Analysis of Genetic Diversity and Clarification of Species Boundaries in *Echinomastus erectocentrus* var. *acunensis* and Close Relatives

by

Alison Willis

A Thesis Presented in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Approved April 2020 by the
Graduate Supervisory Committee:

Shannon Fehlberg, Co-Chair
Martin F. Wojciechowski, Co-Chair
Elizabeth Makings

ARIZONA STATE UNIVERSITY

May 2020

ABSTRACT

Echinomastus erectocentrus (J.M. Coulter) Britton & Rose var. *acunensis* (W.T. Marshall) Bravo, the Acuña cactus, is a small, single-stemmed spherical cactus with a restricted distribution across the Sonoran Desert in southern Arizona and into northern Sonora, Mexico. Populations of *E. erectocentrus* var. *acunensis* are threatened by loss of habitat, climate change, predation, and border related impacts. Due to the severity of these threats and shrinking population sizes, *E. erectocentrus* var. *acunensis* was federally listed as endangered by the United States Fish and Wildlife Service in 2013. The varieties of *Echinomastus erectocentrus*, *E. erectocentrus* var. *acunensis* and *E. erectocentrus* var. *erectocentrus* (J.M. Coulter) Britton & Rose, share many morphological characteristics that make them difficult to distinguish from one another. *Echinomastus johnsonii* (Parry ex Engelm.) E.M. Baxter, a presumed closely related species, also has a high level of morphological overlap that further complicates our understanding of species boundaries and detailed morphological data for these three taxa indicate a geographical cline. The goal of this project is to document the genetic diversity within and among populations of *E. erectocentrus* var. *acunensis*, and its close relatives *E. erectocentrus* var. *erectocentrus* and *E. johnsonii*. To accomplish this, populations of *E. erectocentrus* var. *acunensis, E. erectocentrus* var. *erectocentrus*, *E. johnsonii* and the outgroup *Echinomastus intertextus* (Engelm.) Britton & Rose were sampled. Deoxyribonucleic acid (DNA) was extracted, and data were collected for nine microsatellite regions developed specifically for these taxa, and two microsatellite regions developed for *Sclerocactus*, a closely related genus. Standard population genetic measures were used to determine genetic variation and structure, and this observed

genetic differentiation was then compared to the current morphological understanding of the group. These analyses help improve the knowledge of the genetic structure of *E. erectocentrus* var. *acunensis* and inform the understanding of species boundaries and evolutionary relationships within the group by revealing genetic distinctiveness between all four taxa and hybrid populations between the two varieties. This information also reveals patterns of gene flow and population locations that have the highest conservation priority, which can be incorporated into efforts to conserve and protect this endangered species.

ACKNOWLEDGMENTS

I would first like to start by thanking Shannon Fehlberg for how much effort and time she put into this project and in guiding me through this whole process. Most of what I was able to accomplish in this project was due to her taking the time to teach me the ins and outs of working in a plant conservation genetics lab. I also want to thank the rest of my committee, both current and past, for the part they have played in this. I want to thank Julie Stromberg for encouraging me to apply to this program in the first place and for offering to serve as my co-chair before she had to sadly step down from the position. I want to thank Marty Wojciechowski for his guidance and input, and especially for stepping into the position as my co-chair when Julie had to leave. I want to thank Liz Makings for providing me with feedback and for being a strong, female role model that I could look up to throughout this process.

Thank you to the United States Fish and Wildlife Service for providing the funding for this research. And, thank you to the researchers and staff at the Desert Botanical Garden that provided useful information about the taxa studied and assistance in the field: Marc Baker, Steve Blackwell, Natalie Melkonoff, and Kevin Fehlberg.

I want to thank my Mom and Dad for all of the support they have offered me during my academic career, especially while completing this master's degree. Thanks for letting me rant about all of my stresses and thank you for the care you showed me, even in the small ways. I wouldn't have been able to make it this far without them both. I also want to thank the friends who have supported me throughout this process as well. I cherish the commiserating and fun times we all shared. Lastly, I want to thank JP Solves for all of the support he provided me as we went through this experience together. Thank

you for talking things through with me when I was stressed and for celebrating with me when things went right. Finding and going through this experience with the love of my life was a blessing and I hope I was able to help him just as much as he helped me.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

NATURAL HISTORY

Introduction

Echinomastus erectocentrus (J.M. Coulter) Britton & Rose var. *acunensis* (W.T. Marshall) Bravo, the Acuña cactus, is a small, single-stemmed spherical cactus that is distributed across the Sonoran Desert in Maricopa, western Pima, and Pinal counties of Arizona and northern Sonora, Mexico. Populations of *E. erectocentrus* var. *acunensis* are threatened by loss of habitat due to human development, climate change, predation, and border related impacts. Due to the severity of these threats and their interactions with shrinking population sizes, *E. erectocentrus* var. *acunensis* was federally listed as endangered by the United States Fish and Wildlife Service (USFWS) in 2013, and critical habitat was designated for the species by the USFWS in 2016. The accepted taxonomic treatment for this taxon according to Tropicos (2020) is *Echinomastus erectocentrus* subsp. *acunensis* (W.T. Marshall) U. Guzmán. However, the USFWS (2013) and the *Flora of North America* (Zimmerman and Parfitt 2003) continue to recognize this taxon as *Echinomastus erectocentrus* var. *acunensis* and it will be referred to as such throughout the remainder of this thesis. The goal of my project is to document the genetic diversity within and among populations of *E. erectocentrus* var. *acunensis*, and its close relatives *E. erectocentrus* var. *erectocentrus* (J.M. Coulter) Britton & Rose and *E. johnsonii* (Parry ex Engelm.) E.M. Baxter. This work will improve our knowledge of the genetic structure of *E. erectocentrus* var. *acunensis* and inform our understanding of the level of distinctiveness, species boundaries, and evolutionary relationships within the

group. This information will also be used to reveal patterns of gene flow and identify locations that have the highest conservation priority. There is also the potential for this information to be incorporated into the conservation plan for the variety.

Morphology

The following measurements and descriptions were taken from Heil and Melton (1994), the Arizona Rare Plant Guide (2001), Zimmerman and Parfitt (2003), and the USFWS (2013). The single stem of *E. erectocentrus* var. *acunensis* (Fig. 1.1) is cylindric, reaching up to 40 cm (16 in) tall and 9 cm (3.5 in) wide. It has 11-15 radial spines up to 2.5 cm (1 in) long and 2-5 central spines up to 3.5 cm (1.4 in) long. Central spines are purplish pink or nearly white with distal mauve tips, and the longest central spine is curved toward the apex of the plant. Radial spines are similar in color to central spines and widely spread. Flowering occurs from March to April, and fruiting occurs from April to May. Flowers range in color from pale to bright rose-pink and purple and measure 3.6-6 cm by 4-9 cm (1.4-2.3 in by 1.6-3.5 in). Fruits are pale green, 1.25 cm (0.5 in) long, and contain small, nearly black seeds. Fruits ripen in April and split longitudinally as they dry, exposing the seeds.

Closely related species within the *Echinomastus* genus share common morphological characteristics that can make them difficult to differentiate. In Arizona, *E. erectocentrus* var. *acunensis* is very similar to *E. erectocentrus* var. *erectocentrus*, and *E. johnsonii*, due to morphological and geographical overlap. Populations found north of Tucson, Arizona, are geographically, morphologically, and ecologically intermediate between *E. erectocentrus* var. *acunensis* and *E. erectocentrus* var. *erectocentrus*

(Zimmerman and Parfitt 2003). The *E. erectocentrus* varieties are replaced to the northwest by *E. johnsonii* and the two species are only weakly differentiated (Zimmerman and Parfitt 2003). Morphological analyses show overlap or only slight differences between the length and number of stem, spine, and floral characteristics of the varieties of *E. erectocentrus* and *E. johnsonii* (Baker 2007; Baker and Porter 2016). The mean values for nearly all characteristics measured between the three taxa decrease by population moving from the northwest to the southeast, but the most significant for distinguishing taxa are floral characteristics, and guard spine length and thickness (Baker 2007; Baker and Porter 2016). These results suggest a geographical cline exists among these three taxa from the northern Mojave Desert to the northern Sonoran Desert (Baker 2007; Baker and Porter 2016). *Echinomastus* also shares common morphological characteristics with *Sclerocactus,* a closely related genus (Porter et al. 2000; Baker 2007; Baker and Porter 2016). These morphological similarities have resulted in some taxonomic confusion within *Echinomastus* as well as between the genera *Echinomastus* and *Sclerocactus*.

Taxonomy

Echinomastus erectocentrus var. *acunensis*, like the genus *Echinomastus* as a whole, has a complex taxonomic history and has been recognized under different names under different taxonomic treatments since the genus was first proposed by Britton and Rose in 1922. *Echinomastus* currently contains five species and seven distinct taxa including varieties (Zimmerman and Parfitt 2003). The species that is the focus in this study was first collected in 1948 in Organ Pipe National Monument (OPNM) by the

superintendent at the time (Heil and Melton 1994) and mentioned by Marshall in his first edition (1950) of *Arizona's Cactuses* as *Echinomastus acunensis*. The name was validly published in 1953 (Marshall 1953). In 1969, Lymon Benson transferred *E. acunensis* to the genus *Neolloydia* as *Neolloydia erectocentra* var. *acunensis* (W.T. Marshall) L.D. Benson. The variety *acunensis* was then returned to the genus *Echinomastus* by Bravo in 1980 as *Echinomastus erectocentrus* var. *acunensis* (W.T. Marshall) Bravo. This name was used for the *Flora of North America* treatment by Zimmerman and Parfitt (2003) and for federal listing by the USFWS (2013), although the currently accepted name is *E. erectocentrus* subsp. *acunensis* (W.T. Marshall) U. Guzmán (Guzmán 2003), according to Tropicos (2020).

These taxonomic changes were made in the absence of molecular evidence, but a phylogenetic analysis based on chloroplast sequence data by Porter et al. (2000) later resolved *Echinomastus* and *Sclerocactus* as sister monophyletic groups making decisions between *Echinomastus* and *Sclerocactus* subjective (Porter et al. 2000; Baker 2007; Baker and Porter 2016). Based on the additional morphological evidence reported in Baker and Porter (2016), it has been suggested that *Echinomastus* be included within *Sclerocactus*, even though *Echinomastus* has priority over *Sclerocactus* (Britton and Rose 1922). However, the current consensus is that *Echinomastus* retain its generic rank until further evidence for its inclusion in *Sclerocactus* is found (Baker 2007; Baker and Porter 2016).

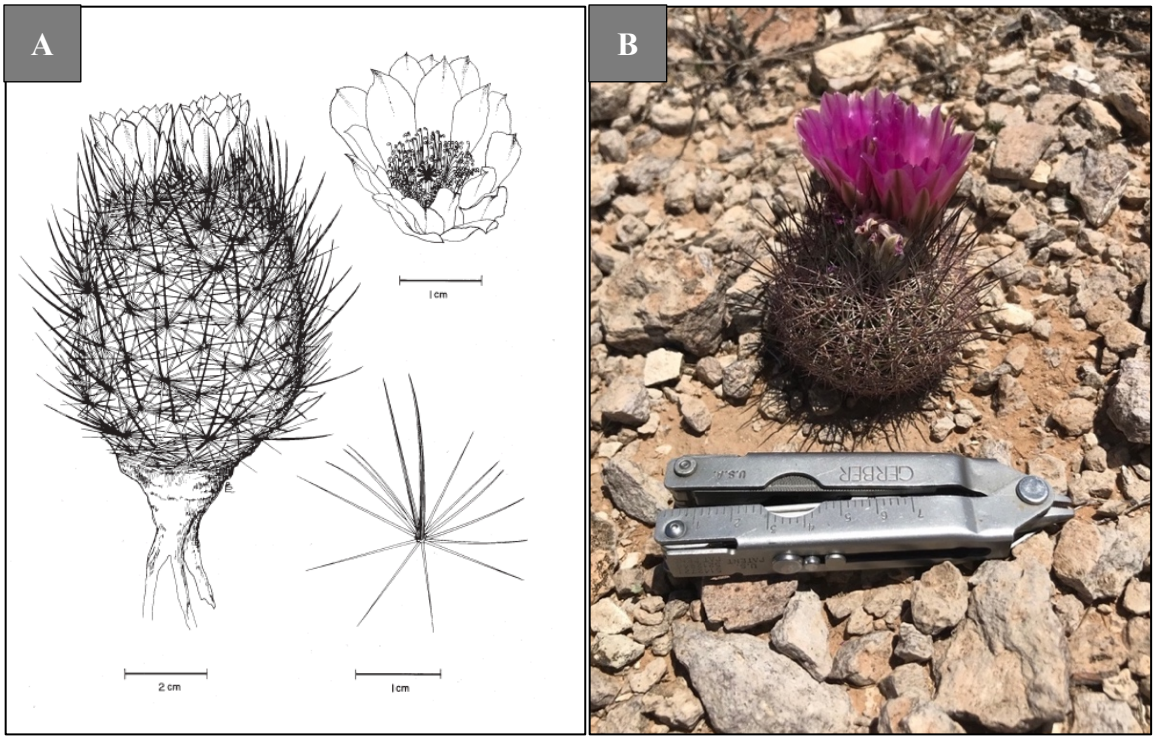


Figure 1.1. Morphology of *Echinomastus erectocentrus* var. *acunensis*. **A**) botanical drawing of *E. erectocentrus* var. *acunensis* taken from the Arizona Rare Plant Guide (2001) **B**) photo of *E. erectocentrus* var. *acunensis* in habitat in the Sonoran Desert National Monument.

Evolutionary Relationships

Echinomastus taxa have been included in molecular phylogenetic studies of the related genus *Sclerocactus* (Porter et al. 2000; Porter et al. 2012), and some limited molecular phylogenetic work has been done within the *Echinomastus* genus as a whole (Baker and Porter 2016). The results have shown that *Echinomastus* is closely related to *Sclerocactus* and that *Sclerocactus* is resolved as a monophyletic group within the paraphyletic group that includes *Echinomastus*, making them sister groups (Baker and Porter 2016). Based on their results, Baker and Porter (2016) suggest including *Echinomastus* within *Sclerocactus*. These authors also suggest treating *E. erectocentrus* var. *acunensis*, *E. erectocentrus* var. *erectocentrus* and *E. johnsonii* as a single species with three varieties based on the morphological cline they observe. Phylogenetic trees based on analyses of morphological characters show that *E. erectocentrus* var. *acunensis* has more morphological characters similar with *E. johnsonii* than *E. erectocentrus* var. *erectocentrus* (Baker 2007; Baker and Porter 2016). Furthermore, results from all of the analyses done on morphological characters indicate that *E. erectocentrus* var. *acunensis* individuals are intermediate between *E. erectocentrus* var. *erectocentrus* and *E. johnsonii* (Baker 2007; Baker and Porter 2016). Phylogenetic results constructed from chloroplast deoxyribonucleic acid (DNA) also show that within *Echinomastus,* the *E. erectocentrus* varieties and *E. johnsonii* are sister taxa, with *Echinomastus intertextus* (Engelm.) Britton & Rose as the sister group to these taxa (Baker and Porter 2016). *Echinomastus intertextus* was included as an outgroup in the analysis done below due to its widespread geography and its close phylogenetic relationship to the other taxa.

Species concepts are often debated, and species boundaries in Cactaceae can be especially difficult to distinguish since taxa within this family are often capable of hybridizing (Powell et al. 1991; Griffith 2003; Tepedino et al. 2010). Population genetic studies have been done within *Sclerocactus* by Schwabe et al. (2015) examining the genetic diversity and potential hybridizations between species within that genus. Due to the close relationship between *Sclerocactus* and *Echinomastus,* a similar approach for studying the genetic diversity and potential hybridization among closely related taxa of *Echinomastus*, and even using some of the same genetic markers, holds promise. Genetic analysis of *E. erectocentrus* var. *acunensis*, as well as neighboring *E. erectocentrus* var. *erectocentrus* and *E. johnsonii* populations will elucidate genetic relationships and provide information to clarify species boundaries. These techniques could also provide support for the observed morphological cline (Baker 2007; Baker and Porter 2016). The goal of my research is to provide molecular evidence that may inform any taxonomic revisions and which, in turn, may have consequences for the conservation plan of *E. erectocentrus* var. *acunensis*.

