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Morphological and Ecological Characterization of Opuntia Miller on the Coast of South Carolina

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MORPHOLOGICAL AND ECOLOGICAL CHARACTERIZATION OF
OPUNTIA MILLER ON THE COAST OF SOUTH CAROLINA

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Plant and Environmental Science

by
Kirk Holmes
August 2016

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ABSTRACT

The genus *Opuntia* Miller is one of the most misunderstood and ignored genera of plants occurring in the southeastern United States. This study focused on the clarification of *Opuntia stricta* (Haw.) Haw., *Opuntia macrarthra* Gibbes, *Opuntia pusilla* (Haw.), *Opuntia dillenii* (Ker Gawl.) Haw. and *Opuntia tunoidea* Gibbes on the coast of South Carolina. A comprehensive literature review was completed to gather an understanding of *Opuntia* biology, species concepts and geographic range. The objectives of the morphological study were to determine if *Opuntia* taxa matching past descriptions of the five species chosen were present, what the morphological characteristics of the individuals collected were, if each could be differentiated into five morphologically distinct groups separate from *Opuntia lindheimeri* (Engelm.), *Opuntia lata* (Small) and *Opuntia mesacantha* (Raf.) ssp. *mesacantha* (Majure), and if eight species analyzed behaved on the level of a morphological species. The results from the statistical analysis revealed that there appears to be five morphologically distinct species on the coast of South Carolina that are different from *Opuntia lindheimeri*. Due to limited habitat *O. dillenii* had to be excluded from the ecological study. The objectives of the ecological and geographic range study were to get a better understanding of *Opuntia* presence of the coast of South Carolina. This involved analyzing ecological variables collected using the protocol developed by the Carolina Vegetation Survey. Correlation graphs revealed sites indicative of *Opuntia* in general. Partition analysis was used to build path maps of variables that have a direct impact on the cover value of *Opuntia*. When interpreted the path maps indicate that four of the chosen study species occupy a weak adaptive zone

ecologically. As an addition to ecological study, distribution maps were constructed.

They show the location of *Opuntia* species at the time of the study.

DEDICATION

I would like to dedicate this thesis to my family, Callie, and everyone who has supported me throughout the process. I am also very thankful to the Lord for keeping me strong through every season of this journey.

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CHAPTER ONE

GENERAL INTRODUCTION AND LITERATURE REVIEW

Introduction

The genus *Opuntia* Mill. is one of the most misunderstood and ignored genera of plants occurring in the southeastern United States. Various taxonomic treatments list the diversity of native species of *Opuntia* in our region as ranging from as few as two taxa (Radford et al., 1968) to as many as 22 (Small, 1922). This confusion is due to the rather inhospitable nature of working on such a well-armed species group, the phenotypic plasticity observed within and among species, the difficulty in preserving meaningful dried specimens, and the formation of polyploid hybrid swarms (Powell and Weedon, 2004; Ward, 2009). There are only two in-depth modern treatments of the genus. The first is that of (Majure and Ervin, 2007), completed on the genus in Mississippi. The second is the recent addition to the Flora of the Southern and mid-Atlantic States contributed by Lucas Majure (Weakley, 2015). To date, there has been no comprehensive modern treatment on the coast of South Carolina that includes a careful study of the ecological and morphological characteristics of the genus.

Other recent studies completed by Lucas Majure (Majure et al., 2012a; Majure et al., 2012b) have begun to clarify phylogenetic relationships for many of the cryptic taxa, particularly in the “*humifusa* clade”. Scientists now have a better understanding of the ploidy differences and genetic divergence of many taxa, but their ranges, habitats, natural enemies, ecological limitations, and morphological distinctions and variance are still in need of clarification.

Classification

Opuntia species are angiosperms that belong to the order Caryophyllales (Powell and Weedin, 2004). Caryophyllales are Eudicots, and are the third youngest group of angiosperms. The group is the predecessor to the Rosids and, subsequently, the Asterids (Ballard 2015).

Morphology

They are succulent-type plants in the family Cactaceae. All *Opuntia* are evergreen perennial plants, they are split into three different subgenera: *Cylindropuntia*, *Grusonia*, and *Platyopuntia* (Powell and Weedin, 2004). The categories are classified based on the shape of the pads and the size of the plant itself. The *Platyopuntia* go by the common name of “prickly pear”, having orbicular, flat cladodes that are referred to as “pads” (individual stem segments). *Cylindropuntia* are known as the tree chollas, and are arborescent in form and have cylindrical shaped stem segments. *Grusonia* are known as the dog chollas, with stem segments that are cylindrical. Unlike *Cylindropuntia*, their habitat is a low prostrate mound that is usually less than 30 cm high.

Stems

The stems of *Opuntia* are segmented and referred to as pads. *Cylindropuntia* pads are more complex than *Platyopuntia* in that they have tubercles and ribs, which protrude from the stems (almost like furrows in the bark of an oak). They are referred to as podaria (Powell et al., 2008), and function as the main photosynthetic structure. The pads of *Opuntia* are covered in areoles, which function like stomata and are areas of gas

exchange (Powell and Weedin, 2004). Areoles are locations in which the spines are attached, vegetative buds form, and flowers bud. Several species of *Opuntia* possess purple pads. *Opuntia* belongs to Caryophyllales, and, therefore, the color is due to the secondary Betalain compounds contained in their tissue. Betalains are water-soluble, vacuolar pigments containing nitrogen in their molecular structures. They consist of betacyanins that produce red colors and betaxanthins that produce yellow colors (Powell and Weedin, 2004). These species include *O. macrocentra* (Engelm.), *O. parva* (Rose), *O. humifusa* (Raf.), etc. *Opuntia* express betacyanin pigments in their flowers (Betaxathins), pads, and even spines. Secondary pigments become visible after the breakdown of chlorophyll. Perennial plants do this as a means of storing nutrients for next year's growth. It is thought that betalain pigments aid in drought and cold tolerance (Ramakrishna and Ravishankar, 2011).

Spines

There are very few species of *Opuntia* that are spineless. Most spineless forms are cultivars of species that have spines in their native form. Spines are an adaptation for protection from herbivores, protection from light, and also serve reproductive purposes, as many species readily dislodge and become entangled in the fur of animals and can be transported as asexual clones. Spines are most often accompanied by glochids, which are sharp trichomes several millimeters in length. These are also an adaptation to herbivory, but may also serve as a water conservation characteristic. A boundary layer is often formed near the surface of leaves or pads, and serves its purpose in keeping the air still in order to prevent rapid evaporation.

Leaves

Although mostly absent, the cone-shaped leaves of *Opuntia* are seen mostly in the spring and are associated with new growth. In appearance, the leaves are more like an enation than an actual leaf, being several millimeters long with no venation (Powell et al., 2008).

Flowers

Opuntia has some of the most amazing flowers in the world, with their coloration due to betalain compounds. Bloom time typically occurs from April through June, depending on the region in which the species dwell. The bee-pollinated, edible flowers are complete hermaphroditic flowers, meaning they are monocious (having both male and female reproductive organs). Each flower contains both stamens and pistils (Powell et al., 2008). The individual flowers of *Opuntia* species only bloom for one to three days, with most in bloom for six or seven hours. Due to the arid areas they inhabit, this is an adaptation to water conservation. *Opuntia* also has fused carpels, free-central placentation, and no true petals, only sepals (Ballard, 2015).

Fruit

Maturing in September and throughout the fall, the fruits of *Opuntia* are predominantly fleshy and are sometimes dry and indehiscent (Powell et al., 2008). Common shapes of the fruit are obovoid, elliptical, and spheroidal. Some fruits are smooth, however, most have glochids and spines.

After fertilization, the fruit forms, which, as mentioned, is fleshy and indehiscent. When fruit forms, it is subsequently eaten by birds and small animals. *Opuntia* have very hard seed coats and inhibiting chemicals that prevent germination under unfavorable conditions. Therefore, ingestion of the seeds helps them overcome dormancy. When the seeds are chewed, they are scarified. This also occurs when they pass through the acid bath of the stomach. (Ueckert, 2015).

Metabolism

There are three different types of plant metabolisms that are related to photosynthesis. The metabolisms are C₃, C₄, and CAM. Each one of these deals with how plants sequester carbon for photosynthesis. *Opuntia* are succulent plants that maintain the CAM (Crassulacean Acid Metabolism) system for sequestering carbon. CAM takes place in single cells of the pad. It involves cytosol, vacuoles, and chloroplasts. This particular type of plant metabolism occurs at night, when temperatures are cooler. When the stomata open, CO₂ diffuses into the cytosol where HCO₃⁻ (bicarbonate) is generated. Phosphoenolpyruvate carboxylase then incorporates HCO₃⁻ into phosphoenolpyruvate. Malic acid is then yielded from the previous process and is stored in the vacuole. This is very important during the day, when temperatures are high and the stomata are closed. When the stomata are closed, CO₂ cannot enter the leaf. The adaptation of CAM allows for the release of malic acid from the vacuole, which is broken down into CO₂ and pyruvate by enzyme activity. The CO₂ then enters the Benson-Calvin cycle (Taiz et al.). This is an adaptation that helps plants perform photosynthesis and survive in arid environments. This metabolism allows for more efficient water conservation, which is

why it occurs at night, when temperatures are cooler. As a result of maintaining this metabolism, succulent plants are also more efficient at nitrogen use because of the reduced need for Rubisco.

Reproduction

Opuntia reproduces both sexually and asexually. In nature, the species have a high degree of clonal growth. In addition to protection against herbivory, the spines are an adaptation to clonal growth. When animals or humans walk by the plants, they assist in dislodging the pads from the plant. *Opuntia* has pads that have the ability to produce new roots in as little as three days (in the right conditions). When pads are broken off of an *Opuntia* plant, they form roots and reproduce asexually. This may also be an adaptation to the short flowering period. The plant can overcome its reduced ability to sexually reproduce due to water conservation. Although *Opuntia* flowers are hermaphroditic, they are herkogamous. The stigmas are positioned above the filaments, making them self-incompatible (Powell and Weedin, 2004). Bees are the main pollinators of *Opuntia*, and are responsible for sexual reproduction. After fertilization, the fruit forms, which, as mentioned, is fleshy and indehiscent. When fruit forms, it is subsequently eaten by birds and small animals. *Opuntia* have very hard seed coats and inhibiting chemicals that prevent germination under unfavorable conditions. Therefore, ingestion of the seeds helps them overcome dormancy. When the seeds are chewed, they are scarified. This also occurs when they pass through the acid bath of the stomach. (Ueckert, 2015).

Natural Enemies

Opuntia species are eaten by many pests. Several include stem sucking *Chelinidea* (Cactus bugs), fruit sucking *Narnia* spp. (Corieds), weevils, red spider mites, Cochineal insects, and *Cactoblastis* moths. The cochineal insects, in particular, are very interesting from an economic standpoint. They harm the plants with their piercing, sucking mouth parts which, in turn, provide us with red food dye. The *Cactoblastis* moth (*Cactoblastis cactorum*) Berg. is threatening the existence of *Opuntia tunoidea* on the South Carolina coast. The insect is native to South America, and specifically to Argentina and Paraguay. In South Carolina, the adults have three flight periods. The first is a spring flight period that takes place from February to May. The second is known as the summer flight, which occurs from June to August. The third flight period is in the fall, and occurs from September to November (Hight and Carpenter, 2009).

During the nine days that the flight periods persist, adults lay eggs in egg stick structures. Each individual female can lay three to four egg sticks during its lifespan. However, it is not uncommon for them to lay eight to twelve egg sticks. The eggs, which are laid in rings starting at the base of a spine and working outward, create the egg sticks. Each egg stick contains up to ninety eggs. As the eggs hatch, the bright orange larvae bore into the tissue of the pad and start consuming it for nutrients. (global invasive species database). This quickly leads to the demise of the cactus.

Not only does the *Cactoblastis* Moth cause damage by feeding, there is also a certain degree of bacterial infection that occurs as well. After larvae have consumed the pads and are developed, they form cocoons for metamorphosis. This is usually done in the leaf litter or the debris of rotting pads on the ground. After the adult emerges, the

cycle is repeated. (University of Florida Entomology & Nematology). Efforts are being made to control the Cactoblastis Moth on *Opuntia tunoidea*. However, the reproductive biology of the moth and the morphology of the cactus itself make efforts difficult. Since larvae feed inside of the cactus and pupate in the ground, treating is best done in the adult stage. This is done with a contact insecticide, but is difficult because you are going after a moving target. Systemic insecticides are used, but are not very effective because they move too slowly through the cactus. This is due to its succulent morphology.

Ecology

In South Carolina, *Opuntia* are known to occupy the coastal beaches. Three zones define the coastal beaches. The high tide zone is where even the highest spring tide never surpasses. The detritus zone or drift line is composed of *Spartina alterniflora* (Aiton) remnants and other debris washed up from nearby marshes. The berm is the area of loose sand between the driftline and dunes. The dunes are mounds formed by wind blown sand accretion in the berm zone. Sand is deposited forming a mound when it hits a windbreak. *Uniola paniculata* L. plays a key role in slowing the wind allowing sand to deposit. Dunes are protected by South Carolina Law to protect them and the species that stabilize them from pedestrian disturbance. Since dunes protect against the forces of the ocean this is very important. *Opuntia* is known to occur in the swale area behind the dunes. This type of habitat is referred to as a maritime grassland (Porcher and Rayner, 2001). It is characterized as being a fairly flat area, protected by wind and ocean waves. It is also protected by pedestrian traffic in that specific habitat. The soil is composed of sand and crushed shell. Here, *Opuntia* is found growing with species such as *Spartina patens* (Aiton), *Smilax ariculata* (Walter), *Gaillardia pulchella* (Fougeroux), *Ilex vomitoria*

(Aiton), *Hydrocotyle bonariensis* (Lamarck), *Yucca gloriosa* (L.), *Juniperous silicicola* (Small), and *Sabel palmetto* (Walter). Maritime grasslands can be very dense; however, they are still very light intensive due to the type of species that grow in them. The cacti, however, do grow in areas of the grassland that are less dense, with larger species such as *Juniperous silicicola* (Small) Bailey. On barrier islands where maritime shrub thickets develop, *Opuntia* species are known to occur on the outer edge and openings as well (Porcher and Rayner, 2001).

