triglochidiatus</i> | | | | |
| | Section: <i>Triglochidiati</i> | | | | |
| | Series: <i>Compacti</i> | | | | |
| | | | <i>arizonicus</i> | subsp. <i>arizonicus</i>* | Rose ex Orcutt |
| | | | | subsp. <i>nigrihorridis-pinus</i>* | W.Blum & Rutow |
| | | | <i>triglochidiatus</i> | subsp. <i>triglochidiatus</i>* | Engelmann |
| * Indicates present in the USA Bold type indicates samples taken from these taxa in the study | | | | | |