Distribution and Habitat Type

Echinomastus erectocentrus var. *acunensis* is known from five populations in Arizona and one in northern Sonora, Mexico (OPNM Report 2006, Fig. 1.2), in patchy distributions across granitic hills, benches and flats at elevations ranging from 400-1200 meters (Heil and Melton 1994). The most consistently monitored populations are those found in Organ Pipe National Monument where plots were established in 1977, with additional plots being added in 1983 and 1988 (OPNM Report 1995; OPNM Report

2006). Other populations can be found in the vicinity of the Sand Tank Mountains in the Sonoran Desert National Monument (SDNM), the peak of Coffeepot Mountain within the Sauceda Mountains, and outside the cities of Ajo, Florence and Wickenburg, Arizona. The possible existence of a population in the Barry M. Goldwater Air Force Range has been suggested in previous reports and the Federal Register references a single individual found in 1997, but this individual was not found when the site was revisited in 2012 (OPNM Report 2006; USFWS 2013). There is also potential habitat in the Tohono O'odham tribal lands, but no populations there have been confirmed (OPNM Report 2006). Other populations could exist in similar habitat types elsewhere in the Sonoran Desert.

Echinomastus johnsonii is found throughout the Mojave Desert and into the northwestern edges of the Sonoran Desert scrub on rocky slopes and gravely hills at 500-1100 m in elevation in Utah, Nevada, Arizona and California (Zimmerman and Parfitt 2003). *Echinomastus erectocentrus* var. *erectocentrus* is found in the grasslands of the Sonoran and Chihuahuan Deserts on low gravelly hills and bajadas on igneous and calcareous substrates in southeastern Arizona (Zimmerman and Parfitt 2003). The two *E. erectocentrus* varieties can be found in the same habitat type (Zimmerman and Parfitt 2003).

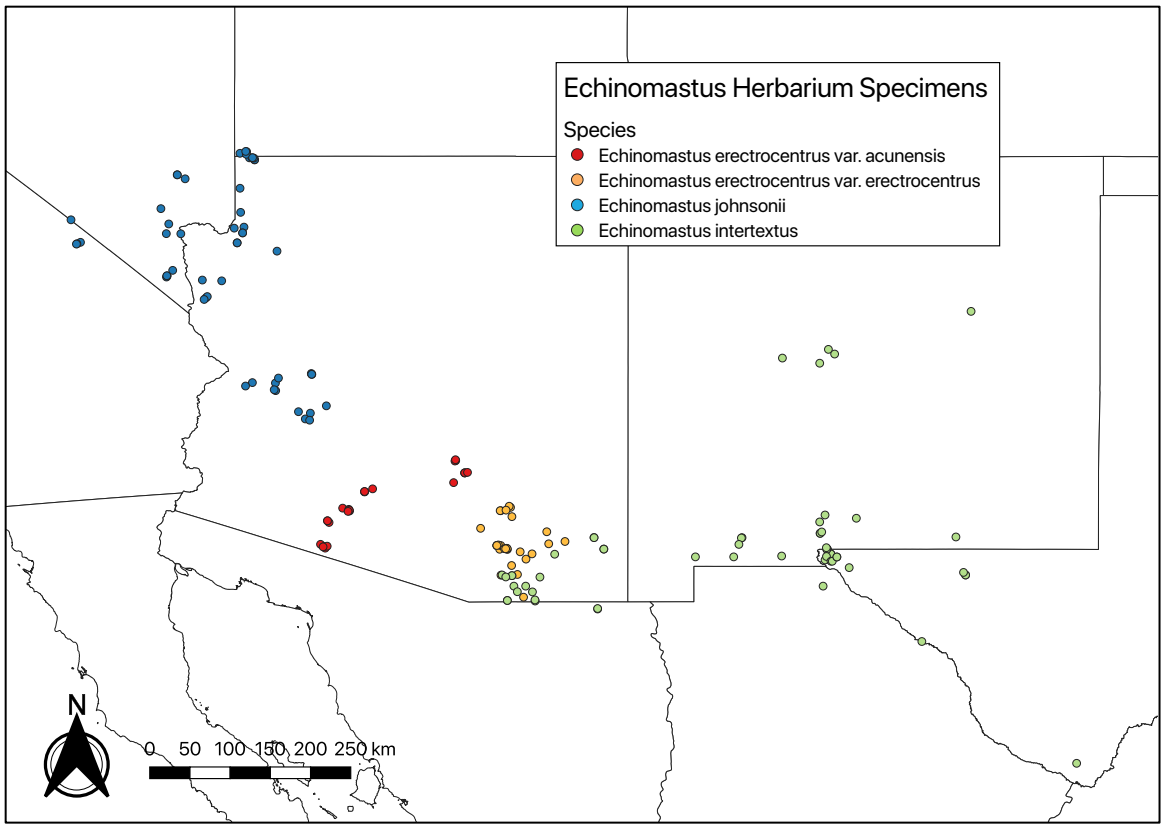


Figure 1.2. Map of collection localities of herbarium specimens of *Echinomastus erectocentrus* var. *acunensis* (red), *E. erectocentrus* var. *erectocentrus* (yellow), *E. johnsonii* (blue), and *E. intertextus* (green) that were sourced from SEINet (ARIZ, ASC, ASU, BRY, DES, MABA, NMC, NMCR, SJNM, UCR herbaria).

Reproduction, Pollination and Insect Predation

The plots at Organ Pipe National Monument have been used to monitor pollinators and the reproduction patterns of *E. erectocentrus* var. *acunensis* since 1977 (OPNM Report 1995; OPNM Report 2006). *Echinomastus erectocentrus* var. *acunensis* is self-incompatible and relies on insect vectors for pollination and appears to rely on numerous bee species, the most abundant of which are *Megachile palmensis* Mitchell and *Diadasia rinconis* Cockerell (Johnson 1991, 1992). The percentage of flowering individuals and number of flowers produced are observed to increase with plant height, with the smallest flowering individual measuring 24 mm tall (Johnson 1992). Flower number is also observed to be correlated with plant height and width, suggesting that water availability limits flower production (Johnson 1992). Seeds germinate primarily during or following summer monsoons, depending on the amount of summer precipitation and timing of rainfall, while juvenile survival is correlated with summer water availability and rainfall (Johnson et al. 1993). Recent declines in population size have been observed in populations of *E. erectocentrus* var. *acunensis*, but it is still unclear if these are signals of long-term decline or natural fluctuations (OPNM Report 2006). It is also unclear if these declines are dependent on precipitation level patterns, as no singular factor can explain all declines (OPNM Report 2006).

Observations of insect herbivory were also collected with the pollinator studies. Larvae of the *Opuntia* borer, *Moneilema gigas* LeConte (Cerambycidae), prey on the roots and later the interiors of larger individual plants, typically ≥ 90-100 mm tall, which kills the plant (Johnson 1992). Fruits and seeds are consumed by larvae of the pyralid moth, *Yosemitia graciella* Hulst, which interferes with successful germination (Johnson

1991; OPNM Report 2006). Another beetle identified as possibly belonging to family *Nitidulidae* is commonly found in *E. erectocentrus* var. *acunensis* flowers and is assumed to consume pollen and other floral parts, but it is not observed to move from flower to flower, suggesting that it is not a pollinator (Johnson 1991, 1992).

Population Sizes of Acuña Cactus

Long-term trends in population size are a significant concern for the survival for *E. erectocentrus* var. *acunensis*. Population sizes and trends are summarized as follows from the 2013 Federal Register in which *E. erectocentrus* var. *acunensis* was listed as endangered, unless otherwise noted. The large area within Organ Pipe National Monument where the long-term monitoring plots were established had only as many as 2,000 individuals across the entire 1,326 ha in 2011. This same population was estimated at 10,000 individuals in 1981 (Heil and Melton 1994). There were 200 individuals recorded at the recently observed population found at the Sand Tank Mountains in the Sonoran Desert National Monument. In the Sauceda Mountains, within the Coffeepot Area of Critical Environmental Concern, a total of 310 individuals were reported in plots that were established for monitoring in 1982, and in 2008 the same population was found to contain 77 individuals. A few populations exist in the Ajo area, the biggest of which is on Indian Village Hill. In 1996 there were 102 individuals, but by 2013 only 33 living plants were found. The Mineral Mountain population near Florence was estimated to have around 100 individuals in the 1990's, and in 2011 when the site was revisited, there were only 33 individuals found alive (USFWS 2013). This noticeable decline in *E. erectocentrus* var. *acunensis* populations during the relatively short period of time that

they have been monitored is due to the various threats they face. These threats will be covered in more detail below.

Echinomastus erectocentrus var. *acunensis* was federally listed as endangered by the USFWS in 2013, and areas of critical habitat for this species, which covers approximately 7,501 hectares in Maricopa western Pima, and Pinal counties, were designated in 2016. While populations of *E. erectocentrus* var. *acunensis* experience significant decreases in size and the threat of extinction, other *Echinomastus* species, such as *E. johnsonii,* have large, steady population numbers and do not appear to be as seriously threatened. In 1993, the status of *E. erectocentrus* var. *erectocentrus* was reviewed along with *E. erectocentrus* var. *acunensis*, but a lack of sufficient data on vulnerability has left it unlisted (USFWS 1993). While *E. erectocentrus* var. *erectocentrus* is not officially endangered, it has been recognized as a species of concern, and its State Rank is designated as S3: Vulnerable by the state of Arizona (AZGFD 2019).

Threats to Acuña Cactus

Threats to *E. erectocentrus* var. *acunensis* are summarized as follows from the 2013 Federal Register listing, unless otherwise noted. The observed declines in population sizes of *E. erectocentrus* var. *acunensis* can be attributed to habitat loss due to human developments including border-related impacts, changes in climate, and predation. Populations such as those near Florence and Ajo have been threatened by the loss of habitat through municipal developments including roads leading in and out of the city, and in Ajo specifically, loss of habitat through mining developments. These same

populations are threatened by recreational human activities such as ORV use. Populations along smuggling or illegal immigration routes of the United States-Mexico border are also under threat from trampling and vehicular traffic. While livestock have not been observed consuming *E. erectocentrus* var. *acunensis*, they have been observed to trample the cacti and their grazing can have a negative impact on the environment. Due to its endangered status and physical characteristics, *E. erectocentrus* var. *acunensis* is a target rare cactus for illegal collecting and evidence of poaching at some locations has been recorded (Buskirk 1981; Phillips and Buskirk 1982; OPNM Report 1995; USFWS 2013). When conducting field work in the Sonoran Desert National Monument for this project, I observed small, semi-shallow holes that had been dug using a tool adjacent to existing individuals of *E. erectocentrus* var. *acunensis*, showing signs of possible poaching.

The plot data from Organ Pipe National Monument show mortality of individuals far outweighs recruitment; most of the mortality occurs at the seedling and juvenile stage and is related to low precipitation levels and timing of rain in the seasons proceeding germination (OPNM Report 1995, 2006). The shifting of rainy seasons and the amount of precipitation are changing along with other climatic factors, and this could have adverse effects on the germination cycles of this species. This along with other stressors such as predation from insects are concerns for long-term survival.

The level of threat that *E. erectocentrus* var. *acunensis* faces has been determined to be severe enough that human interference will be required for the taxon to remain viable. Initial steps, such as designating critical habitat have been put into place, but more information is required to develop long-term plans (USFWS 2013). Determining the level of genetic diversity and level of gene flow currently present in populations of *E.*

erectocentrus var. *acunensis* will provide the USFWS and other members of the scientific community with the information needed to develop these long-term plans. Comparing *E. erectocentrus* var. *acunensis* data to its close relatives will deepen our understanding of the evolutionary history and help clarify species boundaries between and among closely related taxa, identify populations of concern, and inform conservation efforts, such as reintroductions, and other interventions regarding its survival.

CHAPTER 2

GENETIC DIVERSITY AND SPECIES BOUNDARIES

Introduction

Given ongoing changes to climate and the effect this has on global ecosystems, the long-term survival of many species is concerning. More studies are being done to understand the full scope of threats that species are facing, and conservation plans are being developed in order to help reduce these threats. While many animal groups have garnered much attention and research, there remain large gaps of information for others such as plants. This lack of information is concerning, given how dependent most species are on plants in some fashion, including humans (Goettsch et al. 2015, 2018). Organizations such as the International Union for Conservation of Nature (IUCN) are collecting information and assessing threat level for various plant species and of the groups that have been fully assessed by the IUCN, Cactaceae is the fifth most threatened major taxonomic group and the most species rich with approximately 1,500 (Goettsch et al. 2015, 2018; IUCN 2019).

As species are assessed, data such as threats, population sizes, distribution, habitat, and phenology are collected and made available to governing bodies to provide information that may be used to enact and enforce policies that will help conserve the species. In the United States, for example, plants are assessed and protection is enforced by the United States Fish and Wildlife Service (USFWS) based upon the criteria set out by the Endangered Species Act (ESA) of 1973. Due to limited funds, decisions regarding funding are made based on certain criteria such as the importance of the species, the level

of threat, and the timeframe over which results are achieved (Gerber 2016). Morphological variation in populations can further complicate the decision to protect certain species especially when species boundaries are uncertain (Moritz 1999).

Morphologically unique populations have often been a focus for conservation, even though the underlying genetic diversity of these populations may be low (Moritz 1999). The level of genetic diversity between populations is not a specific criterion examined when listing a species as endangered, but it does have an overall effect on the long-term survival and success of a species (Reed and Frankham 2003). Smaller population sizes have been linked to reduced fitness and future adaptability due to the increased level of inbreeding and genetic drift that tends to occur (Reed and Frankham 2003). These findings stress the importance of the conservation of genetic diversity, not just for the survival of current populations, but for the evolutionary potential of the species as well (Rao and Hodgkin 2002; Reed and Frankham 2003). It may be argued that rather than focusing on preserving specific populations that may express certain phenotypic characteristics, conservation efforts should be on the underlying molecular diversity needed for maintaining and restoring evolutionary processes (Moritz 1999; Coates et al. 2018). The level of genetic diversity found in plant taxa is a result of a combination of biological (such as breeding systems and longevity) and ecological factors (such as geographic distances, differences in temperatures, and differences in moisture availability) (Rao and Hodgkin 2002; Rayamajhi and Sharma 2018). Important evolutionary processes like hybridization can also have a positive effect on the level of genetic diversity found in populations through the introduction of new genetic variation (Whitham et al. 1999).

While genetic diversity is not a strict criterion used when assessing threats to species, the overall size and number of populations is an important target for conservation for both the IUCN and the ESA (ESA 1973; IUCN 2019). Our understanding of species boundaries can alter the perception of the size and number of populations, yet even the concept of species can be unclear as definitions change depending on the organism of interest (de Querioz 2007). Differing criteria between these definitions are a result of defining a species before the process of speciation is complete (de Querioz 2007). Hybridization frequently occurs in closely related plant species with overlapping ranges, especially in populations that are located in extreme habitats (Harrison and Larson 2014; Schwabe et al. 2015). The presence of undetected molecular hybrids can muddle boundaries based on morphological species and these hybrids can actually suggest recent speciation events (Harrison and Larson 2014; Schwabe et al. 2015). While better metrics of defining species boundaries are needed, the presence of properties associated with genetically distinct species can be used to support the inference of species boundaries (de Querioz 2007). One property typically associated with separate species is limited gene flow, suggesting that high levels of genetic differentiation between taxa can be used to separate them (de Querioz 2007; Abbott et al. 2013).

When focusing on the conservation of Cactaceae, these evolutionary processes should be even more of a focus since Cactaceae has a more recent evolutionary divergence compared to other plant groups, such as cycads and conifers, that have been fully assessed by the IUCN according to the Red List criteria (Arakaki et al. 2011; Goettsch et al. 2015). While the Cactaceae lineage is thought to have originated in the late Eocene, the high level of extant diversity found in this family stems from its radiation

during the Miocene and Pliocene which occurred during environmental changes that led to the formation of many of our modern desert ecosystems (Arakaki et al. 2011). Novel pollination syndromes also played a part in the diversification of Cactaceae during the Miocene (Hernández-Hernández et al. 2014). Recent diversification also suggests the Cactaceae are actively moving through various evolutionary processes, such as hybridization and speciation. A prominent characteristic in Cactaceae that can be explained by this recent diversification is the predominance of narrow endemism (Duarte et. al 2014).