Another place *Opuntia* species are known to occur is in the more open areas of the maritime forests. These forests are formed by salt spray. They occur inland from the shoreline and on barrier islands. They are composed of plants such as *Qurecus virginana* Miller, *Pinus taeda* L., *Magnolia grandiflora* L., *Ilex opaca* (Aiton), *Zanthoxylum clava-herculis* L., *Ilex vomitoria* (Aiton), *Morella cyrifera* L., *Persia borbonia* L. Sprengel, and other salt tolerant plants (Porcher and Ryner, 2001).

Opuntia species also occur in or on Native American shell mounds, rings or hummocks. These can occur on land or as islands in the salt marsh. They are basically composed of piles of shell. This causes the soil in the area to be very high in calcium. The areas are inhabited with species found in maritime forests, and maritime grasslands along with calcicoles. Calcicoles are plants that love calcium soils. These include species such as *Acer barbatum* Michaux, *Sageretia minutiflora* (Michaux) Mohr, *Tilia heterophyla* (Vent.), and *Cornus asperifolia* Michaux (Porcher and Rayner, 2001)

Geographic Range

Geographic range is the geographic area in which a plant species can be found. There are a number of factors that determine the geographic range of a plant species. It includes things such as climate, soil type, and other plant species. There are also certain indicators that help identify these ranges. The *Opuntia* species of this study seem to have a somewhat endemic distribution within South Carolina. They appear to be restricted to the coastal maritime grassland habitat. This may be due to several reasons. *Opuntia* species are succulent plants. They thrive in dry, warmer, open areas. A desert is a term that can be used to describe such an area. In South Carolina, the coastal beaches meet these requirements. The soil is sandy and porous. The climate stays warmer for longer periods of time than the rest of the state. The soil is very high in calcium, which is also found in West Texas deserts. Salinity is higher, limiting or stunting what species can grow in maritime grassland habitats. This contributes to the openness of the habitat, along with other variables like dry soil, warmer climate, and nutrient status. The counties chosen for this part of the study were known for having this type of habitat.

Species Concepts

When attempting to classify species taxonomically, it is important to think about species concepts. The morphological species concept defines groups of species by gaps in morphological variation. It focuses on the similarities members of a species have (Judd et al., 2008). The issue with classifying on this premise is that species that are genetically or ecologically very distinct, perhaps even with reproductive barriers, may be considered the same species. This method does not account for biological or ecological processes but

rather relies on morphology as a proxy for those. (Species Concepts, 1998). A species defined using this concept is known as a Phenetic Species. Phenetic classification is grouping based on similarity. Matrices of similarities are used to create a Phenogram in order to estimate phylogeny (Mallet, 2007).

The premise behind the Ecological Species Concept is that a species occupies a particular adaptive zone. The biological species concept states that a species is a group of interbreeding populations that are reproductively isolated from other groups (Mayer, 1942). The problem with this is species as well as genera have shown to interbreed. It is generally assumed that if you are a biological species, then you are a species but if you don't satisfy the biological species concept you may still be a species by several definitions.

The Phylogenetic Species Concept states that an evolutionary species has a single lineage of descent from which it maintains its identity (Whitley, 1978). Phylogenetic species correspond to the terminals on a cladogram. The problem arises when determining where to draw the monophyletic line.

The species concepts are human envisioned ideas or theories. Scientists want to try and place everything into neat artificial "boxes". This provides some way to function with the idea of classification. These "boxes" don't always work as planned. Statistical software helps eliminate human error and analyze traditional species concepts with a much more accurate and unbiased approach. The real question that arises in this project is, "Can we classify each of our study species as species based on traditional concepts with the data collected?"

Taxonomic treatment in this study

When this study was initiated, there was much confusion about these taxa and the treatment by Majure included in Weakley (2015) was not completed. The taxonomy of *Opuntia* species is likely to change again in the near future. Included here is a list of the names used in this treatment and their corresponding names in Weakley (2015).

Name of species referred to in this study	Corresponding name in Weakley (2015)
<i>Opuntia dillenii</i> (Ker.) Haw.	<i>Opuntia stricta</i> var. <i>dillenii</i> (Ker. Gawler)
<i>Opuntia pusilla</i> (Haworth) Nuttall	<i>Opuntia drummondii</i> (Graham)
<i>Opuntia stricta</i> (Haw.)	<i>Opuntia stricta</i> (Haw.) Haworth var. <i>stricta</i>
<i>Opuntia macrarthra</i> Gibbes	<i>Opuntia mesacantha</i> spp. <i>australis</i>
<i>Opuntia tunoidea</i> Gibbes	Considered as <i>Opuntia engelmannii</i> var. <i>lindheimeri</i> B.D. Parffit & Pinkava Combined with <i>Opuntia stricta</i> var. <i>dillenii</i> (Ker. Gawler) and <i>Opuntia stricta</i> (Haw.) Haworth var. <i>stricta</i>
<i>Opuntia lindheimeri</i> (Engelm.)	<i>Opuntia engelmannii</i> var. <i>lindheimeri</i> B.D. Parffit & Pinkava
<i>Opuntia lata</i> (Small)	<i>Opuntia mesacantha</i> (Rafinesque) spp. <i>lata</i> (Small) Majure
<i>Opuntia mesacantha</i> (Raf.) ssp. <i>mesacantha</i> (Majure)	<i>Opuntia mesacantha</i> (Raf.) <i>mesacantha</i> ssp. <i>mesacantha</i> (Majure)

Table 1.1 Species names referred to in this study in correspondence to names referred to in Weakley (2015).

Description of Species



Figure 1.1 Flowers and Pads of *Opuntia dillenii*. Coastal South Carolina U.S.A.

Opuntia dillenii (Ker.) Haw. Is currently described as *Opuntia stricta* var. *dillenii* (Ker. Gawler) in Weakley's Flora of the Southern and Mid- Atlantic States (Weakley, 2015). This study describes it from the coastal region of South Carolina. This species has been found to occupy the bank of the Stono River at the very tip of Wadmalaw Island, in a location known as Rockville. This species has also been treated as a variety of the coastal species *Opuntia stricta* as *Opuntia stricta* var. *dillenii* by treatments such as that of L. D. Benson (Bohm, 2008).

John Kunkell Small (J.K.) was the first to describe *Opuntia dillenii* in the southeastern United States in his Manual Of The Southeastern Flora published in 1933.

He described the species in Florida and on the islands of Florida. He described it to occupy the coastal areas, tidal hammocks, and coastal dunes. The habitat site is very similar in its occupancy in South Carolina (Small, 1933).

It is documented for our study as rare growing on bank of Stono River raised 2 – 3 m above water level, growing in association with *Baccharis halimifolia*, *Smilax ariculata*, *Andropogon tenuispathus*, and *Spartina patens*. Plants are growing in sand between rocks and oyster shell used for bank retention. Locations include Site 1 – very end of Maybank Hwy. behind 7129 Maybank Hwy. Wadmalaw Island, SC. Site 2 – 2 individuals spotted near 2451 and 2455 Sea Island Yacht Club Road. 18 March, 2015.

Plants are perennial, succulent and erect. Specimens measured were 47.5 cm tall x 47.5 cm wide with pads 21.5 cm long x 7.5 cm wide and 60 cm tall x 75 cm wide with pads 20 cm long by 12 cm wide. Sinuate elliptic to obovate pads are light green in color and free from any apparent insect damage. The epidermis of the pad is elevated at every areole (area of gas exchange), which is callus like and white in color. Each areole, 4.5 cm apart, on average contains up to 3 flattened golden yellow straw colored spines. Spines are up to 3 cm in length. Accompanying the spines at each areole are glochids (trichome like protrusions) of the same color up to 2 mm long. Flowers are yellow with salmon streaks on the inner tepals. The size of the flowers measured was 8cm wide. The stigmas are yellow with up to 6 lobes on styles up to 1.25 cm long. The anthers are yellow on filaments up to 1 cm long. Up to 8 inner tepals have been recorded with their length up to .75 cm. The Inner tepals were measured up to 2cm in length with an individual presence recorded of 16 tepals. No fruit were found on population in South Carolina.



Figure 1.2 Flowers and Pads of *Opuntia pusilla*. Coastal South Carolina U.S.A.

***Opuntia pusilla* (Haworth) Nuttall** is formally known as and now properly referred to by L. Majure as *Opuntia drummondii* Graham (Weakley, 2015). It is described in this thesis study from the coastal region of South Carolina. Majure (2007) states that the recognized *Opuntia tracyi* Britton in (Small, 1933) may be *O. pusilla* as well. He states it may be a result from phenotypic plasticity. During this study *O. pusilla* was found to occupy 4 counties and 9 different locations with the coastal region. (Weakley, 2015) describes it as occurring from Georgia, North Carolina, South Carolina, Florida to Texas. He quotes it as a southeastern coastal plain endemic. Small included it as *Opuntia drummondii* in the Southeastern United States in his *Manual of the Southeastern Flora* published in 1933 (Small, 1933). His description matches (Weakley, 2015; Majure, 2007; Porcher and Rayner). *Opuntia pusilla* is known for its wound inflicting barbed spines and

disarticulating pads. The plants tend to grow in the areas behind active dunes of the coastal beaches and barrier islands. They are also known to occur on shell mounds such as those found at Hobcaw Barony in Georgetown County.

Plants are perennial, succulent and prostrate, to 10 cm tall x 15 or more cm wide with pads 4 cm long x 1.5 cm wide. Cylindrical stem joints, green in color, during spring and summer, often turn purple in the winter months. Plants were found to be apparently less susceptible to insect damage than the larger species. Spines are usually present in single or in pairs of 2 to 3 at every areole on the stem joint. Spines are light brown to tan and rounded (widest at base), 1 – 3 cm long. Older spines may be grey. Areoles are raised averaging around 1.0 cm to 1.5 cm apart and occur at a density of between 6 and 12 per 25cm² on the pad. Accompanying the spines at each areole are glochids (trichome like protrusions) white in color and averaging 1.0 mm in length. The flowers of *O. pusilla* are a lemon yellow in color, sometimes with salmon colored blotches near the tips of the inner tepals. Flowers range to 1.5 cm L X 3.0 cm W. They are composed of up to 6 to 8 outer tepals (green/yellow with red tips) that are up to 1.5cm in length and have up to 11 inner tepals (yellow) up to 3cm in length. The stigmas are whitish and consist of 4-5 lobes on styles averaging 1.75 cm long. The anthers are very light yellow with whitish filaments averaging 1.25 cm in length. The fruit of *O. pusilla* is red, averaging 2.5 cm L and 1.5 cm W. They contain relatively large seeds on the light pink inside coated in a clear sweet pulp. The seeds that were measured averaged up to 4 – 5 mm in size.



Figure 1.3 Pads and Flowers of *Opuntia stricta*. Coastal South Carolina U.S.A.

***Opuntia stricta* (Haw.)** (Pest Pear) is known from the coastal region of South Carolina. In this study the species was found to occupy 2 counties and 4 different locations within the coastal region of South Carolina. It has been described as occurring from North Carolina, South Carolina, Florida, Texas, Alabama and Mississippi (Weakley, 2015); Majure,2007). Weakley say it occurs in coastal dunes, coastal scrubs, shell middens and salt marsh areas. These types of habitats remain true in the counties of South Carolina. One exception to that is a specimen growing epiphytically 9 m up in a live oak canopy found in Rockville, SC. Weakley also mentions that Small described it as *Opuntia sricta* confused with *Opuntia tunoidea* in the Southeastern United States in his Manual Of The Southeastern Flora published in 1933 (Small, 1933). Descriptions of this species claim it has spines but may be spineless (Powell and Weedon, 2004; Majure 2007;

Weakley, 2015). The plants have been found in this study to grow on shell mounds (ie. Hutchinson Island) and other barrier islands. *Opuntia stricta* plays a key role in the presence of the *Cactoblastis* Moth in SC. The moth was introduced to control the invasive *O. stricta* in Australia. It was eventually introduced in the Caribbean Islands, which is how it made its way here (Majure, 2007).

Plants are perennial, succulent, and have a sprawling/frutescent growth habit. Each plant is on average 26 cm tall x 70 cm wide with pads averaging 13 cm long x 6 cm wide. The stems, referred to as cladodes are elliptic or linear, and are usually broader toward the middle. They are green in color during spring and summer. During the winter they may have purple tinges and a wrinkled appearance. *Chelinidea* (Cactus bugs) have altered the appearance of the pads in some locations. Spines are absent from the cladodes. The areoles are flush with the epidermis of the cladode averaging 3cm apart and occur at a density of between 4 per 25 cm² on the pad. Dark glochids up to 1mm are sometimes present. The flowers of *O. stricta* are a yellow in color, sometimes having salmon colored blotches near the tips of the inner tepals. The size of the flowers is on average 3.5 cm L x 6 cm W. They are composed of up to 6 to 7 outer tepals (green/yellow with red tips) that are up to 1.5 cm in length and have up to 13 inner tepals (yellow) up to 3.5 cm in length. The stigma/style are white consisting of 4-5 lobes and averaging 1.75 to 2 cm long. The anthers are yellow with yellow filaments averaging 1.5 cm in length. The fruit of *O. stricta* are reddish purple, elongated with a wider apex averaging 2.5 cm L and 1.5 cm W. It is not uncommon to see them rippled. They contain relatively large seeds on the lighter inside coated in a pinkish clear pulp. Often times seedless, the seeds measured averaged up to 4 – 5 mm in size.



Figure 1.4 Pads and Flowers of *Opuntia macrarthra*. Coastal South Carolina U.S.A.

***Opuntia macrarthra* Gibbes** is currently included in the concept of *Opuntia mesacantha* *spp. australis* by Lucas Majure and is recognized in Weakley's Flora of the Southern and Mid- Atlantic States (Weakley, 2015). It is known from the coastal region of South Carolina. In our study, the species was found to occupy 3 counties and 7 different locations within the coastal region. It's described as occurring in SC and NC (Small, 1933). Small describes it as occurring in sand-dunes. The original description by Gibbes in the Elliot Society Jan. 1858 describes the species as being within a few miles of Charleston, SC. This goes without any further elaboration. Gibbes wrote that the species was prostrate in habit with pads to 10 – 15 inches long (25 – 38cm), 3 inches wide (7.62 cm) and 1 inch thick (2.54 cm). Fruit were described as being 2.5 inches long (6.34 cm) by 1 inch thick (2.54 cm), slender and clavate (Russell and Jones, 1859). (Small,1933)

gives a more detailed description of the species. He adds that the color of the species was light green with sparse spines. He describes the spines as solitary, brown with a pale tip, and 1.5 – 3.5 cm long. He described the flowers as bright yellow, 6-7 cm wide, having inner tepals 3 – 3.5 cm, etc. The fruits were described as clavate-obovate, red to red-purple, and 4-6cm long. They contained somewhat flat seeds 4 – 4.5 mm in diameter (Small, 1933). The size of the pads, size of the fruit, and the habit from the original description complement the current description (i.e. Small says pads are 15- 33 cm in length, etc.).

Plants described in this study are perennial, succulent, with sprawling/frutescent growth habit. They average 40 cm tall x 70 cm wide with pads 21 cm long x 8.5 cm wide. The stems are very similar to *O. stricta* with exception of raised areoles accompanied by dark tufts of glochids. As with *O. stricta*, Chelinidea (Cactus bugs) have altered the appearance of the pads in some locations. Toothpick-like spines are brown with a pale tip up to 4.25 cm long. Areoles average 2.84 cm apart and occur at a density of 5 per 25 cm² on the pad. The flowers of *O. stricta* a yellow in color, sometimes with salmon colored blotches near the tips of the inner tepals. The size of the flowers averages 8cm L X 8 cm W. They are composed of up to 7 outer tepals (green/yellow) that average 1 cm in length and up to 15 inner tepals (yellow) averaging 3.5 cm in length. The stigmas are white and consist of 4-5 lobes. They are on styles that average 2 cm long. The anthers are yellow on yellow filaments that average 1.5cm in length. The fruit are reddish purple, elongated with a wider apex and more round than *O. stricta*. The fruit averages 3cm long and 1.5 cm wide. The seeds measured averaged 4 mm in size.



Figure 1.5 Pads and Flowers of *Opuntia tunoidea*. Coastal South Carolina U.S.A.

***Opuntia tunoidea* Gibbes** Plants I am including as this species are considered by L. Majure in Weakley's Flora of the Southern and Mid-Atlantic States to be a type of *Opuntia lindheimeri* Engelm. (Weakley, 2015). This has not been validated genetically and the plants seem to differ from plants from Texas. Majure has combined the name *Opuntia tunoidea* with *Opuntia stricta dillenii* and my application of the name is representative of a different interpretation of these populations as potentially native in South Carolina. It is described in this study from the coastal region of South Carolina. This species has been found in this study to occupy 3 counties and 3 different locations. Efforts have been taken to preserve and protect this species from extinction due to the Cactoblastis moth. The moth has really affected the population that occurs in Edisto Beach State Park in Colleton County. Gibbes described *O. tunoidea* as one of 4 different *Opuntia* he found near Charleston. He stated that the erect plants had large ovate pads, and were armed with three quarter inch yellow spines with brown tips. (Russell and Jones, 1859).

Plants in this study are perennial, succulent, and have an erect arborescent/frutescent growth habit. They have been measured up to 90cm tall x 240cm wide with pads to 47 cm long x 39 cm. Pads are large, ovate, and blue-green with a white hue or green in color. The areoles are not raised, but upper marginal and some medial areoles are accompanied with glochids. These glochids are up to 5mm in length and dark golden yellow. Each areole is 3.5 to 4 cm apart on average. They occur at a density of 3 per 25cm² and 7 per 100cm². The spines are the same color as the glochids. They are flat, mostly curved downward, and up to 3cm long. They usually only occur along the upper margin of the pad and may be present at a density of up to 3 per areole. Several of the spines have been seen with a dark, brownish tip. The flowers of *O. tunoidea* are bright deep yellow. The stigmas are green, having up to 6 to 7 lobes with styles averaging 1.5 cm long. The anthers are yellow with filaments averaging 1 cm long. There are usually up to 7 to 10 outer tepals. They are green to greenish-yellow and average 2 cm in length. There are usually up to 12 to 15 inner tepals. They are yellow and average 3.5 cm long. The flower itself can be up to 10.5 cm wide by 8 cm long. The fruits are large and purple, wider at apex. The apex is usually indented like a cup, up to 7 cm long x 4 cm wide. The seeds measured averaged 3 mm in size.

CHAPTER TWO
MORPHOLOGICAL CHARACTERIZATION OF *OPUNTIA* MILLER ON THE
COAST OF SOUTH CAROLINA

Introduction

The goal of this research was to complete a morphological study on *Opuntia* populations on the Coast of South Carolina. The reason for this was to clarify morphological characteristics and species-level distinction. Since the use of dried material is limited, all of the observations and measurements were made from living specimens. As much information as possible was collected from the native populations. However, due to the need for prolonged observation of flowering, fruiting, and winter condition of the taxa, ex-situ individuals were maintained. The plants were grown in both greenhouse and trial beds outdoors. Individuals from sighted populations were collected for growing as live specimens and observation of morphological characteristics. Living individuals included; *Opuntia stricta* (Haw.) Haw., *Opuntia macrarthra* Gibbes, *Opuntia pusilla* (Haw.), *Opuntia dillenii* (Ker Gawl.) Haw. and *Opuntia tunoidea* Gibbes. Note that several *Opuntia* species names referred to in this paper have been updated recently in Weakley's Flora of the Southern and Mid Atlantic States. The following species have been updated: *Opuntia macrarthra* Gibbes is currently included under the concept of *Opuntia mesacantha* ssp. *australis* (L. Majure). *Opuntia dillenii* (Ker Gawl.) Haw. is considered *Opuntia stricta* var. *dillenii* (Ker-Gawler). *Opuntia pusilla* (Haw.) is now properly referred to as *Opuntia drummondii* (Graham) by L. Majure. Also *Opuntia tunoidea* (Gibbes) is considered to be a representative of *Opuntia lindheimeri* Engelm.(Weakley, 2015). However, the appropriate cytology, genetic research, and morphological research have not been completed to confirm the placement of *O. tunoidea*.

When attempting to classify species taxonomically, it is important to think about species concepts. The morphological species concept defines groups of species by gaps in morphological variation. It focuses on the similarities members of a species have (Judd et al., 2008). The issue with classifying on this premise is that species that are genetically or ecologically very distinct, perhaps even with reproductive barriers, may be considered the same species. This method does not account for biological or ecological processes but rather relies on morphology as a proxy for those. (Species Concepts, 1998). A species defined using this concept is known as a Phenetic Species. Phenetic classification is grouping based on similarity. Matrices of similarities are used to create a Phenogram in order to estimate phylogeny (Mallet, 2007).

The premise behind the Ecological Species Concept is that a species occupies a particular adaptive zone. The biological species concept states that a species is a group of interbreeding populations that are reproductively isolated from other groups (Mayer, 1942). The problem with this is species as well as genera have shown to interbreed. It is generally assumed that if you are a biological species, then you are a species but if you don't satisfy the biological species concept you may still be a species by several definitions.

The Phylogenetic Species Concept states that an evolutionary species has a single lineage of descent from which it maintains its identity (Whitley, 1978). Phylogenetic species correspond to the terminals on a cladogram. The problem arises when determining where to draw the monophyletic line.

The species concepts are human envisioned ideas or theories. Scientists want to try and place everything into neat artificial "boxes". This provides some way to function

with the idea of classification. These “boxes” don’t always work as planned. Statistical software helps use eliminate human error and analyze traditional species concepts with a much more accurate and unbiased approach. The real question that arises in this project is, “Can we classify each of our study species as species based on traditional concepts with the data collected?”.

The statistical analysis software used to analyze data collected for morphological measurements was JMP. A combination of cluster analysis, discriminant analysis, analysis of variance, Tukey HSD, and least squared contrast were used to identify groups of like individuals and the morphological measurements that did the best job at separating the groups.

Morphological measurements described were used to create groups of like individuals in cluster analysis. Discriminant analysis was used to identify the important variables that were used to place individuals in the groups. After identification, the means data for identified variables were compared across groups using ANOVA. Pairs of means were compared using the Tukey HSD test.

The purpose of this study was to answer the following questions: Are there *Opuntia* species matching past descriptions of these on the coast of South Carolina? What are their morphological characteristics? Are there morphological characteristics that differ between the five study species that can be used to tell them apart? Do the morphological characteristics differ from other *Opuntia* species described from South Carolina and ones native to Texas? How can they be differentiated from one another? Does each behave on the level of morphological species? The main question was, “Can

each of the study species be classified based on traditional morphology using the data collected?”

The results revealed a set of variables that can be used to differentiate the five species described from coastal South Carolina from one another, as well as from other described species. This confirmed gaps in the morphological characteristics between each one. Therefore, it is inferred that each of the five species obeys the morphological species concept. While providing information on morphological species level distinction, this study has also created a tool that will aid in the identification of the five species in the field.

Materials and Methods

Opuntia individuals for the morphological study were collected in five counties from coastal South Carolina as well as seven counties in coastal North Carolina. South Carolina counties included: Beaufort, Charleston, Colleton, Horry, and Georgetown. North Carolina counties included: Brunswick, New Hanover, Pender, Carteret, Hyde, Hatteras, and Dare. After collection, morphological characteristics were measured for each individual.

The morphometric data were entered into JMP where it was analyzed using a combination of techniques. Hierarchical Clustering was first used to place individuals contained in the data set into groups that were similar in terms of the set of morphological measurements applied. In hierarchical clustering, differences of the variable values are taken and averaged. The smaller the average between each individual (or group), the

more closely related the individuals (or groups) are considered. The desired goal of the cluster analysis was to discover groups to be related to one of the presumed study species.

After cluster analysis, discriminant analysis was used to determine which morphological characteristics were most important in placement of individuals into the clusters. Discriminant analysis predicts group membership based on a linear model of the morphological measurements (characteristics). The group membership and interval variable values are known at the time of analysis. The analysis creates a model of interval variable values for predicting unknown group membership (Stockburger, 1996). Discriminant analysis provides a canonical plot of the data. Hierarchical clustering and discriminant analysis do not provide a representation of the actual data measurements. For this reason, canonical data was used to look at the importance level of the variables that did the best job from the discriminant analysis. In particular, the standardized scoring coefficients were used to determine the variables that had the most negative and most positive weights contributing to the canonical score of each cluster.

The variables with significant weights were chosen for Analysis of Variance (ANOVA). ANOVA was used to compare the means of the morphological measurements between groups. If morphometric variables tested gave a significant p-value, it indicated that at least one of the group means for that variable in relation to each of the tested species was different from the others. In order to determine which mean was different, a Tukey (HSD) or Honest Significant Difference test was performed on each variable (Abdi and Williams, 2010).

The ANOVA and Tukey HSD together provided a way to support the grouping of individuals in the cluster analysis by eliminating any human error caused by categorical data classification.

This process was completed these for three different data sets. These data sets were chosen to have a concentration more on non- ephemeral data rather than ephemeral. The reason for this is because the flowers of *Opuntia* are only present for such a short period of time. The first data set contained features that can be found year-round on the five species of interest on the Coast of South Carolina. The second data set contained ephemeral flower variables. The third data set was the same as the second, but with the addition of 3 other species for comparison.

These species included *Opuntia lindheimeri* (Engelm.), *Opuntia humifusa* (Raf.), *mesacantha ssp. mesacantha* (Majure), and *Opuntia lata* (Small), *mesacantha* (Raf.) spp. *lata* (Small) Majure. *Opuntia lindheimeri* is native to Texas and Mexico. It Occurs in Brewster Co. along the Rio Grande, along the Pecos River, Durango and Chihuahua Mexico, etc. (Powell and Weedin, 2004.) The Texas Prickly Pear, as it's known, occurs in many ornamental landscapes in South Carolina. *Opuntia mesacantha ssp. mesacantha*, formally known as *Opuntia humifusa*, is considered native to the eastern United States. It is known to inhabit rock outcrop areas of the Piedmont in South Carolina as well as areas in the coastal region such as the Ace Basin (Spira, 2011; Whitaker et al., 2004). *Opuntia lata* is also considered a native of the eastern United States also known from South Carolina. The purpose of running the third data set was to see if the three species separated into groups by themselves in the hierarchical cluster analysis. This gives

inference that they are different from the other 5. It also helps build a case for morphological species concept satisfaction.

Results

Figure 2.1 shows a hierarchical cluster tree output from JMP. This dendrogram represents grouping based on morphological variables that can be measured year round on each species. According to the output, the morphometric data used for each of the 89 individuals cleanly separated individuals into five groups. Each of the five groups also appears to be one of the five presumed species of study. The power of the cluster analysis may be explained by four individuals in the group of individuals that are of the type *Opuntia stricta*. These four individuals have MCRCross and Cross in their names. These individuals were thought to be a cross between *Opuntia macrarthra* and *Opuntia stricta* at the time of collection. However, the cluster analysis

Figure 2.2 shows the canonical plot of the variables that did the best job at separating the 89 individuals into the clusters in the dendrogram. The further the groups are from one another on a canonical graph the better job the variables did in separation. Table 2.1 and Table 2.2 show the variables and score summaries for the placement of the 5 clusters. In Table 2.1, the percent misclassified tells the chance of correctly identifying each of the five species based on the 4 variables from the discriminant analysis. Since the percent misclassified is 6.2 there is about a 94% chance of correctly placing individuals into groups. It also infers the chances of identifying one species from another in the field. Table 2.2 shows what was misclassified. The analysis indicates that 4 of the individuals in cluster two belong to

cluster one based on the morphometrics analyzed. It also says that 1 individual from cluster four belongs to cluster three. This misclassification could have been due to several reasons. There could have been a mistake in data collection (human error), the individuals misclassified could be hybrids or the individuals could be a cryptic taxon.