Endemics can be the result of recent speciation events or the final remnants of ancient communities (Payton et. al 2019). Endemic species can offer a glimpse into the past or represent the leading edge of evolutionary processes when the factors that influence its endemism are revealed (Payton et. al 2019). Due to the mix of characteristics in endemic species, such as restricted habitat and low populations levels, studying patterns of genetic diversity can also give insight into how isolation, selection, and drift contribute to speciation (Payton et. al 2019). While endemics are useful in understanding recent evolutionary history, the mix of characteristics that they exhibit also make them prone to going extinct (Hernandez and Gomez-Hinostrosa 2011; Isik 2011). While this is a normal consequence of evolutionary processes, it is important to study these species before they disappear, and the information is lost. Endemic species also tend to have a higher conservation priority, due to their geographic restrictions and rarity (Hernandez and Gomez-Hinostrosa 2011).

The Acuña cactus, *Echinomastus erectocentrus* (J.M. Coulter) Britton & Rose var. *acunensis* (W.T. Marshall) Bravo, is a small, single-stemmed spherical cactus that is

endemic to the Sonoran Desert. It is found in restricted habitats across Maricopa, western Pima, and Pinal counties of Arizona and northern Sonora, Mexico. Populations of *E. erectocentrus* var. *acunensis* are threatened by loss of habitat due to human development, climate change, predation, and border related impacts (USFWS 2013). Due to the severity of these threats and their interactions with shrinking population sizes, *E. erectocentrus* var. *acunensis* was federally listed as endangered by the USFWS in 2013, and critical habitat was designated for the species in 2016. In 1993, the status of *Echinomastus erectocentrus* var. *erectocentrus* (J.M. Coulter) Britton & Rose, the other variety within the *E. erectocentrus* species, was reviewed along with *E. erectocentrus* var. *acunensis*, but a lack of sufficient data on vulnerability left it unlisted (USFWS 1993).

Due to the similar morphology of *E. erectocentrus* var. *acunensis*, *E. erectocentrus* var. *erectocentrus,* and another closely related species *Echinomastus johnsonii* (Parry ex Engelm.) E.M. Baxter taxonomic distinctions between the three taxa are unclear. Detailed morphological data of these varieties and *E. johnsonii* indicate a geographical cline from the northern Mojave Desert to the northern Sonoran Desert (Baker 2007; Baker and Porter 2016). When looking at the populations sampled by Baker, the average values of most morphological characteristics, such as central spine length and ovary length, decrease by taxon from the northwest to southeast of their distributions (Baker 2007). Populations of both varieties of *Echinomastus erectocentrus* found north of Tucson, Arizona, are geographically, morphologically, and ecologically intermediate between *E. erectocentrus* var. *acunensis* and *E. erectocentrus* var. *erectocentrus* (Zimmerman and Parfitt 2003). Both varieties of *E. erectocentrus* are

replaced to the northwest by *E. johnsonii* and the two species are only weakly differentiated suggesting current populations of *E. johnsonii* represent the basal taxa and that future generations radiated out to the southeast, creating a gradient of characteristics across populations of *E. johnsonii* through populations of *E. erectocentrus* var. *acunensis* into populations of *E. erectocentrus* var. *erectocentrus* (Zimmerman and Parfitt 2003; Baker and Porter 2016).

The limited molecular phylogenetic work done within the group shows a close relationship between *Echinomastus* and *Sclerocactus* and that *Sclerocactus* is a monophyletic group within *Echinomastus*, making *Echinomastus* paraphyletic (Baker and Porter 2016). The same molecular phylogenetic work indicates *E. erectocentrus* and *E. johnsonii* are sister taxa within the same clade, with *Echinomastus intertextus* (Engelm.) Britton & Rose a close relative in a separate neighboring clade (Baker 2007; Baker and Porter 2016). Accordingly, some have suggested altering the taxonomy to include the two varieties of *E. erectocentrus* within *E. johnsonii*, or even treating all *Echinomastus* taxa as *Sclerocactus* (Baker 2007; Baker and Porter 2016). While no genetic hybrid populations have previously been identified and there is no evidence that gene flow is occurring across taxa, the evolutionary history of *E. erectocentrus* var. *acunensis* is muddled and morphological intermediates among these three taxa make species boundaries unclear.

The goal of this project is to document the genetic diversity found within and among populations of *E. erectocentrus* var. *acunensis*, as well as between *E. erectocentrus* var. *acunensis* and its close relatives *E. erectocentrus* var. *erectocentrus* and *E. johnsonii*. Population-level samples were collected, ensuring that the geographic

range and morphological variation of each target taxon was adequately represented. Microsatellite data were collected and analyzed to determine evolutionary relationships among populations and taxa, taking current taxonomic and morphological understandings of the group into account. Comparing the genetic diversity of *E. erectocentrus* var. *acunensis* with other closely related taxa will help clarify the evolutionary processes that have been at work at the generic level as well as expand our understanding of the taxon's evolutionary future. Understanding the level of genetic diversity present among and between populations of *E. erectocentrus* var. *acunensis* and its close relatives could alter our current understanding of the species, and in turn alter the focus of conservation efforts.

Methods

Field Sampling

Echinomastus erectocentrus var. *acunensis* is currently known from five general areas in Arizona and one large area in northern Sonora, Mexico (USFWS 2013). For this project, all five populations of *E. erectocentrus* var. *acunensis* were visited and sampled in March-April 2018 (Fig. 2.1, Table 2.1). Populations were sampled from Organ Pipe National Monument (OPNM), just south of the town of Ajo, Coffeepot Mountain within the Sauceda Mountains, Sand Tank Mountains within the Sonoran Desert National Monument (SDNM), Box-O Canyon near the town of Florence, and Mineral Mountain near the town of Kearny. Multiple sites were visited for collecting in Organ Pipe National Monument and Box-O Canyon, with a distance of roughly 2.5 km and 1.5 km respectively between collection sites in both areas. Due to the uncertainty in how far

pollinators can travel and the resulting degree of gene exchange across the distances between sampling locations, individuals sampled in Organ Pipe National Monument and Box-O Canyon were treated as two subpopulations each. At each population, including subpopulations, tepals and/or spines from at least 12 distinct individuals were sampled for a total of 97 *E. erectocentrus* var. *acunensis* individuals.

Populations of *E. erectocentrus* var. *erectocentrus*, *E. johnsonii*, and *E. intertextus* were also visited and sampled in March-April 2016, 2017 and 2018 (Fig. 2.1, Table 2.1). Three populations of *E. erectocentrus* var. *erectocentrus* were sampled from south of the town of San Manuel, west of the city of Wilcox, and near Davidson Canyon southeast of the city of Tucson, all in Arizona. Tepals from at least 14 distinct individuals were sampled at each population for a total of 42 *E. erectocentrus* var. *erectocentrus* individuals. Four populations of *E. johnsonii* were sampled from Date Creek and Vulture Mountain near the town of Wickenburg in Arizona, and northwest of the town of Moapa and northeast of the town of Searchlight in Nevada. Tepals from an average of 15 distinct individuals were collected at each population for a total of 61 *E. johnsonii* individuals. Two populations of *E. intertextus* were sampled from Anthony Gap southeast of Las Cruces and Florida Gap southeast of Deming in New Mexico. Tepals from 11 individuals were collected at each population for a total of 22 *E. intertextus* individuals. The total number of individuals sampled across all four taxa was 222.

All of the sites visited for sampling were selected from information found from herbarium vouchers served on Southwest Environmental Information Network (http://swbiodiversity.org/seinet/index.php) and sites visited by Baker and Porter (2016) for their morphological study, when possible. Sites were documented with photographs

and latitude/longitude coordinates for each sampled plant and linked to previous voucher specimens, as noted in Table 2.1. For each individually sampled plant at each of the sites, 3-5 tepals, a single bud, or spines were carefully removed and placed in silica gel until deoxyribonucleic acid (DNA) extraction. Care was taken when selecting tepals to remove, so as not to harmfully alter the reproductive success of the flower. If spines were collected for DNA extraction, they were fresh spines removed from the top of the plant.

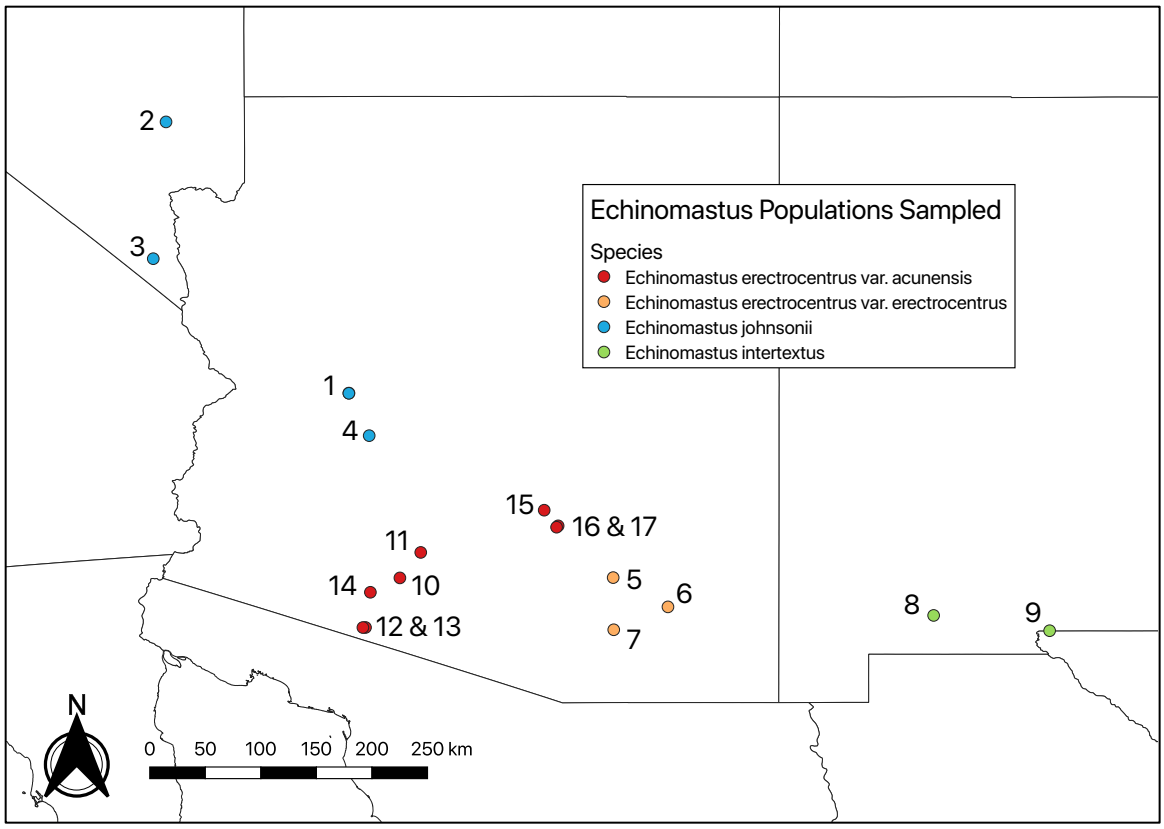


Figure 2.1. Map of sampled populations of *Echinomastus erectocentrus* var. *acunensis* (red), *E. erectocentrus* var. *erectocentrus* (yellow), *E. johnsonii* (blue), and *E. intertextus* (green). Population numbers are defined in Table 2.1.

Table 2.1. The populations of *Echinomastus* taxa sampled in this study with taxon name, location, sample size, corresponding latitude and longitude, elevation, field number and historical voucher used to determine sampling locations.

Taxon	Location	Pop. Sample Size	Latitude-North	Longitude-West	Elevation (m)	Field no. (SDF)	Voucher
Echinomastus johnsonii	Wickenburg/ Date Creek (1)	18	34.22578398	-113.072272	885	31016, 33016	Baker 16144, Hodgson 29918
	North of Moapa (2)	14	36.76486001	-114.782571	672	31216-1	Hodgson 25008A
	NNW of Searchlight (3)	16	35.48516602	-114.902013	1168	31216-2	Baker 16543-1, 2, 4
	Wickenburg/ Vulture Mine (4)	13	33.82959296	-112.882053	683	33018 -1	Baker 16130
Echinomastus erectocentrus var. erectocentrus	S of San Manuel/ FR 4450 (5)	16	32.501641	-110.599546	1115	32516-1	Baker 16117 Hodgson 29597
	W of Wilcox/ Cascabel Rd (6)	14	32.22777598	-110.087052	1474	32516-1	Baker 16119
	Davidson Canyon (7)	12	32.013615	-110.594194	1084	32616-1	Baker 15556-3, Puente 5123
Echinomastus intertextus	Florida Gap (8)	11	32.14773903	-107.60158	1461	30917	Baker 16121
	Anthony Gap (9)	11	32.00476002	-106.516426	1362	31017	Baker 16123
Echinomastus erectocentrus var. acunensis	Coffee Pot (10)	12	32.49878604	-112.594709	721	31418	Baker 15241
	Sonoran Desert National Monument (11)	16	32.73724297	-112.399656	1112	40218	Baker 16148, Holm 20000914-3
	Organ Pipe Population A (12)	13	32.03510997	-112.916376	512	33118-1	Baker 7586, Baker 7718
	Organ Pipe Population B (13)	14	32.03459004	-112.940932	482	33118-2	Baker 7586, Baker 7718
	Ajo (14)	14	32.36439303	-112.870829	567	33018 -2	Rutman 20070619-3
	Kearny (15)	14	33.13226203	-111.244819	629	32618	Baker 16383, Anderson 6-2009
	Box-O Canyon Population A (16)	10	32.98591359	-111.1144118	825	32518	Baker 16120, Hodgson 4479
	Box-O Canyon Population B (17)	4	32.973143	-111.128959	805	32518	Baker 16120, Hodgson 4479

DNA Extraction

DNA was extracted using a modified CTAB Direct Column Cleaning procedure (Doyle and Doyle 1987; Cullings 1992). For each individual sample, 0.030-0.060 g of dried tissue was weighed out and placed into a 2 mL screw top tube with two steel balls, one below the sample and the other above. The screw top tubes were then placed into a five-tube adapter for the Retsch MM200 mixer mill (Retsch GmbH, Haan, Germany). The mixer mill was run at 25 Hz for 2 min, and this step was repeated as necessary to grind all of the samples fully. Once the samples were fully ground, 800 µl of CTAB buffer, which included 4% PVP-40 and 10 µl of 5x proteinase K, was added to each tube. The 5x proteinase K contained 2.5mg/mL of lyophilized proteinase K (Amresco, Solon, OH, USA) in TE Buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Tubes were inverted to mix the contents and then placed in a heat block at 55°C for 120 min of lysis. Contents were mixed by inverting the tubes at least twice during the 120 min incubation.

Following the 120 min of lysis, 500 µl of 24:1 chloroform/isoamyl alcohol solution was added to each tube, and tubes were vortexed vigorously and centrifuged at 18,400 rcf for 10 min. While the tubes were centrifuging, a DNA silica membrane binding column (EconoSpin #1920, Epoch Life Sciences, Missouri City, TX, USA) was prepared for use by placing each column in a 2 mL collection tube and adding 20 µl of 3 M sodium acetate and 450 µl of PB binding buffer (Qiagen, Hilden, Germany). Once the tubes had been centrifuged for 10 min, 450 µl of the supernatant was transferred to the prepared columns. The supernatant and PB binding buffer were gently mixed by pipetting, columns and collection tubes were immediately centrifuged at 18,400 rcf for 1 min, and the filtrate was discarded. Columns were washed by adding 750 µl of PE Wash

Buffer (Qiagen) and centrifuging at 18,400 rcf for 1 min. The filtrate was discarded, and the columns and collection tubes were centrifuged at 18,400 rcf for 2 min to dry. Columns were then transferred to a clean 1.5 mL safe-lock tube and 150 µl of TE Buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), which was heated to 55˚C, was added to each column. The safe-lock tubes and columns were incubated at 55˚C for 15 min and centrifuged at 1,500 rcf for 1 min. These steps were then repeated with an additional 150 µl of TE Buffer for a total of 300 µL. Before storing, each DNA extraction was appropriately labelled and quantified using a NanoDrop Fluorospectrometer (Thermo Scientific, Wilmington, DE, USA). If DNA concentration for an individual sample fell below 3.0 ng/µL, DNA was extracted again.