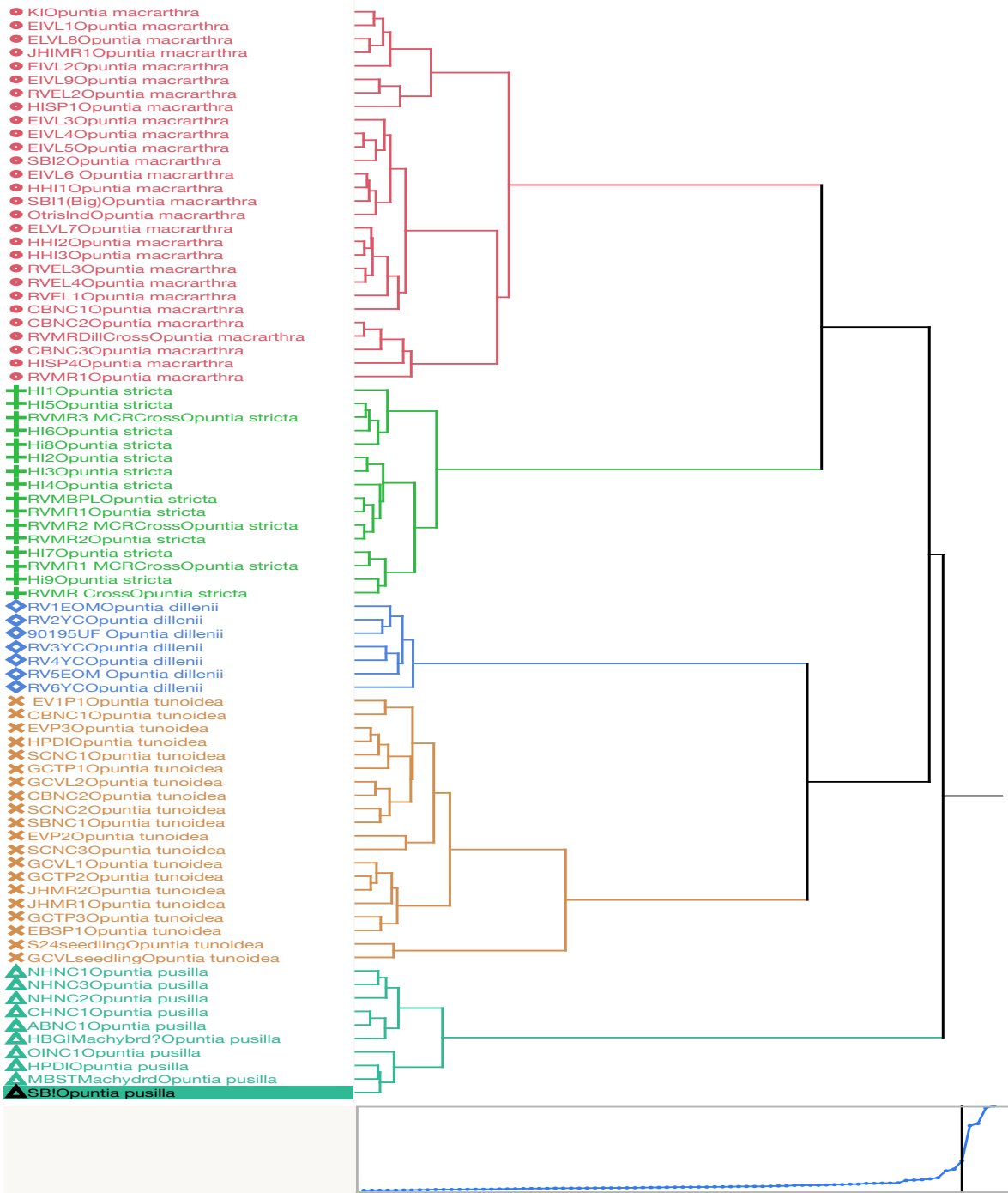


Figure 2.1 Dendrogram (Phenogram) of presumed *Opuntia* species collected on the coast of South Carolina. Individual grouping is based on morphometrics that can be taken year round. The graph at the bottom shows that separating individuals into five clusters seemed statistically appropriate.

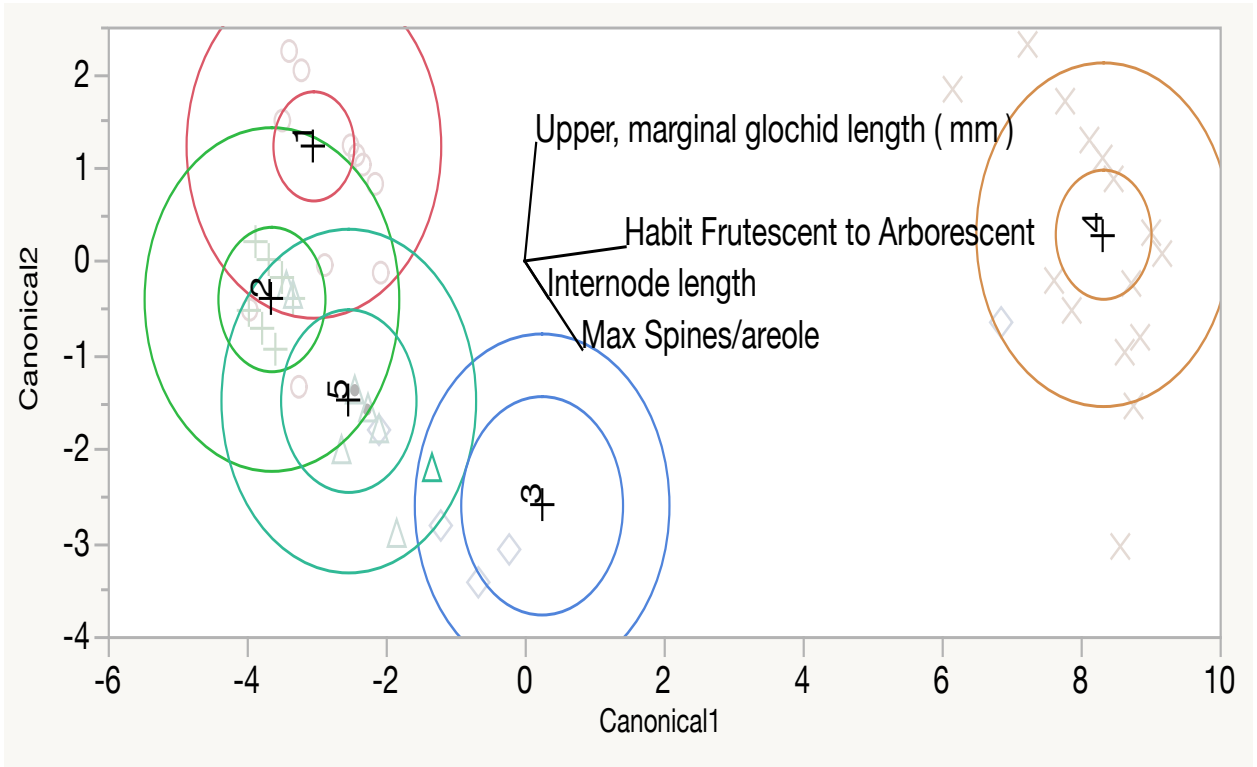


Figure 2.2 Canonical graph formed by discriminant analysis of variables found to be important contributors to group separation of in dendrogram formed using variables found year-round on *Opuntia* types thought to be one of the five study species.

Columns					
Internode length					
Max Spines/areole					
Upper, marginal glochid length (mm)					
Habit Frutescent to Arborescent					

Source	Count	Number Misclassified	Percent Misclassified	Entropy RSquare	-2LogLikelihood
Training	81	5	6.17284	0.62843	90.4582

Table 2.1 Discriminant analysis score summaries of variables found to be important contributors to group separation in dendrogram formed using variables found year round on *Opuntia* types thought to be one of the five study species. Table indicates that the morphometrics and analysis used may be incorrect only 6 percent of the time.

Actual	Predicted				
	1	2	3	4	5
Cluster 2					
1	24	4	0	0	0
2	0	16	0	0	0
3	0	0	6	1	0
4	0	0	0	20	0
5	0	0	0	0	10

Table 2.2 Discriminant analysis score summaries of variables found to be important contributors to group separation in dendrogram formed using variables found year round on *Opuntia* types thought to be one of the five study species. Table indicates what individuals were misclassified.

Figure 2.3 is a dendrogram that represents grouping of individuals based on year round variables as well as flower variables. Each individual collected did not produce a flower. Therefore, only 15 individuals were included in this analysis. Again, the individuals are separated into distinct groups that represent each of the five species.

Figure 2.4 shows the canonical plot for the variables found to do the best job at group separation in the discriminant analysis. Table 2.3 and Table 2.4 show the variables and scores for the cluster placement. By using the variables identified in Table 2.3 it can be inferred that there is a 100% chance that individuals were placed into groups correctly. It also infers that individuals found in the field can be correctly identified and separated into five species.

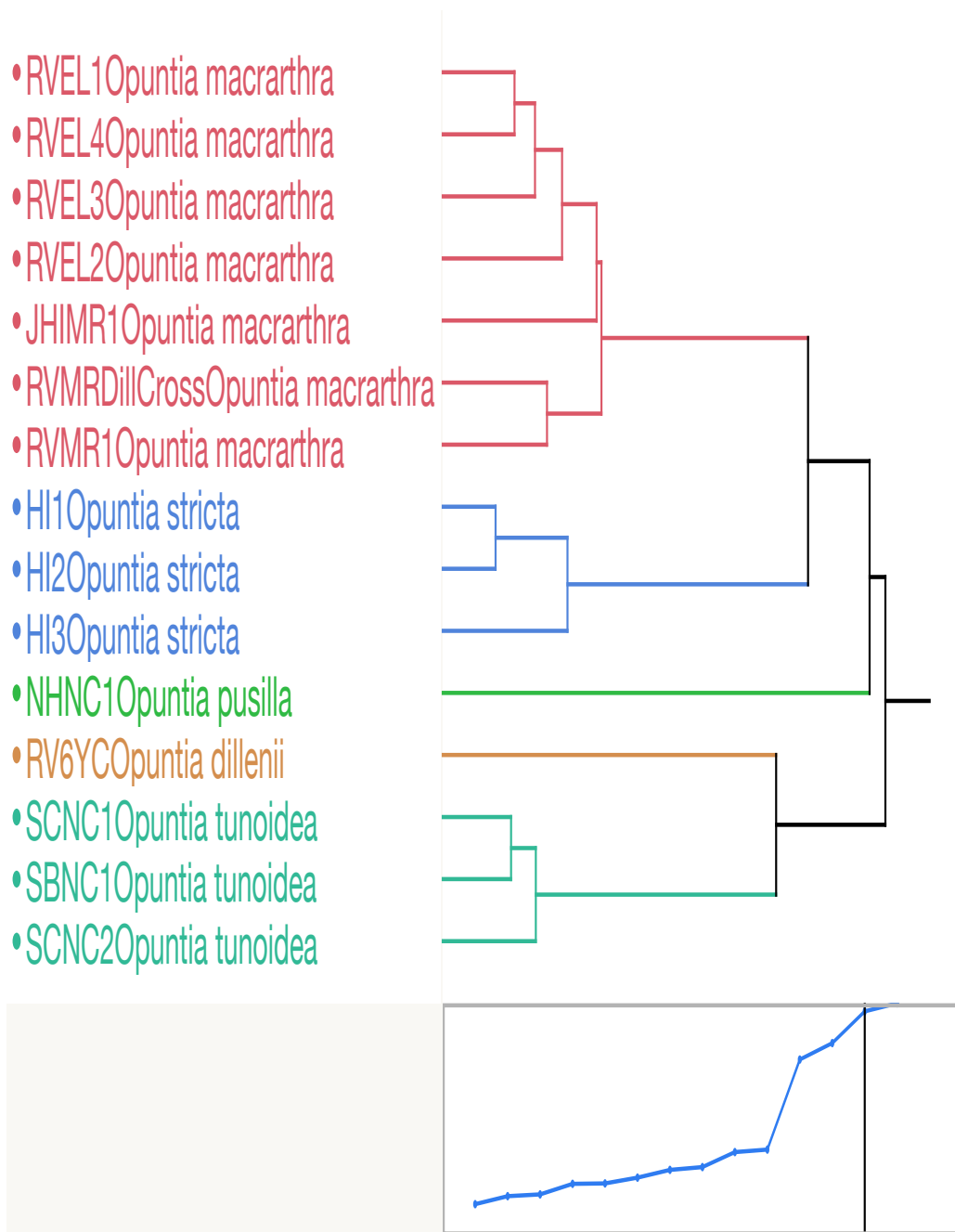


Figure 2.3 Dendrogram (Phenogram) of presumed *Opuntia* species collected on the coast of South Carolina. Individual grouping is based on morphometrics that can be taken year round plus flower variables. The graph at the bottom shows that separating individuals into five clusters seemed statistically appropriate.

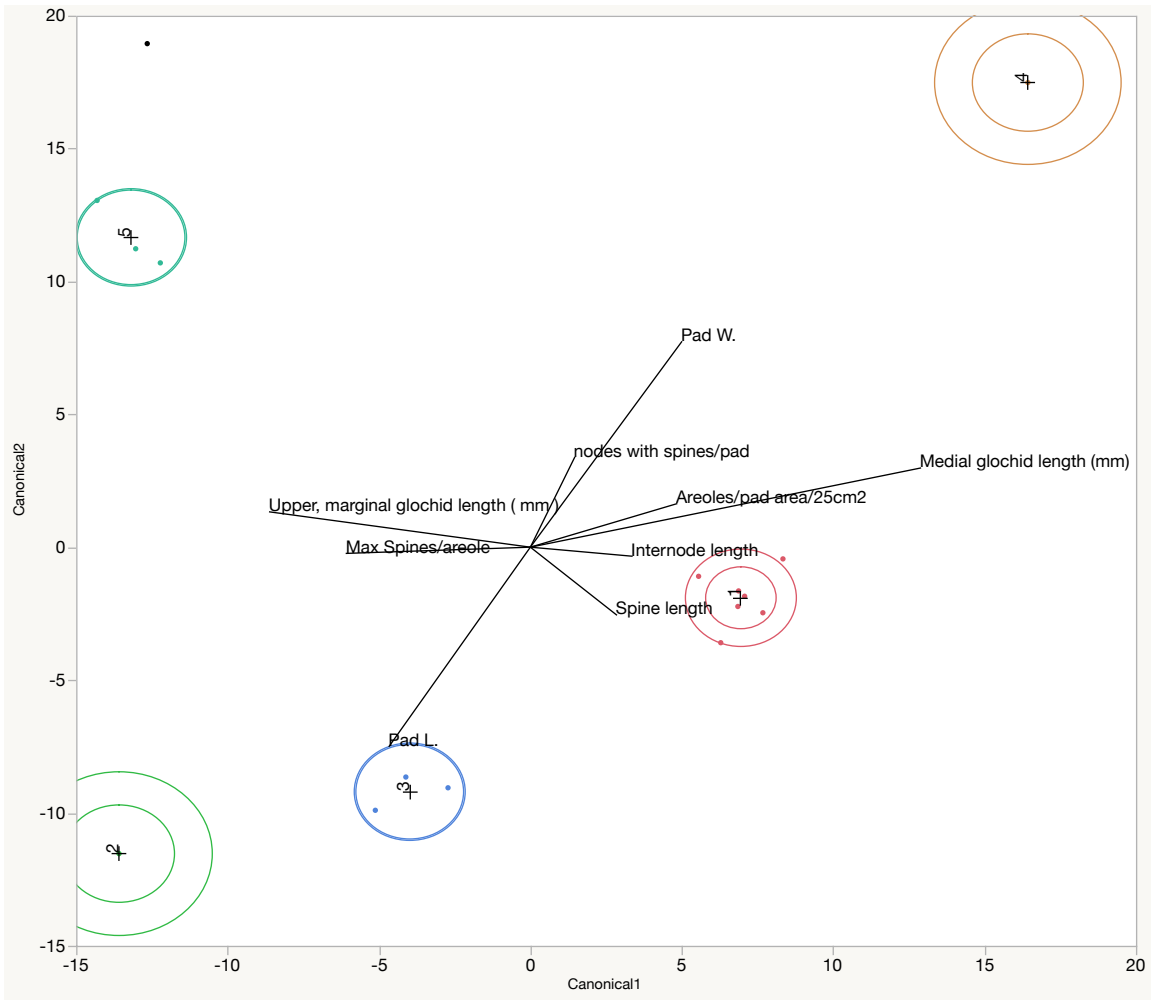


Figure 2.4 Canonical graph formed by discriminant analysis of variables found to be important contributors to group separation in dendrogram formed using variables found year round on *Opuntia* types thought to be one of the five study species.

Columns
Pad L.
Pad W.
Areoles/pad area/25cm ²
Internode length
nodes with spines/pad
Max Spines/areole
Spine length
Upper, marginal glochid length (mm)
Medial glochid length (mm)

Source	Count	Number Misclassified	Percent Misclassified	Entropy RSquare	-2LogLikelihood
Training	15	0	0.00000	1.00000	0
Excluded	1	0	0.00000	.	

Table 2.3 Discriminant analysis score summaries of variables found to be important contributors to group separation in dendrogram formed using variables found year round plus flower variables on *Opuntia* types thought to be one of the five study species. Table indicates 0 percent misclassification.

Cluster	Actual	Predicted				
		1	2	3	4	5
1		7	0	0	0	0
2		0	1	0	0	0
3		0	0	3	0	0
4		0	0	0	1	0
5		0	0	0	0	3

Table 2.4 Discriminant analysis score summaries of variables found to be important contributors to group separation in dendrogram formed using variables found year round on *Opuntia* types thought to be one of the five study species. Table indicates that no individuals were misclassified.

Figure 2.5 shows a dendrogram with eight different clusters instead of five. This analysis included the three other species *Opuntia lindheimeri*, *Opuntia mesacantha ssp. mesacantha*, and *Opuntia lata*. This data set included both year round measurable variables and flower variables for each individual. The individuals from this analysis were also separated into groups of the eight separate assumed species. Figure 2.6 shows the canonical plot of the variables from the discriminant analysis on the data set. Tables 2.5 and 2.6 show the variables and the scores for classifying each cluster. According to the discriminant analysis Table 2.6 shows that there was zero percent misclassification on individual group placement. This indicates that the individuals were placed in groups correctly found in the field can be correctly identified based on the variables found in Table 2.6. It also indicates that the five species of the studied from coastal South Carolina are in different groups than the other three species.

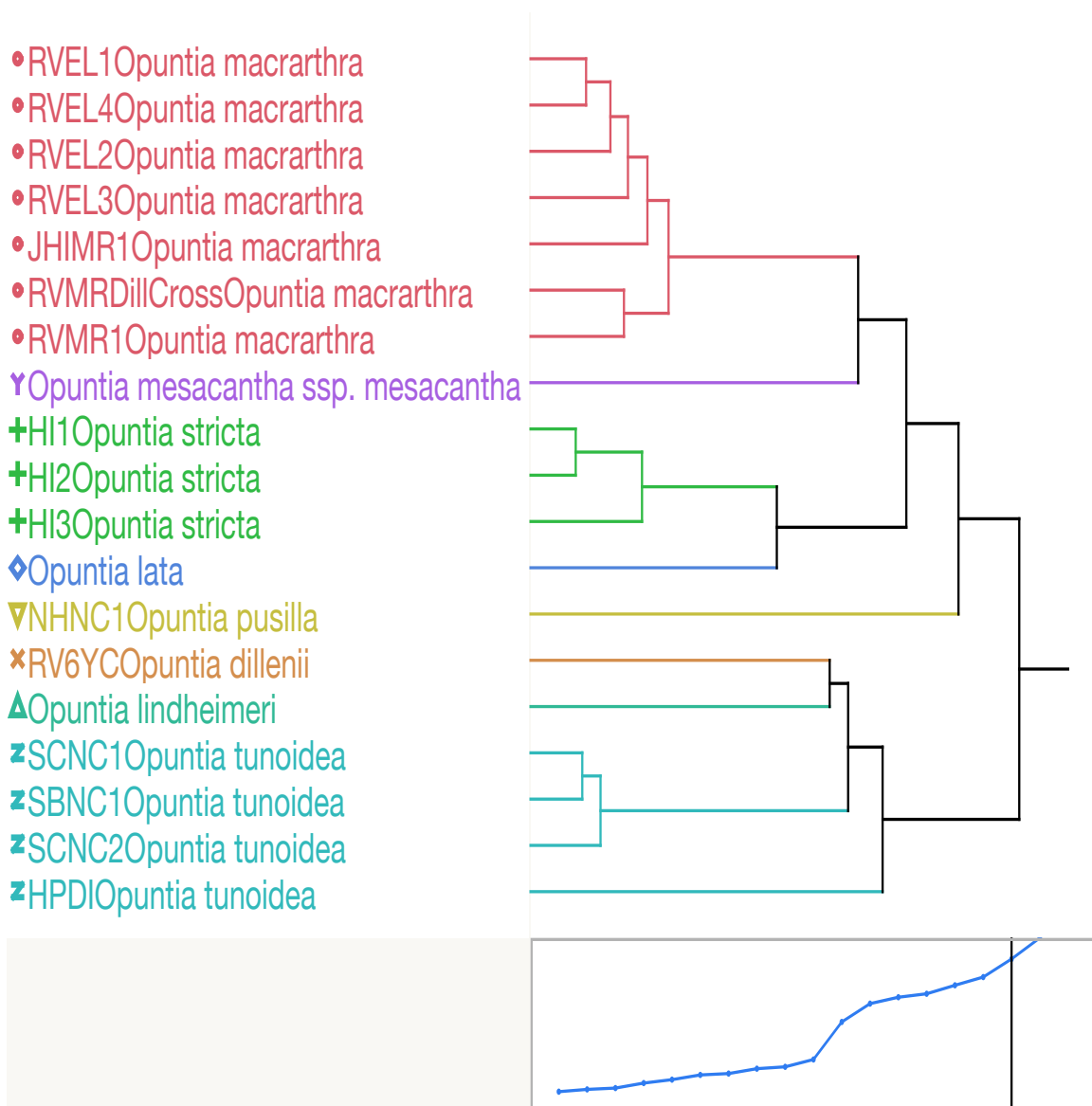


Figure 2.5 Dendrogram (Phenogram) of presumed *Opuntia* species collected on the coast of South Carolina, As well as individuals of the types *O. lindheimeri*, *O. lata*, and *O. mesacantha ssp. mesacantha*. Individual grouping is based on morphometrics that can be taken year round plus flower variables. The graph at the bottom shows that separating individuals into five clusters seemed statistically appropriate.

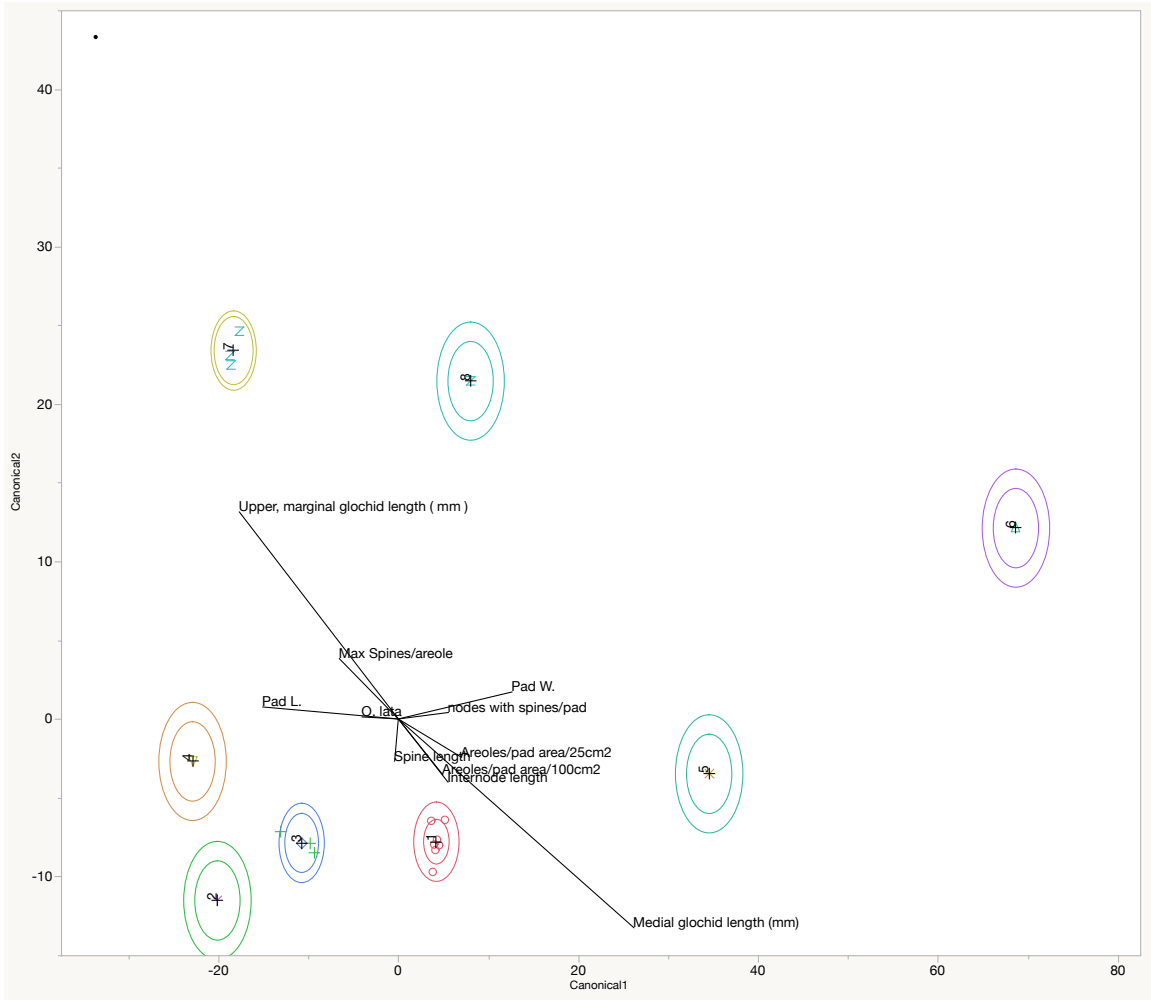


Figure 2.6 Canonical graph formed by discriminant analysis. Variables found to be important contributors to group separation in dendrogram formed using variables found year round plus flower variables on *Opuntia* types thought to be one of the five study species as well as individuals of the types *O. lindheimeri*, *O. lata*, and *O. mesacantha ssp. mesacantha*.

Columns						
O. lata						
Pad L.						
Pad W.						
Areoles/pad area/25cm2						
Areoles/pad area/100cm2						
Internode length						
nodes with spines/pad						
Max Spines/areole						
Spine length						
Upper, marginal glochid length (mm)						
Medial glochid length (mm)						
Source	Count	Number Misclassified	Percent Misclassified	Entropy RSquare	-2LogLikelihood	
Training	19	0	0.00000	1.00000	0	

Table 2.5 Discriminant analysis score summaries of variables found to be important contributors to group separation in dendrogram formed using variables found year round plus flower variables on *Opuntia* types thought to be one of the five study species, as well as individuals of the types *O. lindheimeri*, *O. lata*, and *O. mesacantha* spp. *mesacantha*. Table indicates 0 percent misclassification.

Actual	Predicted							
Cluster 3	1	2	3	4	5	6	7	8
1	7	0	0	0	0	0	0	0
2	0	1	0	0	0	0	0	0
3	0	0	4	0	0	0	0	0
4	0	0	0	1	0	0	0	0
5	0	0	0	0	1	0	0	0
6	0	0	0	0	0	1	0	0
7	0	0	0	0	0	0	3	0
8	0	0	0	0	0	0	0	1

Table 2.6 Discriminant analysis score summaries of variables found to be important contributors to group separation in dendrogram formed using variables found year round plus flower variables on *Opuntia* types thought to be one of the five study species, as well as individuals of the types *O. lindheimeri*, *O. lata*, and *O. mesacantha spp. mesacantha*. Table indicates that no individuals were misclassified.