Microsatellites and DNA Sequencing

Microsatellite regions for this project were selected from regions previously developed by Schwabe et al. (2013) and Zumwalde et al. (2019). Based on our current knowledge of phylogenetic relationships (Baker and Porter 2016), *Sclerocactus* forms a monophyletic group within *Echinomastus*, making *Echinomastus* paraphyletic. This close relationship suggests that microsatellites developed for *Sclerocactus* by Schwabe et al. (2013) could potentially be used in *Echinomastus*. Of the microsatellites developed by Schwabe et al. (2013), initial tests indicated potential utility of SCGL 71, SCGL 346, SCGL 401, SCGL 416, SCGL 446, and SCGL 704. Further testing determined that two regions, SCGL 71 and SCGL 704, resulted in usable data for this study (Table 2.2). Of the microsatellites developed for *Echinomastus* by Zumwalde et al. (2019), nine regions, ECHMA 1, ECHMA 3, ECHMA 4, ECHMA 5, ECHMA 6, ECHMA 10, ECHMA 16,

ECHMA 21 and ECHMA 25, resulted in usable data for this study (Table 2.2). Because three regions ECHMA 1, ECHMA 3, and ECHMA 16, did not amplify in *E. intertextus*, two separate data sets were created: a four-taxa data set that included eight microsatellite regions for all individuals (222) of *E. erectocentrus* var. *acunensis*, *E. erectocentrus* var. *erectocentrus*, *E. johnsonii*, and *E. intertextus*; and a three-taxa data set that included all 11 microsatellite regions for all individuals (200) of *E. erectocentrus* var. *acunensis*, *E. erectocentrus* var. *erectocentrus*, and *E. johnsonii*.

Polymerase chain reactions (PCR) for all microsatellite regions were performed in 12.7 µl volume reactions containing 5.9 µl of nuclease-free water, 1.3 µl Promega 5x PCR buffer, 1.0 µl 25 mM $MgCl_2$, 1.0 µl 10 mM dNTPs, 1.3 µl 10 mM bovine serum albumin, 0.23 µL 10 µM 5'-GTTT-3' tagged reverse primer, 0.02 µL 10 µM 5'-CAGTCGGGCGTCATCA-3' tagged forward primer, 0.2 µL 10 µM 5'-CAGTCGGGCGTCATCA-3' FAM-labeled primer, 0.2 µL GoTaq DNA Polymerase (5U/µl; Promega, Madison, WI, USA), and 1.5 µL DNA template (15 ng/µL maximum). Thermocycling conditions (Mastercycler Pro, Eppendorf, Westbury, NY, USA) consisted of a touchdown protocol with an initial denaturation step of 2 min at 94˚C followed by 20 cycles of 96˚C for 30 sec, 60˚C for 30 sec (decreased 0.5˚C per cycle), and 72˚C for 30 sec; 20 cycles of 96˚C for 30 sec, 50˚C for 30 sec, and 72˚C for 30 sec; and a final elongation step of 10 min at 72˚C.

Amplification products were screened through gel electrophoresis to determine if reactions were successful. If successful, the PCR products were purified and run on an ABI 3730 Capillary Electrophoresis Sequencer at the Arizona State University DNA sequencing facility using a LIZ 600 internal size standard (Applied Biosystem, Waltham,

MA, USA). Microsatellite allele lengths for each individual were determined manually from the raw output of the capillary sequencer using the program GENEMARKER (SoftGenetics, State College, PA, USA). Whole number allele sizes were determined objectively from decimal allele sizes for each microsatellite region across all individuals and taxa using the program TANDEM (Matschiner and Salzberger 2009). Due to incompatibilities with TANDEM, whole number allele sizes for ECHMA 1, ECHMA 3, ECHMA 6, ECHMA 10, and SCGL 704 were determined manually.

Table 2.2 Primer sequences and characteristics for nine microsatellite regions isolated from *Echinomastus* (ECHMA) and two microsatellite regions from *Sclerocactus glaucus* (SCGL).

Region	Primer Sequence (5'-3')	Repeat Motif	Allele size range (bp)	No. of Alleles	T_a (°C)
ECHMA1*	F: GGGGAGCTTGGTGTGTGC R: CCTCTTGGGCTCAATGTTGC	$(TTC)_{39}$	165-220	26	52.3
ECHMA3*	F: TTCCCCAAAACGGACATAGC R: CGTTATTCACACAAAGCGAGC	$(ATT)_{54}$	309-378	21	50.0
ECHMA4	F: CAACTCAACTGCCCATGTCC R: TTTGAGGGGTTGTTTCGAGG	$(TC)_{30}$	249-281	16	50.6
ECHMA5	F: GGGTGTGTGTTGTTGACACG R: CAAAACCCTGAATTTCACACG	$(TC)_{34}$	223-277	23	47.4
ECHMA6	F: CGCGGTTTAATCTCATGTGG R: GCGTAGGAATTAGAAGCATGGC	$(TC)_{30}$	163-207	20	49.2
ECHMA10	F: TGACAATGGGTAAGGGATGC R: ACTCAGGTGATGAGAATGTTGC	$(ATATC)_{35}$	278-308	11	50.1
ECHMA16*	F: AGATGCTTGAAACCAAGGGG R: TCTTAGCAAGGCCCAGATCC	$(TTC)_{45}$	398-461	19	50.5
ECHMA21	F: AAGGGGAGAGTCAAAAGCCC R: TCATCAGTTTCTGCTTAAAGGAACC	$(TC)_{28}$	339-377	19	50.6
ECHMA25	F: GGAAGAATGTCATCATGTTTATTTGG R: GAGTCACACGCAAGAGCACC	$(TC)_{20}$	213-250	27	47.6
SCGL71	F: TCATCTGGTCCAATCAGCAA R: TCAGCGAACAAGAATCATGC	$(CT)_{18}$	163-197	17	52.9
SCGL704	F: GCAAACCATTCAAAGCAGTG R: CTTGCTGGCTGTTGAACTA	$(CT)_{23}$	177-207	25	50.0

*Microsatellite region does not amplify in *Echinomastus intertextus*, and therefore, is excluded from the four-taxa data set.

Microsatellite Data Analyses

To determine the level of genetic diversity within and among populations for all taxa, the data were examined using GENALEX version 6.5 (Peakall and Smouse 2006, 2012). Standard population genetic diversity statistics were calculated, including mean number of alleles per microsatellite region, total number of private alleles, observed and expected heterozygosity, and inbreeding coefficient.

Genetic distances between all possible pairs of individuals and populations were calculated using three different data sets: the four-taxa and three-taxa data sets described above and a two-taxa data set that included all 11 microsatellite regions for all individuals (N=139) of *E. erectocentrus* var. *acunensis* and *E. erectocentrus* var. *erectocentrus*. Several different ways to calculate genetic distance were employed, and two are reported here. First, allele frequency-based distances were calculated between populations as F_{ST} and averaged between taxa. Second, genetic data were converted into a pairwise individual-by-individual genetic distance matrix in GENALEX based on the methods of Smouse and Peakall (1999). This genetic distance matrix was used for downstream analyses including Principal Coordinates Analysis (PCoA) of individual genetic distances, calculation of Φ_{PT} between populations (an analogue of F_{ST} or population level differentiation that is calculated from the individual-by-individual genetic distance matrix rather than allele frequencies), PCoA of Φ_{PT}, analyses of molecular variance (AMOVA), and Mantel tests. PCoA allowed major patterns of genetic relationships among individuals and populations to be visualized and was based on a covariance matrix with data standardization. AMOVA determined the partitioning of genetic variation among taxa, among populations within taxa, and among individuals within populations for all

three data sets and for *E. erectocentrus* var. *acunensis* alone. Mantel tests compared geographic distances to genetic distances for the four-taxa and three-taxa data sets and also for *E. erectocentrus* var. *acunensis, E. erectocentrus* var. *erectocentrus,* and *E. johnsonii* individually. A single geographic point for each population was used to calculate the natural log of geographic distance between each pair of populations in GENALEX. The geographic points used in this calculation correspond to a centrally located individual that was sampled at each population. The matrices of the natural log of geographic distances were compared to the matrices of genetic distances calculated as Φ_{PT} to determine if the geographic distance between populations was positively correlated with the genetic distance between populations.

To understand the patterns of shared genetic variation among individuals and populations, Bayesian clustering analyses were performed in the program STRUCTURE (ver. 2.3; Pritchard et al. 2000) on the four-taxa and three-taxa data sets. The likelihood of K, where K is the number of distinct genetic clusters, was calculated for $K = 1$ to 17 (four-taxa data set) and $K = 1$ to 15 (three-taxa data set) using a model of admixture, correlated allele frequencies, and no prior population information. Each value of K was evaluated with 10 independent runs of 500,000 iterations preceded by a burn-in of 100,000 iterations. To determine the most likely value of K, log probabilities ($L(K)$; Pritchard et al. 2000) and the change in log probabilities ($\Delta L(K)$; Evanno et al. 2005) were examined using the program STRUCTURE HARVESTER (Earl and vonHoldt 2011).

Results

A total of 222 individual samples were successfully collected from 17 populations of all four taxa. A total of 158 alleles were found across eight microsatellite regions in the four-taxa data set, and 214 alleles were found across 11 microsatellite regions in the three-taxa data set. A summary of genetic diversity statistics for all populations is shown in Table 2.3. These results are based on analyses of the four-taxa data set for *E. intertextus* and the three-taxa data set for *E. erectocentrus* var. *acunensis*, *E. erectocentrus* var. *erectocentrus*, and *E. johnsonii*.

For the three-taxa data set, mean number of alleles per microsatellite region averaged across taxa was 5.7, with a low of 4.2 in Ajo (*E. erectocentrus* var. *acunensis*) and a high of 7.1 in Wilcox (*E. erectocentrus* var. *erectocentrus*). A total of 52 private alleles were observed among all three taxa: 18 in *E. erectocentrus* var. *acunensis*, eight in *E. erectocentrus* var. *erectocentrus*, and 26 in *E. johnsonii*. Mean observed heterozygosity averaged across taxa was 0.622, with a low of 0.465 observed in Ajo (*E. erectocentrus* var. *acunensis*) and a high of 0.811 observed in Box-O Canyon, population B (*E. erectocentrus* var. *acunensis*). Mean expected heterozygosity averaged across taxa was 0.643, with lows of 0.559 expected in Searchlight (*E. johnsonii*) and 0.560 in Ajo (*E. erectocentrus* var. *acunensis*) and highs of 0.736 expected in Box-O Canyon, population A (*E. erectocentrus* var. *acunensis*) and 0.731 in Wilcox (*E. erectocentrus* var. *erectocentrus*). The mean inbreeding coefficient averaged across taxa was low, 0.026, with populations ranging from a low of -0.205 in Box-O Canyon, population B (*E. erectocentrus* var. *acunensis*) to a high of 0.186 in Ajo (*E. erectocentrus* var. *acunensis*).

For the four-taxa data set, mean number of alleles per microsatellite region averaged across taxa was 4.9 with a low of 2.5 in Florida Gap (*E. intertextus*) and a high of 7.1 in Wilcox (*E. erectocentrus* var. *erectocentrus*), as seen in the three-taxa data set. A total of 67 private alleles were observed between all four taxa: 24 in *E. erectocentrus* var. *acunensis*, eight in *E. erectocentrus* var. *erectocentrus*, 25 in *E. johnsonii*, and eight in *E. intertextus*. Mean observed heterozygosity averaged across taxa was 0.562, with a low of 0.211 observed in Florida Gap (*E. intertextus*) and a high of 0.771 observed in the Box-O, population B (*E. erectocentrus* var. *acunensis*). Mean expected heterozygosity averaged across taxa was 0.578, with lows of 0.274 expected in Florida Gap (*E. intertextus*) and 0.478 in Anthony Gap (*E. intertextus*) and highs of 0.714 expected in Box-O Canyon, population A (*E. erectocentrus* var. *acunensis*) and 0.703 in Moapa (*E. erectocentrus* var. *erectocentrus*). The mean inbreeding coefficient averaged across taxa was low, 0.017, with populations ranging from a low of -0.213 in Box-O Canyon, population B (*E. erectocentrus* var. *acunensis*) to a high of 0.225 in Ajo (*E. erectocentrus* var. *acunensis*).

Table 2.3. Localities and population genetic diversity statistics for 17 populations of *Echinomastus erectocentrus* var. *acunensis*, *E. erectocentrus* var. *erectocentrus*, *E. johnsonii*, and *E. intertextus*. Population name, number of samples included in the genetic analysis (N), mean number of alleles per microsatellite region (N_a), total number of private alleles (P_a), observed heterozygosity (H_o), expected heterozygosity (H_e), and inbreeding coefficient (F) are included.

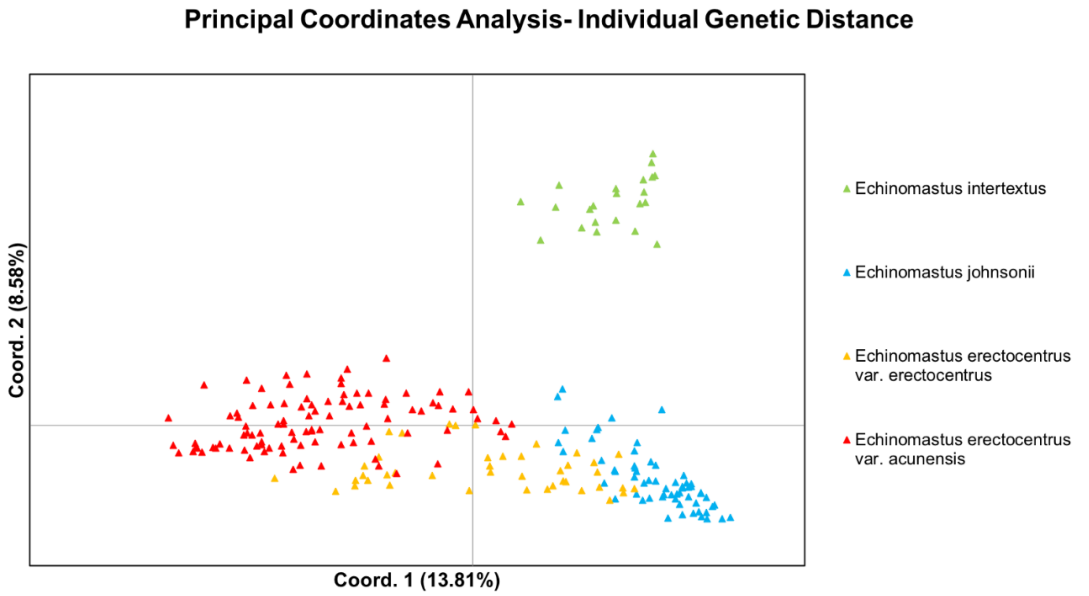
Taxon	Population	N	N_a	P_a	H_o	H_e	F
Echinomastus johnsonii	Date Creek (1)	18	6.5	8.0	0.592	0.59	-0.016
	Moapa (2)	14	7.0	6.0	0.644	0.687	0.105
	Searchlight (3)	16	5.7	3.0	0.491	0.559	0.113
	Vulture Mine (4)	13	5.3	9.0	0.538	0.624	0.134
Echinomastus erectocentrus var. *erectocentrus*	San Manuel (5)	16	6.4	2.0	0.566	0.649	0.125
	Wilcox (6)	14	7.1	3.0	0.658	0.731	0.073
	Davidson Canyon (7)	12	6.2	3.0	0.681	0.682	-0.036
Echinomastus intertextus	Florida Gap (8)	11	2.5	3.0	0.211	0.274	0.099
	Anthony Gap (9)	11	3.9	5.0	0.401	0.478	0.172
Echinomastus erectocentrus var. *acunensis*	Coffee Pot (10)	12	5.2	2.0	0.63	0.62	-0.032
	SDNM (11)	16	4.8	3.0	0.601	0.594	-0.024
	OPNM Population A (12)	13	5.2	1.0	0.636	0.626	-0.031
	OPNM Population B (13)	14	4.9	1.0	0.591	0.622	0.045
	Ajo (14)	14	4.2	0.0	0.465	0.56	0.186
	Kearny (15)	14	5.9	7.0	0.724	0.689	-0.072
	Box-O Canyon Population A (16)	10	6.6	3.0	0.708	0.736	0.032
	Box-O Canyon Population B (17)	4	4.4	1.0	0.811	0.679	-0.205

PCoA of genetic distances between individuals for all taxa showed that the outlying taxon, *E. intertextus*, was indeed separated from the other three taxa (Fig. 2.2a). The PCoA of the four taxa also showed an overlap of *E. erectocentrus* var. *acunensis, E. erectocentrus* var. *erectocentrus,* and *E. johnsonii.* The PCoA created from the three-taxa data set showed that *E. johnsonii* was distinct and that overlapping only occurred between populations of *E. erectocentrus* var. *acunensis* and *E. erectocentrus* var. *erectocentrus* (Fig. 2.2b). The PCoA of the two-taxa data set showed distinctively that overlapping occurred between the populations of *E. erectocentrus* var. *acunensis* found in Kearny and Box-O Canyon, and the populations of *E. erectocentrus* var. *erectocentrus* found in San Manuel and Wilcox (Fig. 2.2b, c). The PCoA of the two-taxa data set also showed that individuals found at the Sonoran Desert National Monument were more genetically distant from other *E. erectocentrus* var. *acunensis* individuals (Fig. 2.2c). The PCoA of genetic distances between population of all four taxa indicated that populations of *E. intertextus* were genetically distinct from the other taxa (Fig. 2.3a). The PCoA of population genetic variation of *E. erectocentrus* var. *acunensis, E. erectocentrus* var. *erectocentrus,* and *E. johnsonii* indicated that populations of *E. johnsonii* were distinct from populations of *E. erectocentrus* var. *acunensis* and *E. erectocentrus* var. *erectocentrus* (Fig. 2.3b). The PCoA of *E. erectocentrus* var. *acunensis* and *E. erectocentrus* var. *erectocentrus* populations showed that San Manuel was more closely related to the populations found in Kearny and Box-O Canyon than the other *E. erectocentrus* var. *erectocentrus* populations (Fig. 2.3c). This PCoA also showed that the population found at the Sonoran Desert National Monument was more genetically distinct compared to other populations of *E. erectocentrus* var. *acunensis* (Fig. 2.3c).