The ANOVA/Tukey results reflect the variables identified by the discriminant analyses run on the three data sets. All of the variables run in the ANOVA/Tukey test were non-ephemeral. Figure 2.7 shows the variability in the means of upper marginal glochid length for the five study species. The bars indicate that the means for each species are different. Even though the bars indicate each mean is different the Tukey HSD only indicates one significant separation. Using just the upper marginal glochid length variable only *O. tunoidea* or *O. macrarthra* may be separated from *O. stricta*, *O. pusilla*, or *O. dilenii*. Using the scale for the means the five species can be separated into two groups. One group is under 1.5 mm and the other group is over 1.5 mm.

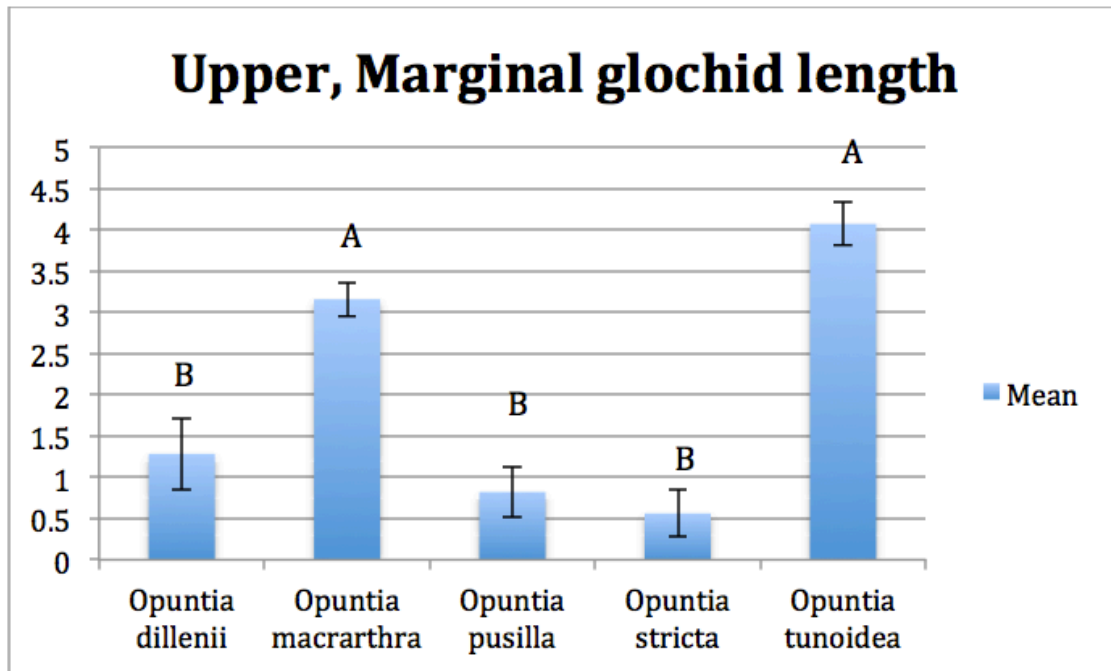


Figure 2.7 Bar graph representing the mean variance and Tukey HSD test results for Upper, Marginal glochid length in millimeters on the included species.

Figure 2.8 shows the mean variability of internode length for the five study species. The Tukey HSD indicates two significant separations. Using just the internode length morphometric variable *O. tunoidea* or *O. dillenii* may be separated from *O. macrarthra* or *O. stricta* and *O. pusilla* can be separated cleanly from any of the other species. Using the scale for the means the five species can be split into 3 different groups. The first group has a mean internode length under 1.5 cm, the second group has a mean internode length of less than 3.5 cm, and the third has a mean internode length of greater than 3.5 cm. Table 2.7 shows each of the five species classified with the use of

both Upper, Marginal glochid length and Internode length. The table indicates with the use of both morphological measurements each of the five species is classified as being distinct morphologically. This provides supporting evidence to the cluster analysis separation. It also suggests that each group behaves on the level of a morphological species.

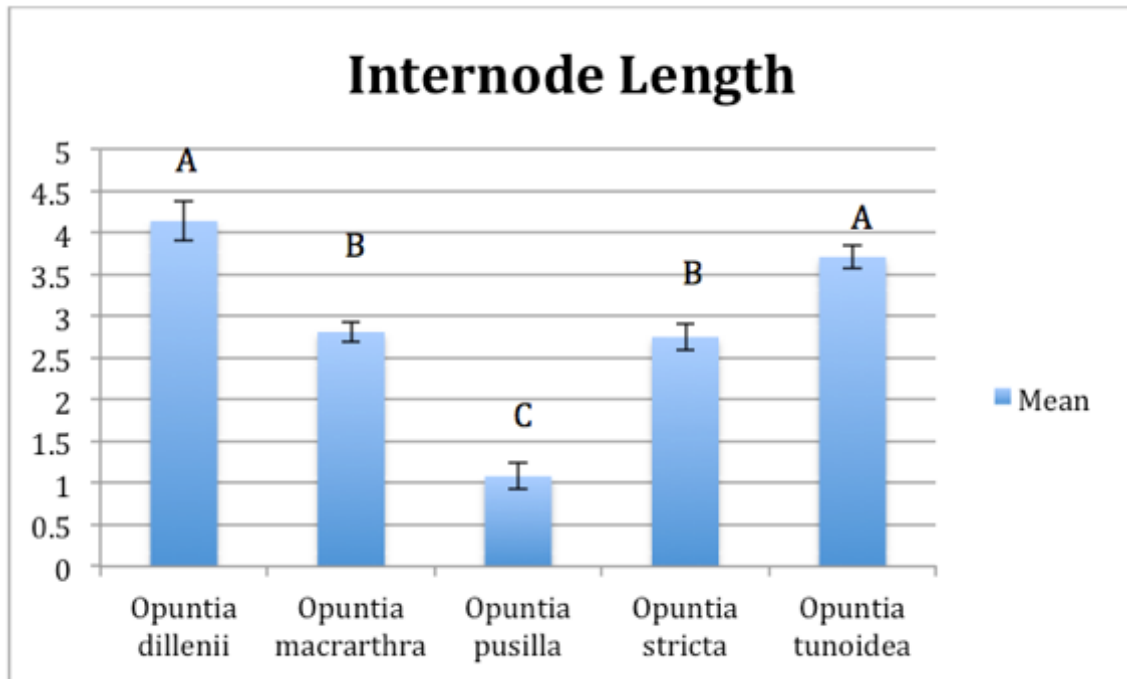


Figure 2.8 Bar graph representing the mean variance and Tukey HSD test results for internode length in centimeters of the included species.

Variable 1 Upper, Marginal glochid length	Species grouping 1	Species grouping 2 and 3	Species grouping 4 and 5
UMGL > 1.5 mm		<i>Opuntia macrarthra</i>	<i>Opuntia tunoidea</i>
UMGL < 1.5 mm	<i>Opuntia pusilla</i>	<i>Opuntia stricta</i>	<i>Opuntia dillenii</i>
Variable 2 Internode Length	IL < 1.5 cm	IL < 3.5 cm	IL > 3.5 cm

Table 2.7 Representative species individuals grouped by mean Upper, Marginal glochid length and mean Internode Length.

The statistical analysis of both Upper, marginal glochid length and Internode length indicated that each of the five groups of species is morphologically distinct. Identification in the field may require more measurements to reduce variation and error. The use of more variables is helpful in creating a process of elimination. Therefore, two more morphological characteristics found to be important in the discriminant analysis were analyzed using ANOVA and Tukey HSD. Figure 2.9 represents the variability in the means of the number of areoles per 100cm² of pad area. The Tukey HSD indicates three separations. *Opuntia stricta* and *O. pusilla* form a group that may be identified from the other 3 species using this variable. *Opuntia dillenii* appears to be in a group by itself

although the error may suggest it is similar to the other four groups. Then *O. tunoidea* and *O. macrarthra* form a group morphologically different than the others.

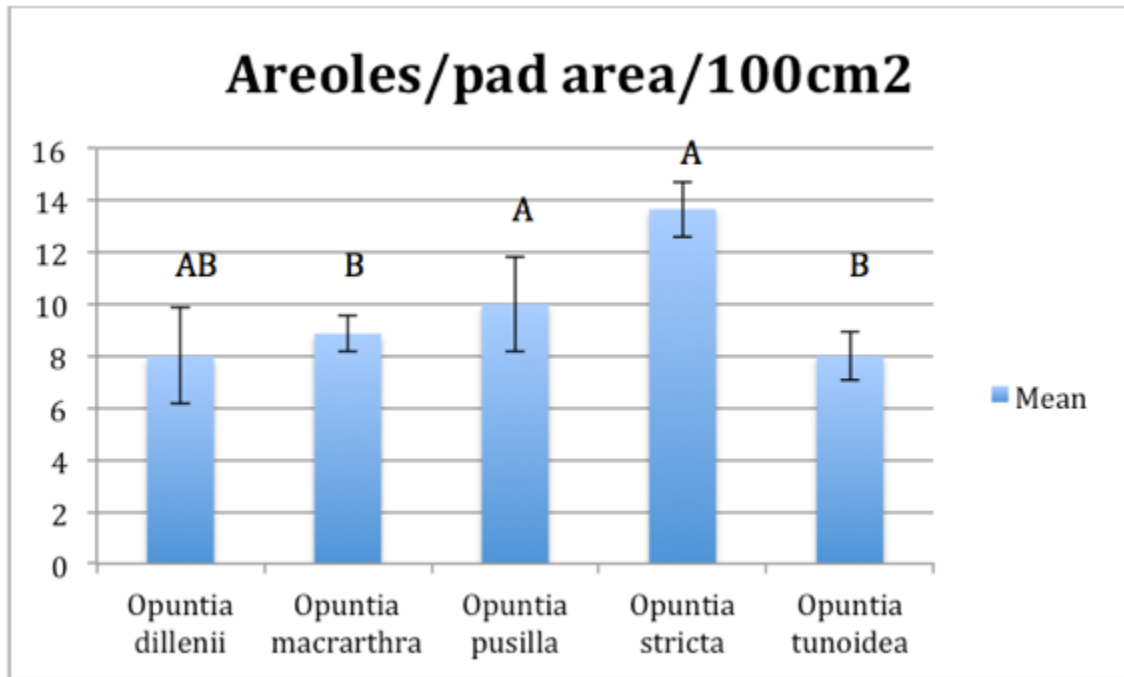


Figure 2.9 Bar graph representing the mean variance and Tukey HSD test results for areoles/ pad area/ 100cm² on the included species.

The means for pad width between species are shown in figure 2.10. There is a noticeable difference between each species. The Tukey HSD test indicates that based on this morphometric *O. dillenii* and *O. tunoidea* are distinctly separated from the other three species. The test implies that *O. macrarthra* could be significantly different or it could be an *O. dillenii* type. *O. pusilla* could be significantly different than the others or it could be like *O. macrarthra*, *O. stricta* or *O. tunoidea*. As well the case is the same for *O. stricta*. Pad width is more helpful when combined with upper marginal glochid length

and internode length. When combined, *O. dillenii* and *O. macrarthra* separate making each species statistically different.

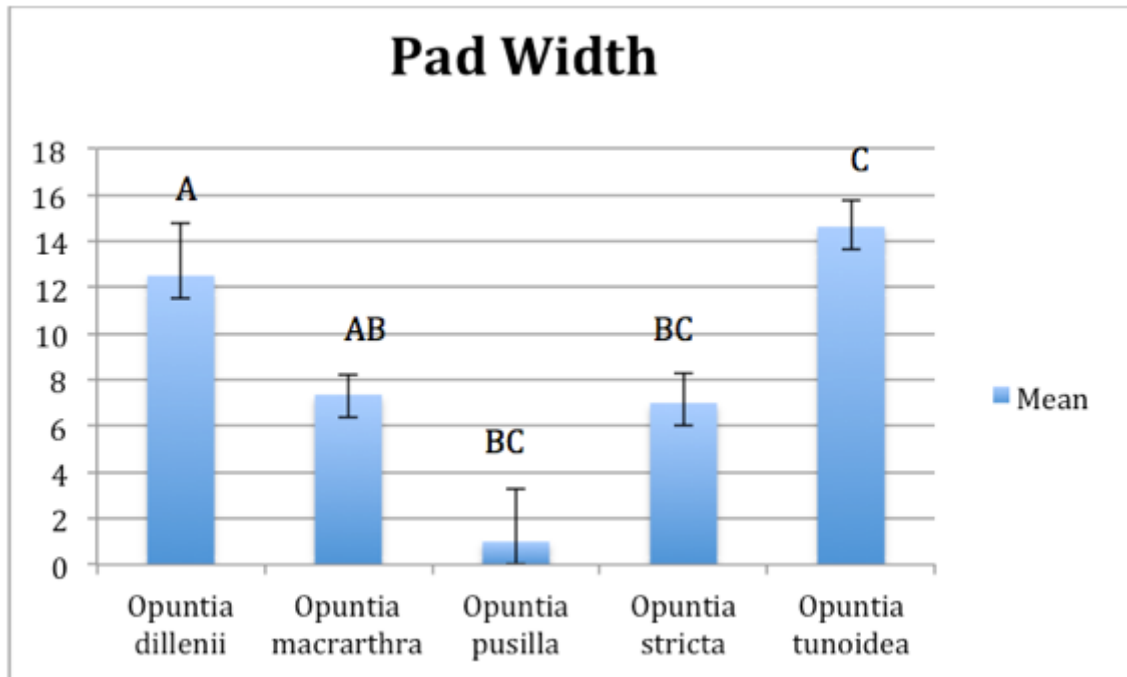


Figure 2.10 Bar graph representing the mean variance and Tukey HSD test results for pad width on the included species.

The next three figures represent measurements from the five coastal species of the study as well as the three comparison species *O. lindheimeri*, *O. mesacantha ssp. mesacantha*, and *O. lata*. Sample size and inconsistent variability made it difficult for the Tukey HSD test to clearly distinguish between these groups. Figure 2.11 shows the upper marginal glochid length means for included species. Note that the *O. pusilla* individuals used in this data set did not have upper marginal glochids. This data set had a reduced number of individuals due to the inclusion of flower data. The bars in the graph show that the means appear to be each slightly different. However, by looking at the Tukey HSD

results and the scale only *O. lindheimeri* appears to be different from the five coastal species. This was confirmed in JMP using least squared contrast.