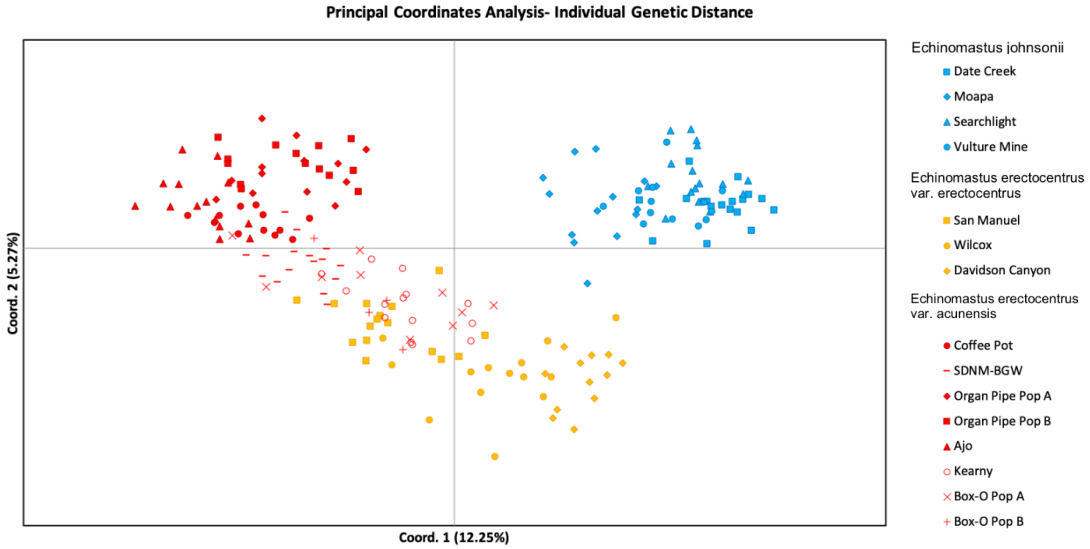
Results from AMOVA indicated that when all four taxa were considered, most of the observed genetic variation was due to differences among individuals within populations (55% rather than differences among populations (24%) or among taxa (21%; Table 2.4). This same pattern of distribution of genetic variation was seen for the three-taxa and two-taxa data sets and for *E. erectocentrus* var. *acunensis* alone, with 59%, 64%, and 69% of observed genetic variation attributable to differences among individuals within populations, respectively (Table 2.4).

Mantel tests confirmed the geographic patterns observed in PCoA of genetic distances (Figs. 2.2, 2.3) and indicated that geographic and genetic distances between populations were significantly, positively correlated for three of the five comparisons (Table 2.5). This positive correlation was strongest when comparing genetic and geographic distances for populations of *E. erectocentrus* var. *acunensis* alone and was negative and/or insignificant when comparing populations of *E. erectocentrus* var. *erectocentrus* or *E. johnsonii* alone (Table 2.5).

a)


Principal Coordinates Analysis- Individual Genetic Distance
Coord. 2 (8.58%)
Coord. 1 (13.81%)
Echinomastus intertextus
Echinomastus johnsonii
Echinomastus erectocentrus var. erectocentrus
Echinomastus erectocentrus var. acunensis

b)


Principal Coordinates Analysis- Individual Genetic Distance
Coord. 2 (5.27%)
Coord. 1 (12.25%)
Echinomastus johnsonii
Date Creek
Moapa
Searchlight
Vulture Mine
Echinomastus erectocentrus var. erectocentrus
San Manuel
Wilcox
Davidson Canyon
Echinomastus erectocentrus var. acunensis
Coffee Pot
SDNM-BGW
Organ Pipe Pop A
Organ Pipe Pop B
Ajo
Kearny
Box-O Pop A
Box-O Pop B

c)

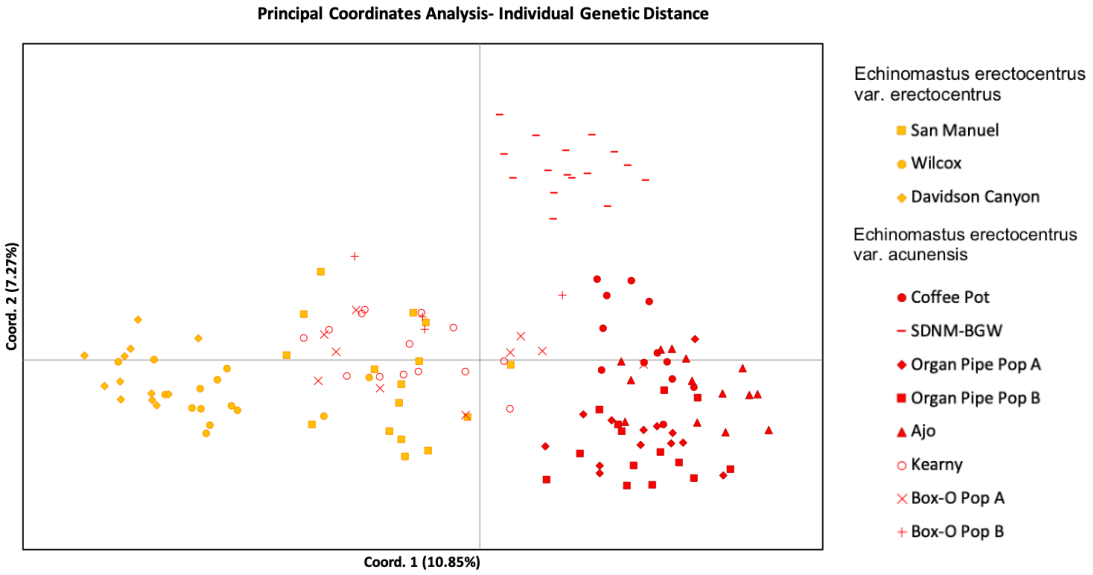
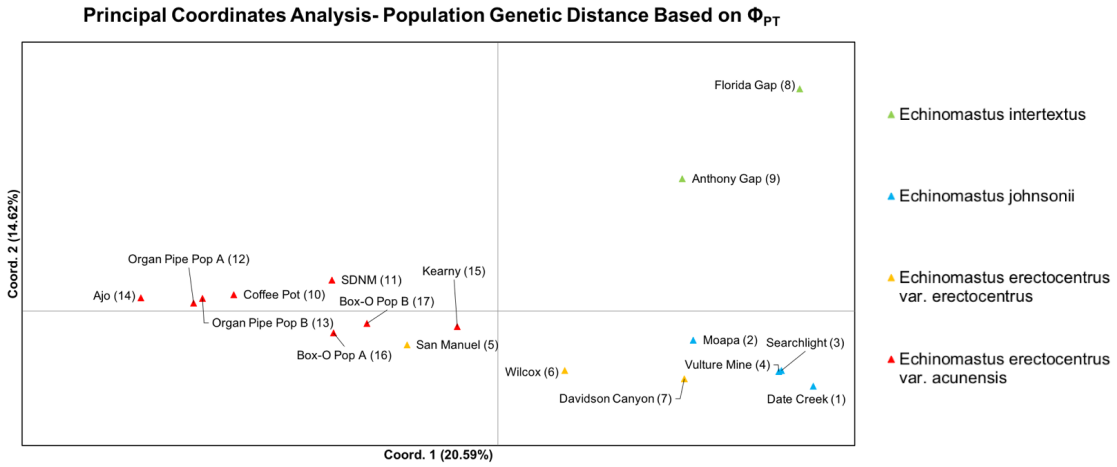
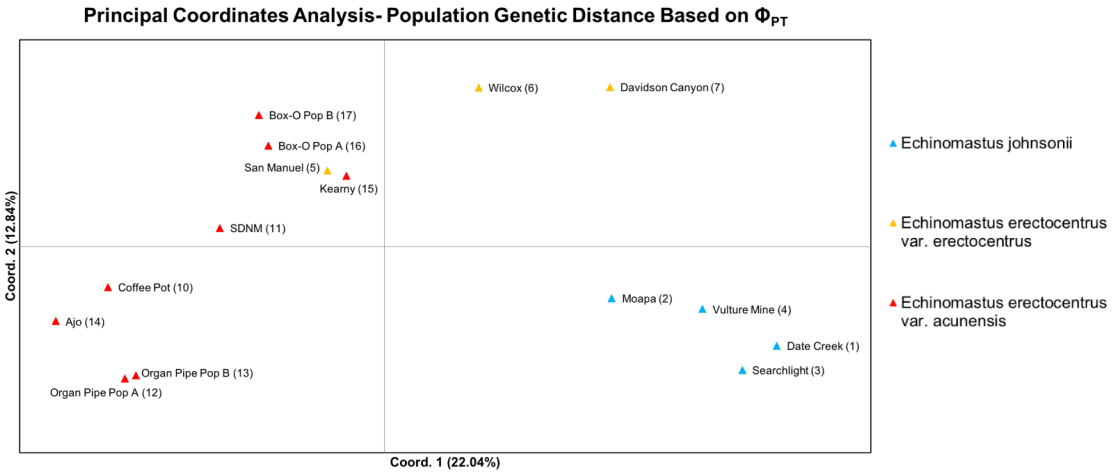


Figure 2.2. Principal coordinates analysis of genotypic genetic distances based on microsatellite allele differences between all possible pairs of individuals from a) four taxa – *Echinomastus erectocentrus* var. *acunensis* (red), *E. erectocentrus* var. *erectocentrus* (yellow), *E. johnsonii* (blue), and *E. intertextus* (green); b) three taxa – *E. erectocentrus* var. *acunensis* (red), *E. erectocentrus* var. *erectocentrus* (yellow), and *E. johnsonii* (blue); and c) two taxa – *E. erectocentrus* var. *acunensis* (red) and *E. erectocentrus* var. *erectocentrus* (yellow).

a)


Principal Coordinates Analysis- Population Genetic Distance Based on Φ_{PT}
Florida Gap (8)
Anthony Gap (9)
Organ Pipe Pop A (12)
Ajo (14)
SDNM (11)
Coffee Pot (10)
Kearny (15)
Box-O Pop B (17)
Organ Pipe Pop B (13)
Box-O Pop A (16)
San Manuel (5)
Moapa (2)
Searchlight (3)
Vulture Mine (4)
Wilcox (6)
Davidson Canyon (7)
Date Creek (1)
Coord. 2 (14.62%)
Coord. 1 (20.59%)
Echinomastus intertextus
Echinomastus johnsonii
Echinomastus erectocentrus var. erectocentrus
Echinomastus erectocentrus var. acunensis

b)


Principal Coordinates Analysis- Population Genetic Distance Based on Φ_{PT}
Wilcox (6)
Davidson Canyon (7)
Box-O Pop B (17)
Box-O Pop A (16)
San Manuel (5)
Kearny (15)
SDNM (11)
Coffee Pot (10)
Ajo (14)
Organ Pipe Pop B (13)
Organ Pipe Pop A (12)
Moapa (2)
Vulture Mine (4)
Date Creek (1)
Searchlight (3)
Coord. 2 (12.84%)
Coord. 1 (22.04%)
Echinomastus johnsonii
Echinomastus erectocentrus var. erectocentrus
Echinomastus erectocentrus var. acunensis

c)

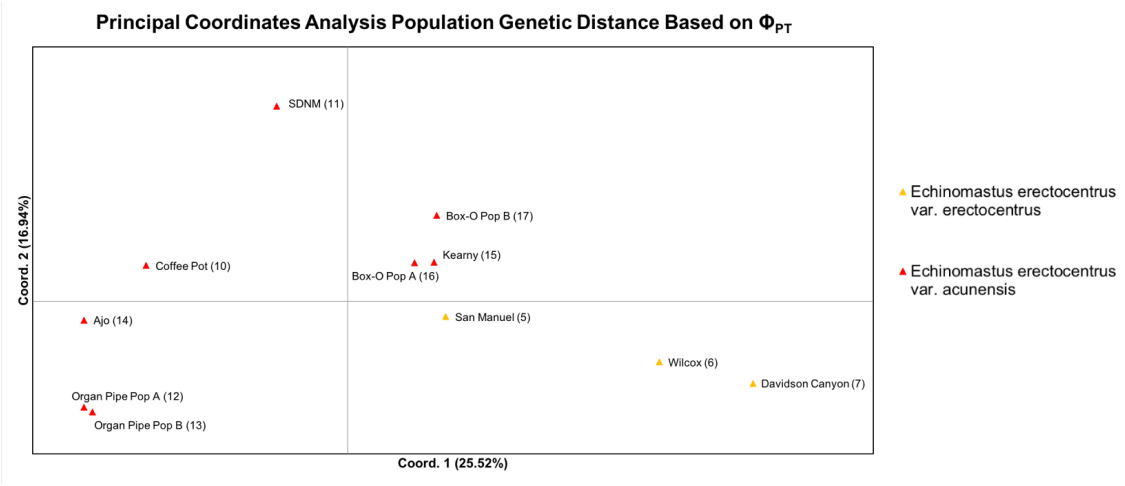


Figure 2.3. Principal coordinates analysis of genetic distances (Φ_{PT}) based on microsatellite allele differences between all possible pairs of populations from a) four taxa – *Echinomastus erectocentrus* var. *acunensis* (red), *E. erectocentrus* var. *erectocentrus* (yellow), *E. johnsonii* (blue), and *E. intertextus* (green); b) three taxa – *E. erectocentrus* var. *acunensis* (red), *E. erectocentrus* var. *erectocentrus* (yellow), and *E. johnsonii* (blue); and c) two taxa – *E. erectocentrus* var. *acunensis* (red) and *E. erectocentrus* var. *erectocentrus* (yellow).

Table 2.4. Hierarchical population structure based on AMOVA (Φ_{PT}) among four taxa of *Echinomastus*. All values are significant at $P = 0.01$.

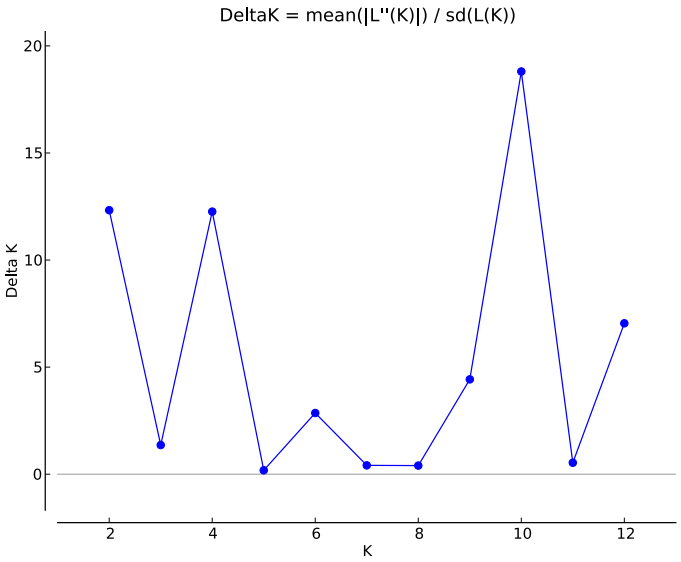
Grouping Source of Variation	d.f.	Sum of Squares	Variance components	Percentage of variation
Four taxa - *E. erectocentrus* var. *acunensis, E. erectocentrus* var. *erectocentrus, E. johnsonii, E. intertextus*				
Among taxa	3	454.058	2.218	21
Among populations	13	486.579	2.446	23
Within populations	205	1185.462	5.783	55
Three taxa - *E. erectocentrus* var. *acunensis, E. erectocentrus* var. *erectocentrus, E. johnsonii*				
Among taxa	2	369.702	2.027	15
Among populations	12	639.156	3.459	26
Within populations	185	1469.875	7.945	59
Two taxa - *E. erectocentrus* var. *acunensis* and *E. erectocentrus* var. *erectocentrus*				
Between taxa	1	129.276	1.315	10
Among populations	9	428.047	3.185	25
Within populations	128	1028.531	8.035	64
E. erectocentrus* var. *acunensis				
Among populations	7	340.546	3.418	31
Within populations	89	684.862	7.695	69

Table 2.5. Results from Mantel tests evaluating the relationship between genetic distance (Φ_{PT}) and the natural log of geographic distance between pairs of populations for four-taxa and three-taxa data sets and also for *E. erectocentrus* var. *acunensis, E. erectocentrus* var. *erectocentrus,* and *E. johnsonii* individually.

Taxa/taxon included in Mantel test	**R_{xy}**	***P* value (R_{xy}-rand ≥ R_{xy}-data)**	**R^2**
Four taxa – *E. erectocentrus* var. *acunensis, E. erectocentrus* var. *erectocentrus, E. johnsonii*, and *E. intertextus*	0.520	0.010	0.2699
Three taxa – *E. erectocentrus* var. *acunensis, E. erectocentrus* var. *erectocentrus,* and *E. johnsonii*	0.467	0.010	0.2185
E. erectocentrus var. *acunensis*	0.621	0.010	0.3858
E. erectocentrus var. *erectocentrus*	-0.896	0.260 ns	0.8035
E. johnsonii	0.632	0.210 ns	0.4000

Results from Bayesian clustering analyses were in agreement with patterns observed from the PCoA of genetic distances and further revealed cohesive genetic groupings of individuals and populations. For the four-taxa data set, $K = 4$ and $K = 10$ were supported as the optimal number of genetic clusters based on evaluation of log probabilities and change in log probabilities from 10 replicate runs at each value of K (Fig. 2.4a). For $K = 4$, genetic clusters correspond to taxonomic identities, with the exception of the *E. erectocentrus* var. *acunensis* populations of Box-O Canyon and Kearny, which clustered with *E. erectocentrus* var. *erectocentrus* populations (Fig. 2.5a). For $K = 10$, genetic clustering revealed substructure within taxa and identified unique populations (San Manuel, Sonoran Desert National Monument, Kearny, and Box-O Canyon; Fig. 2.5b). For the three-taxa data set, $K = 3$ was supported as the optimal number of genetic clusters based on evaluation of log probabilities and change in log probabilities from 10 replicate runs at each value of K (Fig. 2.4b). For $K = 3$, genetic clusters again corresponded to taxonomic identities, with the exception of the *E. erectocentrus* var. *acunensis* populations of Box-O Canyon and Kearny, which clustered with *E. erectocentrus* var. *erectocentrus* populations (Fig. 2.5c). Taken together these clustering analyses support that *E. erectocentrus* var. *acunensis, E. erectocentrus* var. *erectocentrus, E. johnsonii*, and *E. intertextus* are genetically distinct and the *E. erectocentrus* var. *acunensis* populations of Box-O Canyon and Kearny are genetically similar to *E. erectocentrus* var. *erectocentrus* (Fig. 2.5).

a)



b)

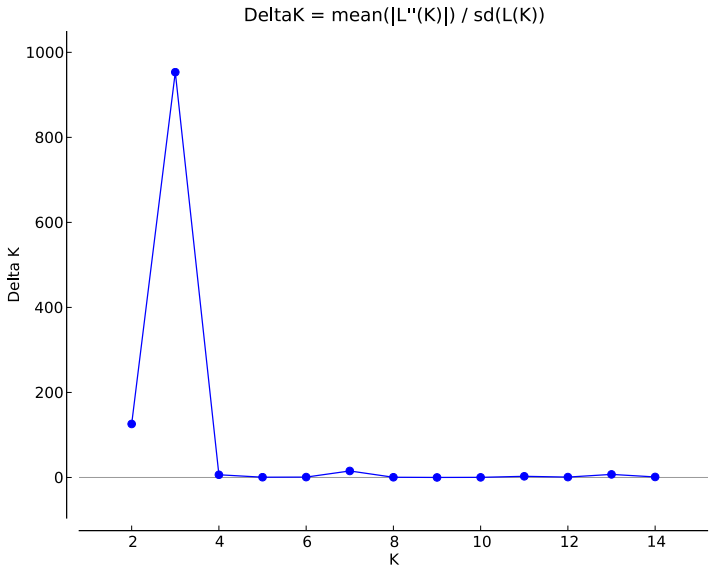
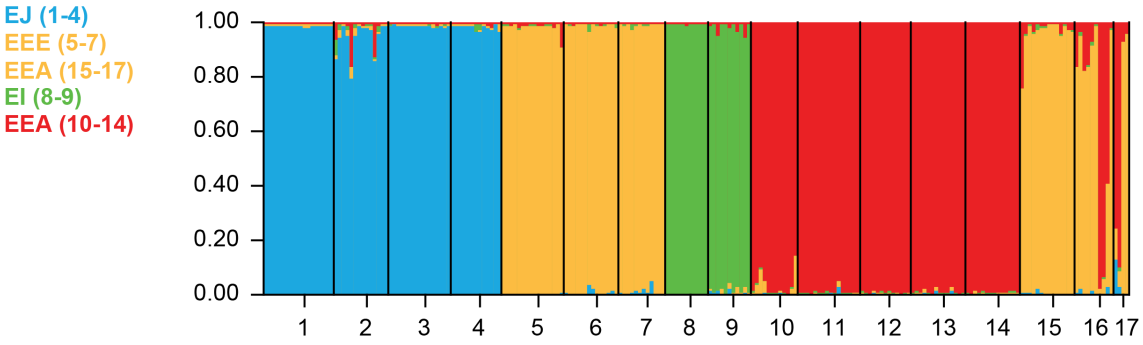
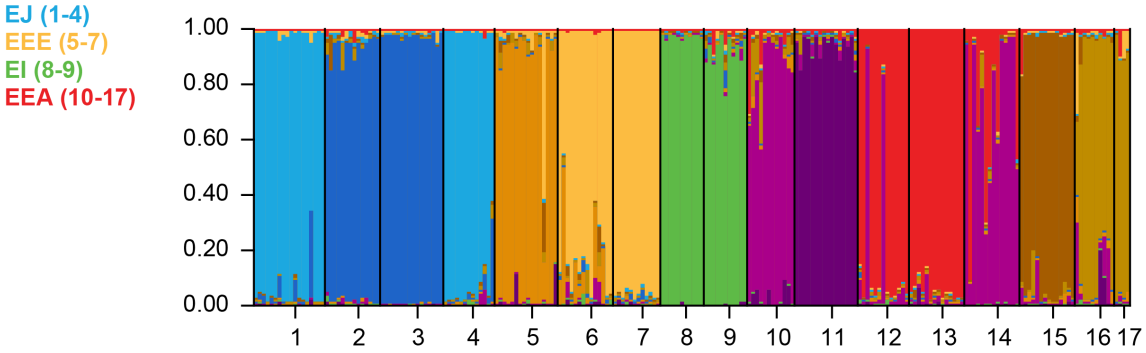


Figure 2.4. Significant Delta K values for a) four-taxa data set and b) three-taxa data set.

a) $K = 4$



b) $K = 10$



c) $K = 3$

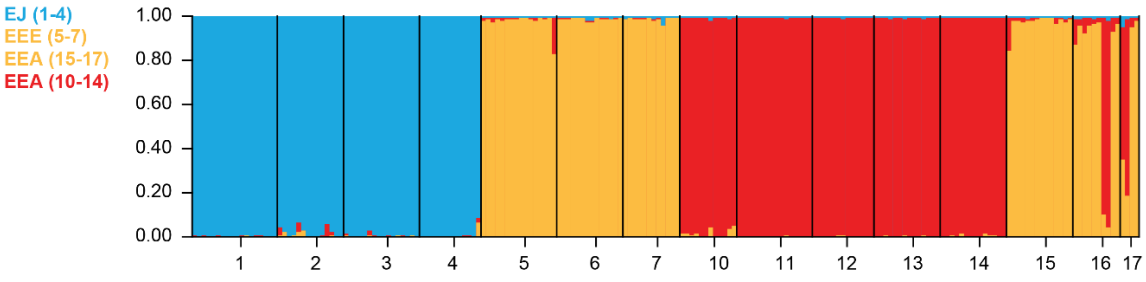


Figure 2.5. Bayesian clustering analyses of population structure in *Echinomastus* for the a) four-taxa data set where $K = 4$, b) four-taxa data set where $K = 10$, and c) three-taxa data set where $K = 3$. Population numbers shown along the bottom of each graph are defined in Table 2.1.

Discussion

The goals of this study were to determine the level of genetic diversity within and between populations of *E. erectocentrus* var. *acunensis*, and better define species boundaries between *E. erectocentrus* var. *acunensis* and its close relatives. Understanding the level of genetic diversity currently present within and between populations of *E. erectocentrus* var. *acunensis* may help focus conservation efforts on vulnerable populations as well as clarify our current understanding of species boundaries, and the evolutionary history of the genus. Understanding where *E. erectocentrus* var. *acunensis* currently lies in the process of speciation, whether it has recently diverged from *E. erectocentrus* var. *erectocentrus* or if these taxa are genetically distinct species, will also help to further inform conservation efforts.

Genetic Diversity within *E. erectocentrus* var. *acunensis*

Looking at the overall patterns of genetic diversity of *E. erectocentrus* var. *acunensis*, there are high levels of heterozygosity and low levels of inbreeding found within populations. When comparing the statistics of *E. erectocentrus* var. *acunensis* with population genetic statistics from other globose cacti, the values of observed (0.65) and expected (0.64) heterozygosity are similar to the average values for all rare cacti (0.58 and 0.67 respectively) included in the table and fit within Hardy-Weinberg expectations (Table 2.6). The expected values of heterozygosity for *E. erectocentrus* var. *acunensis* also match with the expected heterozygosity for the *Sclerocactus* taxa (0.66; 0.59; 0.52) included in Table 2.6. When comparing observed values of heterozygosity between the two genera, values were much lower in *Sclerocactus* (0.47; 0.37; 0.26) than in *E.*

erectocentrus var. *acunensis*, or in *E. erectocentrus* var. *erectocentrus* and *E. johnsonii*. The average inbreeding coefficient for *E. erectocentrus* var. *acunensis* (-0.018) is much lower than the average for all the globose cacti included Table 2.6, even when only looking at the rare cacti (0.139 and 0.151 respectively). The low inbreeding coefficient for *E. erectocentrus* var. *acunensis* suggests that populations are experiencing low levels of inbreeding, which is important for maintaining high levels of genetic diversity in populations. These average levels of heterozygosity and low levels of inbreeding suggest that populations of *E. erectocentrus* var. *acunensis* are in a state of stable equilibrium, where a lack of inbreeding in populations leads to heterozygosity values that fit within Hardy Weinberg expectations. Higher than average levels of heterozygosity would only be expected if outside individuals were suddenly introduced into populations (Moritz 1999). If conservation efforts ever include introducing new individuals into populations, possibly through relocation, the values obtained here could be used as a point of reference and comparison. When comparing these values within the populations of *E. erectocentrus* var. *acunensis* sampled here, elevated levels of heterozygosity and low levels of inbreeding are observed in the populations of Box-O Canyon and Kearny, specifically. These populations will be discussed in more detail later, but these values may indicate that the populations of Box-O Canyon and Kearny are experiencing hybridization with another taxon (Arnold et al. 1999; Moritz 1999).

Table 2.6. The number of populations sampled (N), average of individuals sampled across populations (N_i), number of microsatellites used (N_m), average number of alleles (N_a), average observed heterozygosity (H_o), average expected heterozygosity (H_e), and average inbreeding coefficient (F_{is}) for species of globose cacti. Species with asterisks next to their name are not listed as endangered or rare.

Species	N	N_i	N_m	N_a	H_o	H_e	F_{is}	Citation
Echinomastus erectocentrus var. *acunensis*	8	12	11	5.2	0.65	0.64	-0.018	This study
Astrophytum asterias	5	28	7	9.9	0.64	0.70	0.082	Terry et al. 2012
Coryphantha robbinsorum	6	33	8	6.2	0.70	0.66	-0.074	Fehlberg et al. unpubl.
Coryphantha robustispina subsp. *robustispina*	3	10	10	7.9	0.60	0.66	0.103	Butterworth 2010
Echinocereus arizonicus subsp. *arizonicus*	11	11	5	4.7	0.73	0.67	-0.106	Fehlberg et al. unpubl.
Mammillaria huitzilopochtli	5	30	8	11.8	0.55	0.80	0.308	Solorzano et al. 2014
Mammillaria supertexta	5	21	8	9.3	0.69	0.76	0.132	Solorzano et al. 2014
Polaskia chende (wild)	5	20	7	5.2	0.73	0.68	-0.073	Contreras-Negrete et al. 2015
Sclerocactus glaucus	27	24	13	7.2	0.47	0.66	0.280	Schwabe et al. 2015
Sclerocactus brevihamatus subsp. *tobuschii*	9	25	7	6	0.37	0.59	0.390	Rayamajhi and Sharma 2018
Sclerocactus brevihamatus subsp. *brevihamatus*	1	30	7	6	0.26	0.52	0.510	Rayamajhi and Sharma 2018
Echinomastus erectocentrus var. *erectocentrus**	3	14	11	2.7	0.64	0.69	0.054	This study
*Echinomastus johnsonii**	4	15	11	6.5	0.57	0.62	0.084	This study
*Echinomastus intertextus**	2	11	11	4	0.31	0.38	0.135	This study
Polaskia chichipe (natural populations only)*	3	30	5	6.1	0.63	0.68	0.071	Otero-Arnaiz et al. 2005
*Sclerocactus parviflorus**	8	20	13	6.2	0.39	0.62	0.380	Schwabe et al. 2015
*Melocactus curvispinus**	18	48	19	1.54	0.07	0.10	0.315	Nassar 2001
MEAN RARE	7.73	22.18	8.27	7.22	0.58	0.67	0.139	
MEAN ALL	7.11	21.44	9.22	6.35	0.54	0.58	0.151	

The AMOVA tests support that a majority of genetic variation present is found within populations rather than between populations. This pattern is typical for taxa with similar life history characteristics, though endemic taxa typically have lower levels of variation within populations than their widespread counterparts (Nybom 2004). This means that populations are well differentiated from one another and there is a high level of variation present among individuals in the same population. This was expected considering the distance between most populations and the bee pollinators for this taxon (Johnson 1991, 1992; Johnson et al.1993). In the study done by Rayamajhi & Sharma (2018), *Sclerocactus brevihamatus* subsp. *tobuschii* had AMOVA values of 10% among populations and 90% within populations. These AMOVA values suggest a much higher level of genetic exchange between populations of *Sclerocactus* taxa than is found in *E. erectocentrus* var. *acunensis* (Table 2.4). There is limited information about pollinators and fruit distribution within these groups, but both appear to be pollinated by native ground-nesting bees and rely on gravity to disperse seeds (Johnson 1992; Schwabe et al. 2015; Rayamajhi and Sharma 2018). Considering the similarities in pollinators and seed dispersal, another undefined difference in evolutionary history or life history characteristics could be behind the differences in level of gene exchange documented in these two closely related taxa.