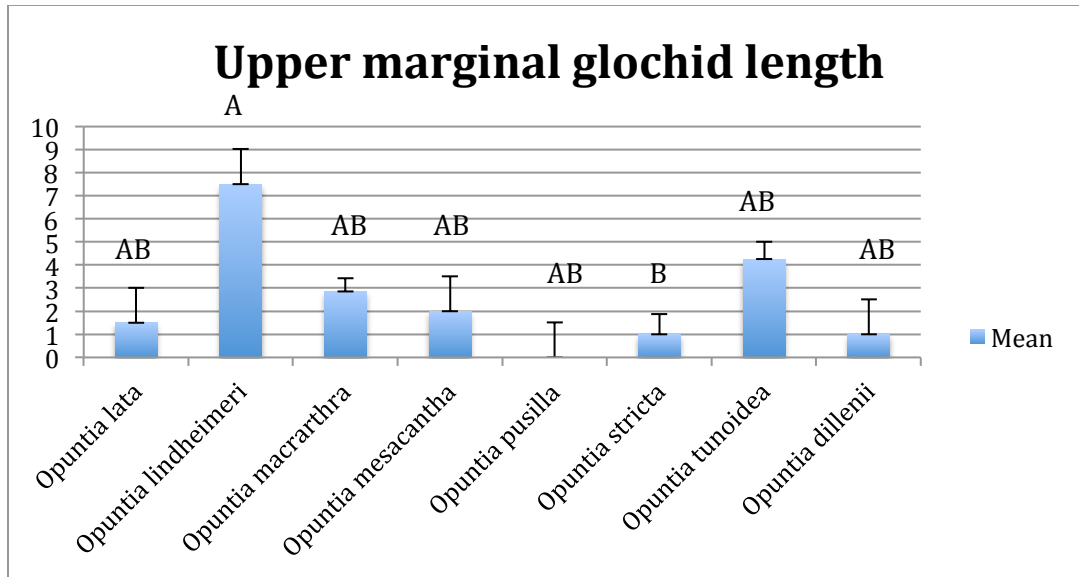


Figure 2.11 Bar graph representing the mean variance and Tukey HSD test results for upper marginal glochid length on the included species. In this graph *Opuntia mesacantha* = *Opuntia mesacantha ssp. mesacantha*.

Pad length means for eight presumed species are represented by the graph in figure 2.12. Again as indicated by the bars, there appear to be differences between the species means. The one that stands out is *O. lindheimeri*. Least squared contrast confirmed that it is the only one statistically different from the five coastal species based on this morphometric. *Opuntia lata* and *O. mesacantha* did not indicate significance when tested by least squared contrast.

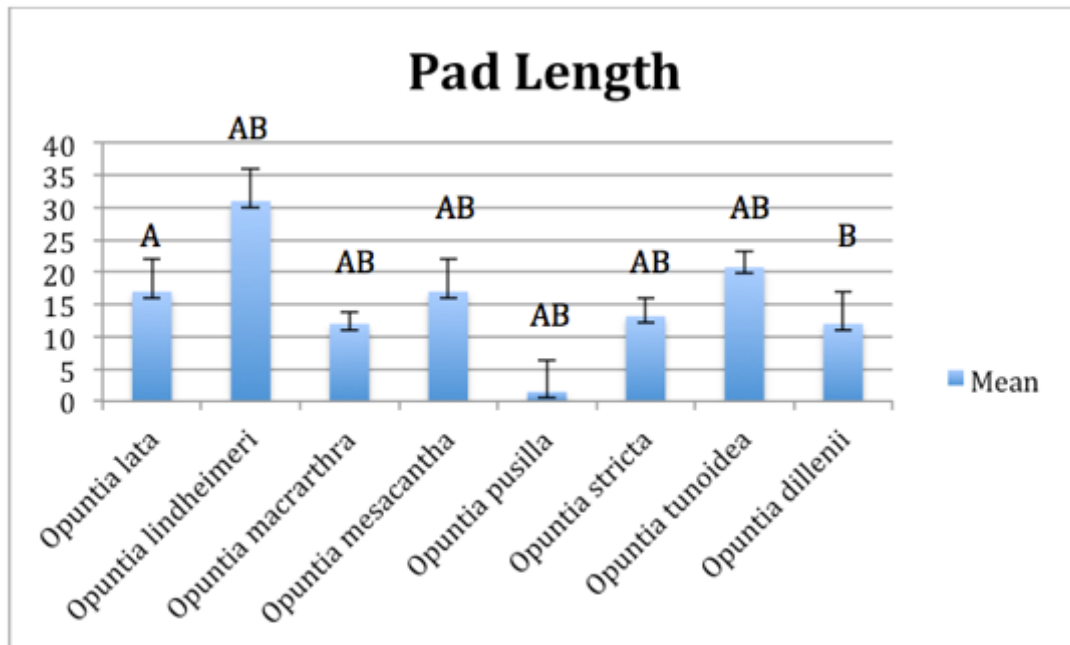


Figure 2.12 Bar graph representing the mean variance and Tukey HSD test results for pad length on the included species. In this graph *Opuntia mesacantha* = *Opuntia mesacantha ssp. mesacantha*.

Figure 2.13 depicts the variance for pad width means of the eight species include. As shown by the bars in the graph, it can be seen that the mean for each species varies slightly. Again least squared contrast only clearly identifies *O. lindheimeri* as being statistically different based on this morphometric. *Opuntia lata* and *O. mesacantha* contrast results showed no significance.

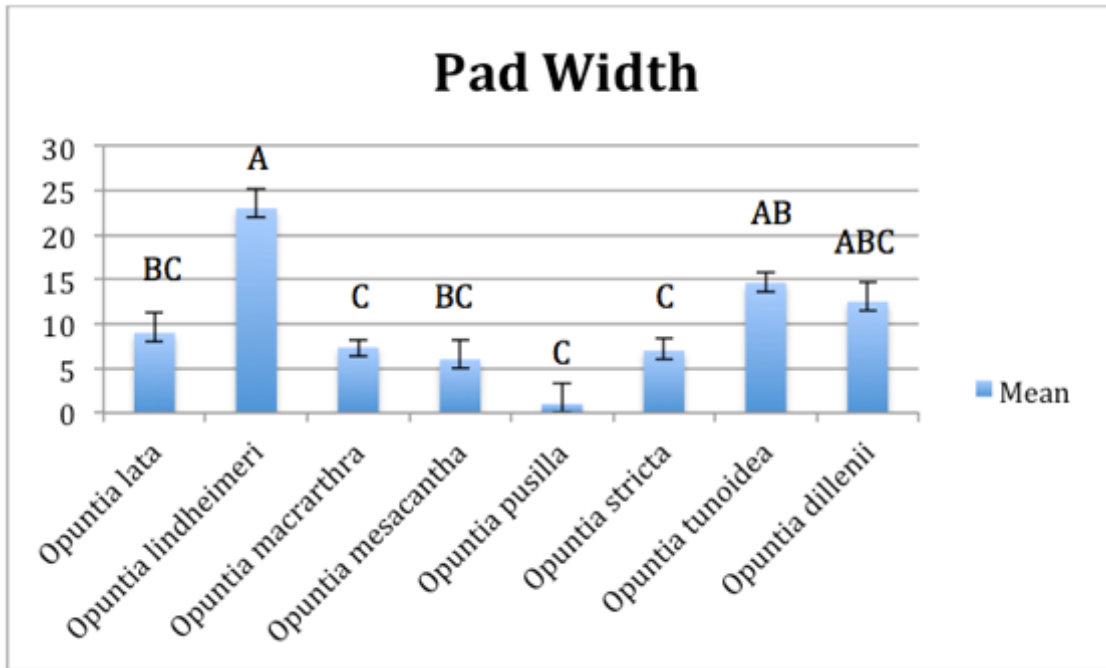


Figure 2.13 Bar graph representing the mean variance and Tukey HSD test results for pad width on the included species. In this graph *Opuntia mesacantha* = *Opuntia mesacantha ssp. mesacantha*.

Discussion

The purpose of this part of the study was to clarify morphological characteristics of *Opuntia* individuals collected on the coast of South Carolina. The goal was to identify characteristics that would separate each individual into groups different from others. The human eye can pick up on differences without the use of a computer. In relation to the *Opuntia*, these characteristics include things such as stem color, flower color, stamen color, stigma color, fruit color, spine color, spine shape, growth habit, raised areoles, and stem shape. These types of variables were run in the statistical software program (JMP) as yes/no variables. This type of data, however, is known as categorical and has no numerical value. For example, if one assumed species has raised areoles, yellow flowers,

and flat spines, it gets a 1 for each category. If another presumed species has raised areoles, yellow flowers, and round spines, it gets a 1 in these categories and a 0 in the flat spine category. The species either has the characteristic or it doesn't. These types of variables are helpful in telling one *Opuntia* from another, but statistically they can only go so far in backing the case. Since there are no numbers to compare, the discriminant analysis is as far as categorical data can go.

When these *Opuntia* species were first described, there was no statistical analysis software. The use of JMP allowed for a more precise clarification of the individuals found. It allowed a deeper look at what was on the South Carolina Coast based off of past descriptions classified by traditional morphological techniques. A question was, "Based on morphology, are *Opuntia dillenii*, *Opuntia pusilla*, *Opuntia stricta*, *Opuntia macrarthra*, and *Opuntia tunoidea* separate species occurring on the coast of South Carolina?" It was also important to know if these five species differed from *Opuntia lindheimeri*, *Opuntia lata*, and *Opuntia mesacantha ssp. mesacantha*. In order to better answer these questions, variables with numerical values needed to be further analyzed.

Phylogenetic analysis may tell a different story about species level distinction. However, in the hierarchical cluster analysis individuals thought to be hybrids were tested with no supporting evidence found, each of the five presumed coastal species could be separated, which included categorical data. They were then separated by ANOVA and Tukey HSD based off of discriminant analysis results. The graphs from the ANOVA/Tukey HSD provided visuals of hard evidence of gaps in morphological measurements between groups of individuals. This is the very definition of the Morphological Species Concept. Individuals of *Opuntia lindheimeri*, *Opuntia lata*, and

Opuntia mesacantha ssp. *mesacantha* were grouped separately from the five coastal species in the cluster analysis. The ANOVA, Tukey HSD, and least squared contrast were only able to provide supporting evidence that *Opuntia lindheimeri* was different. The study provides strong evidence for the argument that there appears to be at least five species of *Opuntia* that occur on the coast of South Carolina. Morphological evidence also suggests that the five coastal species *Opuntia dillenii*, *Opuntia pusilla*, *Opuntia stricta*, *Opuntia macrarthra*, and *Opuntia tunoidea* are different from *Opuntia lindheimeri*. They can be identified using a set of morphometrics, even with some misclassification by the analysis.

CHAPTER THREE

ECOLOGICAL CHARACTERIZATION AND GEOGRAPHIC RANGE OF *OPUNTIA*

MILLER ON THE COAST OF SOUTH CAROLINA

Introduction

In South Carolina, *Opuntia* are known to occupy the coastal beaches. Three zones define the coastal beaches. The high tide zone is where even the highest spring tide never surpasses. The detritus zone or drift line is composed of *Spartina alterniflora* (Aiton) remnants and other debris washed up from nearby marshes. The berm is the area of loose sand between the driftline and dunes. The dunes are mounds formed by wind blown sand accretion in the berm zone. Sand is deposited forming a mound when it hits a wind break. *Uniola paniculata* L. plays a key role in slowing the wind allowing sand to deposit. Dunes are protected by South Carolina Law to protect them and the species that stabilize them from pedestrian disturbance. Since dunes protect against the forces of the ocean this is very important. *Opuntia* is known to occur in the swale area behind the dunes. This type of habitat is known as a maritime grassland (Porcher and Ryner, 2001). It is characterized as being a fairly flat area, protected by wind and ocean waves. It is also protected by pedestrian traffic in that specific habitat. The soil is composed of sand and crushed shell. Here, *Opuntia* is found growing with species such as *Spartina patens* (Aiton), *Smilax ariculata* (Walter), *Gaillardia pulchella* (Fougeroux), *Ilex vomitoria* (Aiton), *Hydrocotyle bonariensis* (Lamarck), *Yucca gloriosa* (L.), *Juniperous silicicola* (Small), and *Sabel palmetto* (Walter). Maritime grasslands can be very dense, however, they are still very light intensive due to the type of species that grow in them. The cacti, however, do grow in areas of the grassland that are less dense, with larger species such as *Juniperous silicicola* (Small) Bailey. On barrier islands, where maritime shrub thickets develop, *Opuntia* species are known to occur on the outer edge and openings as well (Porcher and Ryner, 2001).

Another place *Opuntia* is known to occur is in the more open areas of the maritime forests. These forests are formed by salt spray. They occur inland from the shoreline and on barrier islands. They are composed of plants such as *Quercus virginiana* Miller, *Pinus taeda* (L.), *Magnolia grandiflora* (L.), *Ilex opaca* (Aiton), *Zanthoxylum clava-herculis* (L.), *Ilex vomitoria* (Aiton), *Morella cyrifera* (L.), *Persia borbonia* (L.) Sprengel, and other salt tolerant plants. (Porcher and Ryner, 2001).

Opuntia also occurs in or on Native American shell mounds, rings or hummocks. These can occur on land or as islands in the salt marsh. They are composed of piles of shell. This causes the soil in the area to be very high in calcium. The areas are inhabited with species found in maritime forests, and maritime grasslands along with calcicoles. Calcicoles are plants that love calcium soils. These include plants such as *Acer barbatum* Michaux, *Sageretia minutiflora* (Michaux) Mohr, *Tilia heterophylla* (Vent.), and *Cornus asperifolia* Michaux (Porcher and Rayner, 2001).