This data set shows further evidence that low levels of gene flow are occurring between populations that are geographically distant or isolated, perhaps dependent on pollinators and seed dispersal traits. The Mantel test indicates a significant relationship between genetic and geographic distances within *E. erectocentrus* var. *acunensis*. The more distant populations are from one another the greater their genetic distinctiveness

(Table 2.5). This relationship between genetic distance and distinction is also reflected in the number of private alleles found in each population (Table 2.3). Populations which are closer geographically have high levels of gene flow, and alleles were commonly shared, while more distant and isolated populations contained more private alleles. Another interesting result from the PCoA was the distinction of the SDNM population from other *E. erectocentrus* var. *acunensis* populations (Figs. 2.2c, 2.3c). This means that individuals in this population have a particular lack of gene flow with other populations most likely due to geographic isolation.

Understanding genetic diversity within *E. erectocentrus* var. *acunensis* gives us important insight into which populations should be a focus for conservation efforts. Populations with high levels of genetic diversity, such as Kearny and Box-O Canyon, could be considered populations of high concern. The population found in Ajo, though, exhibits lower levels of genetic diversity and high levels of inbreeding. While this population should not be abandoned, these values should be kept in mind when making conservation decisions. Any plans for possible introductions of new individuals, which could help increase the level of heterozygosity and genetic diversity, should be focused on this population first. Further research could also be done to better understand the relationship between the populations found in Organ Pipe National Monument, Ajo, and Coffee Pot. Looking at the STRUCTURE results for all four taxa when K=10, the population in Ajo appears to be a genetic mix of the population found in Coffee Pot and OPNM. This makes sense considering their proximity, but a better understanding of gene flow is important to elucidate the taxon's evolutionary history.

Species Boundaries

A geographic cline has been suggested between *E. erectocentrus* var. *acunensis*, *E. erectocentrus* var. *erectocentrus*, and *E. johnsonii* (Baker 2007; Baker and Porter 2016). Populations found north of Tucson have also been identified as intermediates between the two varieties (Zimmerman and Parfitt 2003). The similarity of morphological characteristics across these taxa has created unclear boundaries in taxonomic distinctions. Detailed genetic data, as provided in this study, can help clarify evolutionary relationships in this group and be used to better clarify the boundaries between these species.

The results confirm the genetic distinction of *E. intertextus* as a separate species, as expected. If these four taxa were genetically distinct, four distinct clusters of points would be expected in the PCoA results, with each cluster corresponding to a specific taxon. The PCoA results clearly show that *E. intertextus* points are distinct and separated from the other three taxa (Fig. 2.2a, 2.3a). The results also show that *E. johnsonii* is separate from *E. erectocentrus* var. *acunensis* and *E. erectocentrus* var. *erectocentrus* (Figs. 2.2b, 2.3b). This separation is further backed by the STRUCTURE results for K=4, wherein the populations were shown to contain four genetically distinct taxa (Fig. 2.6a). Results showing four distinct taxa indicate that a genetic cline does not exist between *E. johnsonii, E. erectocentrus* var. *acunensis* and *E. erectocentrus* var. *erectocentrus* as suggested by morphology, and that *E. johnsonii* is in fact genetically distinct from the other two taxa. While *E. johnsonii* and *E. intertextus* appear genetically distinct, populations and individuals of *E. erectocentrus* var. *acunensis* and *E. erectocentrus* var.

erectocentrus overlap according to the PCoA results, suggesting that they are very closely related (Figs. 2.2, 2.3).

The data set of *E. erectocentrus* var. *acunensis* and *E. erectocentrus* var. *erectocentrus* gives further insight into the relationship between these two taxa and the overlap between the Box-O Canyon, Kearny, and San Manuel populations (Figs. 2.2c, 2.3c). While the Wilcox population appears distinct in the PCoA when looking at the populations as a whole, the PCoA of individuals showed that a few of the Wilcox individuals can be found in the area of overlap between Box-O Canyon, Kearny, and San Manuel (Figs. 2.2c, 2.3c). These overlaps indicate low levels of genetic distinction compared to the other populations sampled and suggest that geneflow is occurring between these populations. The STRUCTURE analysis gives further insight into the overlapping populations, with the Box-O Canyon and Kearny populations having a combination of the genetic diversity found in *E. erectocentrus* var. *acunensis* and *E. erectocentrus* var. *erectocentrus* (Fig. 2.5). The Kearny population appears to align more closely with *E. erectocentrus* var. *erectocentrus* populations, even though it is currently considered to be *E. erectocentrus* var. *acunensis* (USFWS 2013). The Box-O Canyon populations appear to align more closely to *E. erectocentrus* var. *erectocentrus*, but there are also individuals that appear to be closer to *E. erectocentrus* var. *acunensis*. The Box-O Canyon populations also appear to contain individuals that have a combination of the genetic diversity found in *E. erectocentrus* var. *acunensis* and *E. erectocentrus* var. *erectocentrus* alleles. These populations could indicate the presence of a cline between *E. erectocentrus* var. *acunensis* and *E. erectocentrus* var. *erectocentrus*, or they could be evidence of hybridization, as discussed below.

The close genetic relationship between all four taxa is further supported and explored in the AMOVA results, which show the highest percentage of variation is found within populations at 55%, followed by among populations at 23%, and the lowest amount of variation is found between taxa at 21% (Table 2.4). This suggests that populations are diverse with genetically distinct individuals within populations. Populations are fairly well differentiated from one another, but still share some of the same alleles that are present among all taxa. As you move through data sets, the proportion of variation between taxa decreases as the number of taxa examined decreases. The data set including *E. erectocentrus* var. *acunensis*, *E. erectocentrus* var. *erectocentrus*, *E. johnsonii*, and *E. intertextus* showed 21% of the variation between taxa, the data set including *E. erectocentrus* var. *acunensis*, *E. erectocentrus* var. *erectocentrus*, and *E. johnsonii* showed 15% of the variation between taxa, and the data set including only *E. erectocentrus* var. *acunensis* and *E. erectocentrus* var. *erectocentrus* showed 10% of the variation between taxa (Table 2.4). This decrease is expected as one continues to remove the taxa with the largest genetic differences from the analysis. The percentage of variation between *E. erectocentrus* var. *acunensis* and *E. erectocentrus* var. *erectocentrus* is slightly higher than the percentage of variation seen between similar taxa in other studies (Schwabe et al. 2015). Two closely related *Sclerocactus* taxa had average AMOVA values of 8.11% among groups, 9.65% among populations and 82.24% within populations (Schwabe et al. 2015). Schwabe et al. (2015) suggests that the lower level of genetic differentiation found as one moves along groups could indicate more recent divergence times between certain *Sclerocactus* taxa that were included in the study. Similar patterns of change in genetic differentiation between the number of taxa included

in the analysis (all four taxa vs. three taxa vs. two taxa) are found in this study. Working from this, while *E. erectocentrus* var. *erectocentrus* and *E. erectocentrus* var. *acunensis* are genetically similar, the level of genetic differentiation between them could indicate that they are recently diverged, and their genetic differentiation could increase over time.

The Mantel tests show a significant relationship between genetic and geographic distances (Table 2.5). This supports that populations are more genetically distinct the more distant they are from one another. The STRUCTURE analysis further verifies this by showing that populations within the same taxa resemble each other to a higher degree the closer they are geographically, as seen between the populations of Ajo, Coffee Pot and OPNM (Fig. 2.3b). There was no significant relationship between genetic and geographic distance within *E. erectocentrus* var. *erectocentrus* or *E. johnsonii*. This may be due to the limited number of populations sampled for both taxa, and further work could be done to expand the data set and clarify these relationships.

Hybrids

Hybridization can readily occur between recently diverged species, especially when the species share ranges that occur in extreme habitats, like the Sonoran Desert (Harrison and Larson 2014; Schwabe et al. 2015). Hybridization plays an important role in the process of speciation and can give vital insight into the evolutionary history of a species (Whitham et al. 1999; Nolte and Tautz 2009). Hybrids can colonize previously unfilled niches and develop into a new species (Nolte and Tautz 2009). While the presence of genetic hybrids was unknown before this study, morphology suggests that gene exchange is occurring between *E. johnsonii* and the *E. erectocentrus* varieties.

When collecting samples, Baker (2007) noted the population found near Vulture Mine as a possible hybrid between *E. johnsonii* and *E. erectocentrus* var. *acunensis*. The results above show that this is not actually a hybrid population, even if morphological characteristics might imply otherwise. All of the populations of *E. johnsonii* were actually found to be genetically distinct from *E. erectocentrus* var. *acunensis* and *E. erectocentrus* var. *erectocentrus*.

Comparisons of *E. erectocentrus* var. *acunensis* and *E. erectocentrus* var. *erectocentrus* suggest that gene flow is occurring between these two taxa. The level of genetic exchange could suggest that these two taxa are currently going through the process of speciation and that they will one day be more genetically distinct from one another (Abbott et al. 2013; Abbott 2017). The hybrids found in the Box-O Canyon and Kearny populations could also suggest later secondary contact between the two genetically distinct taxa, or remnant genes from previous contact during early points of speciation (Abbott et al. 2013; Abbott 2017). This would suggest that the process of speciation between these two taxa is complete, or nearly so, and that genetic remnants of this recent evolutionary history still remain (Smissen et al. 2014; Ramos-Ortiz et al. 2016). More detailed research into the relationships in these populations could clarify if genes are actively being exchanged between *E. erectocentrus* var. *acunensis* and *E. erectocentrus* var. *erectocentrus*.

Working with the evidence gathered in this study, it is likely that *E. erectocentrus* var. *acunensis* and *E. erectocentrus* var. *erectocentrus* are undergoing speciation and that the hybrids found in Box-O Canyon and Kearny are due to secondary contact (Smissen et al. 2014; Ramos-Ortiz et al. 2016). The distribution of individuals within these

populations, along with the distribution of the populations as a whole, show that there is not a clear gradient of genetic continuity moving from *E. erectocentrus* var. *acunensis* to *E. erectocentrus* var. *erectocentrus* across the landscape. The PCoA analysis of individuals (Fig. 2.2c), shows that intermediate populations overlap and could possibly be forming a gradient, but when taken in context with the STRUCTURE analysis, these populations also contain individuals that are strictly *E. erectocentrus* var. *acunensis* and *E. erectocentrus* var. *erectocentrus* respectively (Fig. 2.5a). This arrangement aligns with a mosaic hybrid zone, with genetically distinct parental individuals residing in the same location as hybrid offspring and suggests that secondary contact is occurring between the two genetically distinct taxa (Ramos-Ortiz et al. 2016).

Evidence from additional studies reinforce the notion that local hybridization and secondary contact between populations of *E. erectocentrus* var. *acunensis* and *E. erectocentrus* var. *erectocentrus* is occurring. In ongoing research in the genus *Echinomastus*, shallow genomic sequencing data from a few individuals for all populations sampled were acquired and used to assemble nuclear ribosomal and whole chloroplast sequences (Fehlberg and Willis 2019, unpublished report). In the phylogenetic analyses done with these data, the Kearny and Box-O Canyon populations behave differently than expected. In the phylogeny built from the nuclear ribosomal region, these populations were placed in an unresolved relationship with *E. erectocentrus* var. *acunensis* and the *E. erectocentrus* var. *erectocentrus* population of San Manuel. This complicated relationship was mirrored in the PCoA analyses from this study (Figs. 2, 3). In the phylogeny reconstructed from the whole chloroplast genome sequence, the Box-O Canyon and Kearny populations aligned with the remaining *E. erectocentrus* var.

acunensis populations. This suggests that the individuals in these populations have *E. erectocentrus* var. *acunensis* mothers and an *E. erectocentrus* var. *acunensis* lineage, but that genes from *E. erectocentrus* var. *erectocentrus* are being contributed due to recent hybridization events.

Conservation Implications and Future Research

Maintaining current levels of biodiversity is one facet of conservation, and genetic variation and the boundaries between species play an important part in our understanding of biodiversity (Moritz 1999; Coates et al. 2018). Species definitions are based on a variety of criteria including morphology, ecology, and genetics, and lack of information in any one area can blur the boundaries between species and may lead to inaccurate placement of taxa (Coates et al. 2018). Both varieties of *E. erectocentrus* have been assessed by the USFWS but only E. *erectocentrus* var. *acunensis* was officially listed as endangered in 2013 (USFWS 1993, 2013). If the suggested morphological cline was supported by the results above, it would have substantiated the reclassification of *E. erectocentrus* var. *acunensis* and *E. erectocentrus* var. *erectocentrus* under *E. johnsonii*, which could alter the protected status of *E. erectocentrus* var. *acunensis* (Baker 2007; Baker and Porter 2016). The results above show there are four distinct genetic taxa (Fig. 2.5) and that *E. erectocentrus* var. *acunensis* should retain its endangered status.

Genetic diversity data may also inform conservation efforts to prioritize protection of those populations with high levels of heterozygosity, the highest number of private alleles, and lowest levels of inbreeding, and to possibly introduce genetically different individuals to those populations with low heterozygosity (Reed and Frankham

2003; Rayamajhi and Sharma 2018). Working from this standard, the populations of *E. erectocentrus* var. *acunensis* found in Kearny and Box-O Canyon would initially appear to be populations of focus, but these populations have also been shown to contain hybrid individuals. This complicated relationship between *E. erectocentrus* var. *acunensis* and *E. erectocentrus* var. *erectocentrus* may explain the elevated levels of heterozygosity and low levels of inbreeding that were observed in the populations of Box-O Canyon and Kearny (Table 2.3). There is a concern that hybrid individuals may end up replacing the rare taxon, which influences how populations and reintroductions are treated by government organizations (Todesco et al. 2016). The results presented here do not clarify if the hybrid individuals present are a result of current genetic exchange between *E. erectocentrus* var. *acunensis* and *E. erectocentrus* var. *erectocentrus*, or if the shared alleles are due to historical contact between the two taxa at some point in their recent evolutionary history. Further research into the relationships among these populations is important for informing USFWS efforts. If the genetic exchange noted here was due to a historical event, then the populations of Kearny and Box-O Canyon could be populations for USFWS to prioritize without fear of jeopardizing the *E. erectocentrus* var. *acunensis* individuals.

While the level of genetic diversity in populations is important to know when making conservation decisions, it is also important to preserve the genes and populations that allow for the preservation and maintenance of evolutionary processes as a whole (Moritz 1999; Coates et al. 2018). It is therefore important to conserve these populations of interest, not only to maintain whatever processes are occurring here, but also to

maintain the possibility of more detailed research in the future. A more detailed look into these populations could further our understanding of hybrids and speciation processes.

The *E. erectocentrus* var. *acunensis* populations found in Coffee Pot, the SDNM and OPNM (Population A) also showed high levels of heterozygosity, low levels of inbreeding and some of the higher levels of private alleles compared to all of the *E. erectocentrus* var. *acunensis* populations sampled (Table 2.3). Due to this, the populations in OPNM should continue to see the same amount of monitoring and care that they already are by researchers and park officials. The populations of Coffee Pot and SDNM are more remote and harder to get to than some of the other populations, like OPNM and Ajo, which may hinder active restoration efforts. These levels of isolation may also be beneficial to the safety of the individuals found here, but USFWS should assess these populations more fully to create a better conservation plan. As mentioned before, there appears to be a higher level of gene flow occurring between OPNM, Ajo and Coffee Pot populations (Fig. 2.5). While Ajo has the highest level of inbreeding among the *E. erectocentrus* var. *acunensis* populations sampled here, along with the lowest level of heterozygosity, further research should be done into the relationship between the populations found in these three locations. The populations found in SDNM also appeared to be more genetically distant from other *E. erectocentrus* var. *acunensis* populations, suggesting a higher degree of isolation (Fig. 2.2c and 2.3c). The unique state of this population could further support that the SDNM population should see a higher level of conservation focus by the USFWS. For all populations of *E. erectocentrus* var. *acunensis*, genetic variation appears to be strongly influenced by geography. Due to this,

conservation efforts should work to maintain conditions that facilitate gene flow among adjacent populations and across the landscape. Using the results presented above should allow the USFWS to identify future research that is needed and create a conservation plan for *E. erectocentrus* var. *acunensis* that will help current populations survive.

REFERENCES

Abbott, R., D. Albach, S. Ansell, J. W. Arntzen, S. J. E. Baird, N. Bierne, J. Boughman, A. Brelsford, C. A. Buerkle, R. Buggs, R. K. Butlin, U. Diekmann, F. Eroukhmanoff, A. Grill, S. Helms Cahan, J. S. Hermansen, G. Hewitt, A. G. Hudson, C. Jiggins, J. Jones, B. Keller, T. Maczewski, J. Mallet, P. Martinez-Rodriguez, M. Most, S. Mullen, R. Nichols, A. W. Nolte, C. Parisod, K. Pfennig, A. M. Rice, M. G. Ritchie, B. Seifert, C. M. Smadja, R. Stelkens, J. M. Szymura, R. Vainola, J. B. W. Wolf, and D. Zinner. 2013. Hybridization and speciation. *Journal of Evolutionary Biology* 26: 229–246.

Abbott, R. 2017. Plant speciation across environmental gradients and the occurrence and nature of hybrid zones. *Journal of Systematics and Evolution* 55: 238-258.

Arakaki, M., C. Pascal-Antonie, R. Nyffeler, A. Lendel, U. Eggli, R. M. Ogburn, E. Spriggs, M. J. Moore, and E. J. Edwards. 2011. Contemporaneous and recent radiations of the world's major succulent plant lineages. *Proceedings of the National Academy of Sciences USA* 108: 8379–8384.

Arizona Game and Fish Department. 2019. Special Status Species by Taxonomic Group, Scientific Name. *Heritage Data Management System*. Online. Available: https://www.azgfd.com/wildlife/planning/wildlifeguidelines/specieslists/

Arizona Rare Plant Committee. 2001. *Arizona rare plant field guide: a collaboration of agencies and organizations*. United States Government Printing Office, Washington.

Arnold, M. L., M. R. Bulger, J. M. Burke, A. L. Hempel, and J. H. Williams. 1999. Natural hybridization: how long can you go and still be important? *Ecology* 80: 371-381.

Baker, M. 2007. A comparison of morphology among populations of acuña cactus, *Echinomastus erectocentrus* (J. M. Coulter) Britton & Rose var. *acunensis* (W.T. Marshall) H. Bravo and its relatives: *E. erectocentrus* var. *erectocentrus*, and *E. johnsonii* (Parry ex Engelm.) E. M. Baxter. Unpublished report submitted to USFWS. 22 pp.

Baker, M. and J. M. Porter. 2016. The use of multivariate analyses of morphological characters and DNA analyses for assessing the taxonomic ranking of rare plant taxa: an example comparing populations of *Echinomastus erectocentrus* var. *acunensis* with those of its relatives *E. erectocentrus* var. *erectocentrus* and *E. johnsonii*. pp. 19-61. In: *Endangered Species: Threats, Conservation and Future Research*. Melinda Quinn (ed.). Nova Science Publishers, Inc., Hauppauge, New York.

Benson, L. D. 1982. *The Cacti of the United States and Canada.* Stanford (Calif.), Stanford University Press.

Britton, N. L. and J. N. Rose. 1922. The Cactaceae: Descriptions and illustrations of the plants of the cactus family. 3 vols. Carnegie Institute of Washington, Washington, D.C.

Buskirk, W. H. 1981. Status of the Acuña cactus, (*Neolloydia erectocentra var. acunensis*) at Organ Pipe Cactus National Monument, Arizona: a progress report. Report to the National Park Service.

Butterworth, C. 2010. Genetic study of Pima pineapple cactus (*Coryphantha robustispina* ssp. *robustispina*) and phylogenetic study of the genus *Coryphantha*. Final Report prepared for the U. S. Department of the Interior Bureau of Reclamation, Phoenix, Arizona. 30 pp.

Coates, D. J., M. Byrne and C. Moritz. 2018. Genetic diversity and conservation units: dealing with the species-population continuum in the age of genomics. *Frontiers in Ecology and Evolution* 6:165.

Contreras-Negrete, G., M. E. Ruíz-Durán, D. Cabrera-Toledo, A. Casas, O. Vargas, and F. Parra. Genetic diversity and structure of wild and managed populations of *Polaskia chende* (Cactaceae) in the Tehuacán-Cuicatlán Valley, Central Mexico: insights from SSR and allozyme markers. *Genetic Resources and Crop Evolution* 62: 85-101.

Cullings, K. W. 1992. Design and testing of a plant-specific PCR primer for ecological and evolutionary studies. *Molecular Ecology* 1: 233-240.

De Querioz, K. 2007. Species concepts and species delimitation. *Systematic Biology* 56: 879-886.

Doyle, J. J. and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11-15.

Duarte, M., P. C. Guerrero, G. Carvallo, and R. O. Bustamante. 2014. Conservation network design for endemic cacti under taxonomic uncertainty. *Biological Conservation* 176: 236-242.

Earl, D. A. and B. M. von Holdt. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 4: 359-361.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14: 2611-2620.

Fehlberg, S. D. and A. Willis. 2019. Assessing genetic diversity in Acuña cactus (*Echinomastus erectocentrus* var. *acunensis*) and its close relatives using microsatellites and DNA sequences. Report prepared for United States Fish and Wildlife Service, Tucson, Arizona.

Fehlberg, S. D. 2015. Population genetic study of the Cochise pincushion cactus (*Coryphantha robbinsorum*) in Arizona. Report prepared for United States Fish and Wildlife Service, Tucson, Arizona.

Fehlberg, S. D. 2012. Population genetic study of the Arizona hedgehog cactus in support of multiple recovery plan objectives. Report prepared for United States Fish and Wildlife Service, Phoenix, Arizona.

Gerber, L. R. 2016. Conservation triage or injurious neglect in endangered species recovery. *Proceedings of the National Academy of Sciences* 113: 3563-3566.

Goettsch, B., C. Hilton-Taylor, G. Cruz-Piñón, et al. 2015. High proportion of cactus species threatened with extinction. *Nature Plants* 1: 15142.

Goettsch, B., A. Paz Durán, K. J. Gaston. 2018. Global gap analysis of cactus species and priority sites for their conservation. *Conservation Biology* 33: 369-376.

Griffith, M. P. 2003. Using molecular evidence to elucidate reticulate evolution in *Opuntia* (Cactaceae). *Madroño* 50: 162-169.

Guzmán Cruz, L. U. 2003. Cactaceae Systematics Initiatives: Bulletin of the International Cactaceae Systematics Group 16: 17.

Harrison, R. G., and E. L. Larson. 2014. Hybridization, introgression, and the nature of species boundaries. *Journal of Heredity* 105:795–809.

Heil, K.D., and B. Melton. 1994. Status Report for *Echinomastus erectocentrus* (J.M. Coulter) Britton & Rose var. *acunensis* (W.T. Marshall) H. Bravo. Report prepared for United States Fish and Wildlife Service, Phoenix, Arizona.

Hernández, H. M. and C. Gómez-Hinostrosa. 2011. Areas of endemism of Cactaceae and the effectiveness of the protected area network in the Chihuahuan Desert. *Fauna and Flora International* Oryx 45: 191–200.

Hernández-Hernández, T., J. Brown, B. Schlumpberger, L. Eguiarte, and S. Magallón. 2014. Beyond aridification: multiple explanations for the elevated diversification of cacti in the New World succulent biome. *New Phytologist,* 202: 1382-1397.

Holm, P. 2006. Ecological monitoring program report 1997 – 2005. Organ Pipe Cactus National Monument, Arizona.

Hunt, D. and N. Taylor. 1986. The genera of the Cactaceae: towards a new consensus. *Bradleya* 4:65-78.

Isik, K. 2011. Rare and endemic species: Why are they prone to extinction? *Turkish Journal of Botany* 35: 411-417.

IUCN (International Union for Conservation of Nature). 2019. The IUCN red list of threatened species. Version 2012-3.1. IUCN, Gland, Switzerland. Available from www.iucnredlist.org.

Johnson, R. A. 1991. Reproductive ecology and natural history of Acuña cactus, *Echinomastus erectocentrus* var. *acunensis*. Final Report to Southwestern Parks and Monuments Association and the National Park Service.

Johnson, R. 1992. Pollination and reproductive ecology of Acuña cactus, *Echinomastus erectrocentrus* var. *acunensis* (Cactaceae). *International Journal of Plant Sciences,* 153: 400-408.

Johnson, R. A., M. A. Baker, D. J. Pinkava, and G. A. Ruffner. 1993. Seedling establishment, mortality, and flower production of the acuna cactus, *Echinomastus erectocentrus* var. *acunensis*. Pp. 170-180 in Proceedings, Southwestern Rare and Endangered Plant Conference.

Marshall, W. T. 1950. Arizona's cactuses, first edition. Desert Botanical Garden of Arizona, Science Bulletin 1:89-91.

Marshall, W. T. 1953. *Echinomastus acunensis*. *Saguaroland Bulletin* 7: 33-34.

Matschiner, M. and W. Salzburger. 2009. TANDEM: integrating automated allele binning into genetics and genomics workflows. *Bioinformatics* 25: 1982-1983.

Moritz, C. 1999. Conservation units and translocations: strategies for conserving evolutionary processes. *Hereditas* 130: 217-228.

Nassar J. M., J. L. Hamrick, T. H. Fleming. 2001. Genetic variation and population structure of the mixed-mating cactus, *Melocactus curvispinus* (Cactaceae). *Heredity* 87: 69–79

National Park Service Cooperative Park Studies Unit (Tucson, A.Z., United States. National Park Service., Organ Pipe Cactus National Monument (Ariz.). Division of Natural and Cultural Resources Management. 1995. *Organ Pipe Cactus National Monument, Ecological Monitoring Program, Annual Report 1993.* Tucson, Ariz.: National Biological Service, Cooperative Park Studies Unit, The University of Arizona.

Nolte, A. W. and D. Tautz. 2009. Understanding the onset of hybrid speciation. *Trends in Genetics* 26: 54-58.

Nybom, H. 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology* 13: 1143-1155.

Otero-Arnaiz, A., A. Casas, J. L. Hamrick, and J. Cruse-Sanders. 2005. Genetic variation and evolution of *Polaskia chichipe* (Cactaceae) under domestication in the Tehuacán Valley, central Mexico. *Molecular Ecology* 14: 1603 – 1611.

Payton, A. C., A. A. Naranjo, W. Judd, M. Gitzendanner, P. S. Soltis, and D. E. Soltis. 2019. Population genetics, speciation, and hybridization in *Dicerandra* (Lamiaceae), a North American Coastal Plain endemic, and implications for conservation. *Conservation Genetics* 20: 531-543.

Peakall, R., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288-295.

Peakall, R., and P. E. Smouse. 2012. GENALEX 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28: 2537-2539.

Phillips III, A. M., and W. H. Buskirk. 1982. Status of the Acuña cactus (*Neolloydia erectocentra* var. *acunensis*) and Ajo rock daisy (*Perityle ajoensis*) at Organ Pipe Cactus National Monument, Arizona. Report to the National Park Service.

Porter, J. M., M. S. Kinney, and K. D. Heil. 2000. Relationships between *Sclerocactus* and *Toumeya* (Cactaceae) based on chloroplast *trnL-trnF* sequences. *Haseltonia* 7: 8–23.

Porter, J. M., J. Cruse-Sanders, L. Prince, L. Robert. 2012. Species status of *Sclerocactus brevispinus*, *S.wetlandicus*, and *S. glaucus*: inferences from morphology, chloroplast DNA sequences, and AFLP markers. *Aliso: A Journal of Systematic and Evolutionary Botany*. 30: 69-83.

Powell, A. M., A. D. Zimmerman, and R. A. Hilsenbeck. 1991. Experimental documentation of natural hybridization in Cactaceae: origin of Lloyd's Hedgehog cactus, *Echinocereus* x *lloydii*. *Plant Systematics and Evolution* 178: 107-122.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959.

Rayamajhi, N., and J. Sharma. 2018. Genetic diversity and structure of a rare endemic cactus and an assessment of its genetic relationship with a more common congener. *Genetica* 146: 329-340.

Reed D. H., and R. Frankham. 2003. Correlation between fitness and genetic diversity. *Conservation Biology* 17: 230–237.

Ramos-Ortiz, S., K. Oyama, H. Rodríguez-Correa, and A. González- Rodríguez. 2016. Geographic structure of genetic and phenotypic variation in the hybrid zone between *Quercus affinis* and *Q. laurina* in Mexico. *Plant Species Biology* 31: 219-232.

Roa, V. R., and T. Hodgkin. 2002. Genetic diversity and conservation and utilization of plant genetic resources. *Plant Cell, Tissue and Organ Culture* 68: 1–19.

Schwabe, A. L., A. R. Hubbard, J. R. Neale, and M. E. McGlaughlin. 2013. Microsatellite loci development for rare Colorado *Sclerocactus* (Cactaceae). *Conservation Genetics Resources* 5: 69–72.

Schwabe, A. L., J. R. Neale, and M. E. McGlaughlin. 2015. Examining the genetic integrity of a rare endemic Colorado cactus (*Sclerocactus glaucus*) in the face of hybridization threats from a close and widespread congener (*Sclerocactus parviflorus*). *Conservation Genetics* 16: 443-457.

Solórzano, S., P. D. Cuevas-Alducin, V. García-Gómez and P. Dávila. 2014. Genetic diversity and conservation of *Mammillaria huitzilopochtli* and *M. supertexta*, two threatened species endemic of the semiarid region of central Mexico. *Revista Mexicana de Biodiversidad* 85: 565-575.

Smissen, R. D., S. J. Richardson, C. W. Morse and P. B. Heenan. 2014. Relationships, gene flow and species boundaries among New Zealand *Fuscospora* (Nothofagaceae: southern beech). *New Zealand Journal of Botany* 52: 389-406.

Tepedino, V. J., T. L. Griswold, and W. R. Bowlin. 2010. Reproductive biology, hybridization, and flower visitors of rare *Sclerocactus* taxa in Utah's Uintah Basin. *Western North American Naturalist* 70: 377-386.

Terry, M. K., A. E. Pepper, A. W. Strong, D. M. Tarin, D. M. Price and J. R. Manhart. 2012. Genetic structure of a population of the endangered Star Cactus (*Astrophytum asterias*) in Southern Texas. *The Southwestern Naturalist* 57: 182-188.

Todesco M., M. A. Pascual, G. L. Owens, K. L. Ostevik, B. T. Moyers, S. Hübner, S. M. Heredia, M. A. Hahn, C. Caseys, D. G. Bock and L. H. Rieseberg. 2016. Hybridization and extinction. *Evolutionary Applications* 9: 892–908.

Tropicos.org. Missouri Botanical Garden. 20 Apr 2020 <http://www.tropicos.org/Name/100438785>

United States Fish and Wildlife Service. 1993. Endangered and threatened wildlife and plants; review of plant taxa for listing as endangered or threatened species. Federal Register 58: 51144-51190.

United States Fish and Wildlife Service. 2013. Endangered species status for *Echinomastus erectocentrus* var. *acunensis* (Acuna Cactus) and *Pediocactus peeblesianus* var. *fickeiseniae* (Fickeisen Plains Cactus) throughout their ranges. Federal Register 78: 60608-60652.

Whitham, T., G. Martinsen, P. Keim, K. Floate, H. Dungey, and B. Potts. 1999. Plant hybrid zones affect biodiversity: Tools for a genetic-based understanding of community structure. *Ecology* 80: 416-428.

Zimmerman, A. D. and B. D. Parfitt. 2003. *Echinomastus*. pp 194-195. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. Vol. 4. Oxford University Press, New York, NY.

Zumwalde, B. A., J. K. Dahir, A. B. Shaw, A. Willis, and S. D. Fehlberg. 2019. Characterization and development of microsatellite markers for *Echinomastus johnsonii* (Cactaceae) and congeneric taxa. *Applications in Plant Sciences* 7: e11302.