When attempting to classify species taxonomically, it is important to think about species concepts. The morphological species concept defines groups of species by gaps in morphological variation. It focuses on the similarities members of a species have (Judd et al., 2008). The issue with classifying on this premise is that species that are genetically or ecologically very distinct, perhaps even with reproductive barriers, may be considered the same species. This method does not account for biological or ecological processes but rather relies on morphology as a proxy for those. (Species Concepts, 1998). A species defined using this concept is known as a Phenetic Species. Phenetic classification is grouping based on similarity. Matrices of similarities are used to create a Phenogram in order to estimate phylogeny (Mallet, 2007).

The premise behind the Ecological Species Concept is that a species occupies a particular adaptive zone. The biological species concept states that a species is a group of interbreeding populations that are reproductively isolated from other groups (Mayer, 1942). The problem with this is species as well as genera have shown to interbreed. It is generally assumed that if you are a biological species, then you are a species but if you don't satisfy the biological species concept you may still be a species by several definitions.

The Phylogenetic Species Concept states that an evolutionary species has a single lineage of descent from which it maintains its identity (Whitley, 1978). Phylogenetic species correspond to the terminals on a cladogram. The problem arises when determining where to draw the monophyletic line.

The species concepts are human envisioned ideas or theories. Scientists want to try and place everything into neat artificial "boxes". This provides some way to function with the idea of classification. These "boxes" don't always work as planned. Statistical software helps use eliminate human error and analyze traditional species concepts with a much more accurate and unbiased approach. The real question that arises in this project is, "Can we classify each of our study species as species based on traditional concepts with the data collected?".

Environmental variables were measured in order to better understand the presence of *Opuntia*. Information on habitat, ecological associations/natural communities where each of the five study species was found needed clarification. This was done using the methodology developed for the Carolina Vegetation Survey (Peet et al., 1998).

Partition analysis in the statistical analysis software, JMP, was used to analyze the ecological variables associated with the species of the study. Path maps were then constructed with the partition analysis data. This was done to better describe habitat, indicator species, and soil characteristics, as well as to determine any specific niche associated with location of 4 of the five study species. Due to limited habitat space, *Opuntia dillenii* had to be omitted from the ecological characterization study. The other four species were studied to get a better clarification of the sites where they occur. Carolina Vegetation Protocol was used to record vegetation associated with the *Opuntia*, soil characteristics, site exposure, and the shape of the terrain where they occur. The data collected were then used in partition analyses to determine significant variables associated with the presence of each species and if these variables revealed an ecological niche.

The purpose of the study was to answer the following questions: Where do the species occur? What do they occur with? What are the conditions like where they occur? Do they follow the ecological species concept? When the path maps were interpreted, they gave a better understanding of site characteristics as well as associated species that are good indicators of the presence of each species. The evidence from the maps may or may not imply that each obeys the ecological species concept. Within the dunes system, it depends on one's perception of space. The space between the dune zones has been described as being between 10 and 500 years old, one year for every meter away from the ocean. That seems like a much larger gap than 10 and 500 meters. It presents a fine line of separation that can be backed with morphological evidence implying that they behave like a species. However, phylogenetic research is needed to form a clear answer.

As a continuum of the ecological data, geographic range was also documented. Geographic range is the geographic area in which a plant species can be found. There are a number of factors that determine the geographic range of a plant species. It includes things such as climate, soil type, and other plant species. There are also certain indicators that help identify these ranges. The *Opuntia* species of this study seem to have a somewhat endemic distribution in South Carolina. They appear to be restricted to the coastal maritime grassland habitat. This may be due to several reasons. First of all, *Opuntia* are succulent plants. They thrive in dry, warmer, open areas. A desert is a term that can be used to describe an area as such. In South Carolina, the coastal beaches meet these requirements. The soil is sandy and porous. The climate stays warmer for longer periods of time than the rest of the state. The soil is very high in calcium, which is also the case in West Texas deserts. Salinity is higher, limiting or stunting which species can grow in maritime grassland habitats. This contributes to the openness of the habitat, along with other variable like dry soil, warmer climate, and nutrient status. The counties chosen for this part of the study were known for having this type of habitat.

The geographic range was documented by a series of distribution maps. Each species is documented as to which county it occurs in and where in the county it occurs. These maps are based mostly on morphological characteristics. They provide information as to where individuals from each species were found at the time of the study.

Materials and Methods

A modified Carolina Vegetation Survey (Peet et al., 1998) Protocol was used to collect comprehensive data from field plots constructed in known maritime grassland habitats. A total of 22 plots were sampled from five counties along the coast of South Carolina including Beaufort, Charleston, Colleton, Georgetown, and Horry. Assigned with GPS coordinates, the 10 X 10 m plots were sampled at a depth level of five. The level five indicates thorough plot investigation with the implementation of sub-quadrants. Each plot had five nests with five sets of sub-quadrants. Each corner contained a nest, as well as the center of the 10 X 10 m plot. Species presence was recorded for the sub-quadrants (10 cm, 33 cm, 1.0 m, 3.3 m and 10 m). The sub-quadrants were sampled in increasing order from smallest to largest. Species were given a number corresponding to the sub-quadrant in which they were first seen. If a certain species was seen in the first sub-quadrant it received a five, and for each sub-quadrant the numbers decreased by one until the number two was reached at 3.3 m. If the species previously recorded was seen again in the same nest, it was not assigned another number. Only new species not seen in the previous sub-quadrant received a new number. This was repeated for each of the five nests. After the center sub-quadrant was recorded, anything not captured in the nested sub-quadrants was recorded, denoted by the number 1 for its presence in the 10 m sub-quadrant. After the presence for every species in the plot was recorded, a cover number (1- 10) was assigned according to the amount of each species present. The cover number assigned to each species was associated with a percentage (1=>1%, 2= 0-1%, 3= 1-2%, 4= 2-5%, 5=5-10%, 6=10-25%, 7=25-50%, 8=50-75%, 9=75-95%, and *=100%). For “woody” species, stem count was recorded for all stems in 3 categories (0-1 cm, 1-2.5

cm, and >2.5 cm). A clinometer was used to record McNab indices. These include Landform Index and Terrain shape Index at 8 different angles (aspect, 45, 90, 135, 180, 225, 270, and 315). Landform Index (LFI) is the regional protection of an area or sampled plot. Therefore, the higher a reading on the clinometer the more protected a site is from environmental factors such as wind and light. The Terrain Shape Index (TSI) refers to localized site protection in relation to the grade or slope of the plot. If area in a plot has a negative value for TSI then that area is concave. Concave areas receive more runoff water, are more protected from wind, and are more protected from light. Representative soil samples were collected from each plot as well. The Clemson University soils lab tested the soil samples. The samples were tested for the following content: pH, buffer pH, P, K, Ca, Mg, Zn, Mn, B, Cu, Na, S, CEC, acidity, base saturation, Ca%, Mg%, K%, and Na%.

In JMP, variables from the data were first run against the *Opuntia* variables for inquiry of correlations. Correlation analysis confirmed several observations of variables in the field. However, further investigation was required to better identify prediction of *Opuntia* presence. Partition analysis was then completed for the five assumed species of the study. The purpose of the partition analysis was to describe the location of the species with ecological parameters. Path maps were constructed from the analysis output to show what variable or variables best predicted the presence of each of the species. Due to the very limited space and location, *Opuntia dillenii* could not be included in this study. There was not enough area surrounding the species to construct a plot.

Results

The correlation analysis results show that *Opuntia* density is more when natural stems present in a plot are lower. Density of *Opuntia* also appears to be greater in areas more open to light, and areas that have flatter terrain. This appears to be true for each of the presumed species of the study. Figure 3.1 represents stems found in the plots with each of the 4 study species. The data points represent the plots that contain the *Opuntia* species and the density associated with the presence. It appears that the data points are more concentrated the fewer the stems.

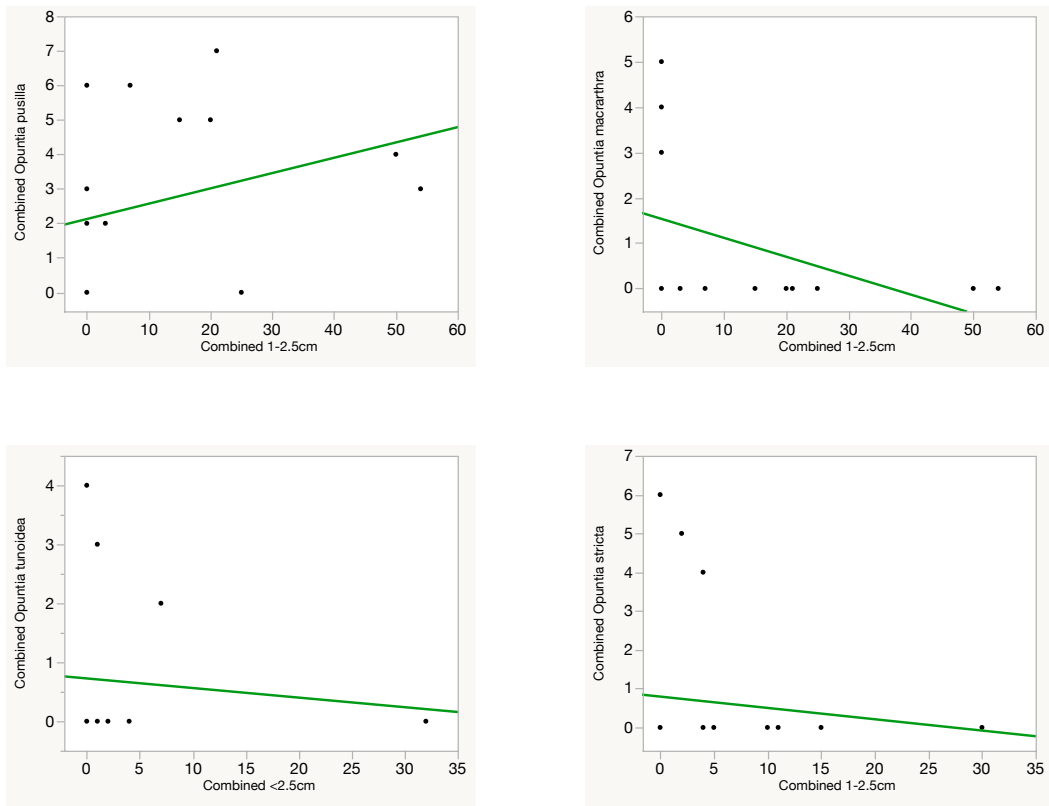


Figure 3.1 Correlation graphs between amount of *Opuntia* species cover and the number of individual stems recorded from 22 plots that ranged from 1 cm in diameter to greater than 2.5 cm in diameter. Each graph indicates how many plots contained what species at a particular cover value in relation to the number and size of “woody” stems present.

Landform Index is displayed for each of the study species in Figure 3.2. Each graph does a fairly sufficient job at indicating that the more open the plot is to light, the more dense *Opuntia* presence is. The graphs suggest which species seem to be more tolerant of lower light levels. *Opuntia pusila* seems to be present when a plot has a clinometer reading of 15 at the aspect. It is suspected that this is due to its common placement further away from the ocean.

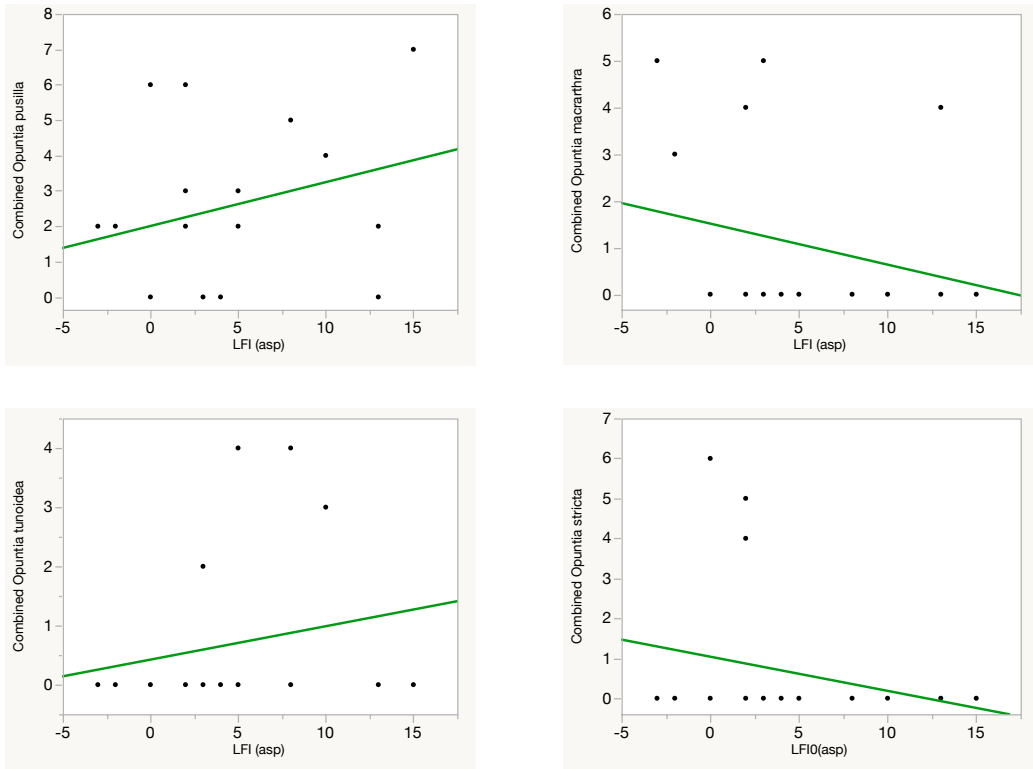


Figure 3.2 Correlation graphs between amount of *Opuntia* species cover and Landform Index (point of light entry) taken at the slope of each plot (aspect) from 22 sampled plots on the coast of South Carolina. Each graph indicates the amount of a particular *Opuntia* species and how many plots contained that species at a particular Landform Index.

The changes in terrain for each plot were recorded using Terrain Shape Index. Figure 3.3 reveals the slopes for the terrain at 45 degrees. This is shown for each species. For the most part *Opuntia* presence was highly correlated with level or slightly convex sites. One

important observation to note is that a data points represents the slope at a particular angle and the *Opuntia* concentration within the plot. It does not mean that the presence of *Opuntia* was indicative of the slope taken at that particular angle.

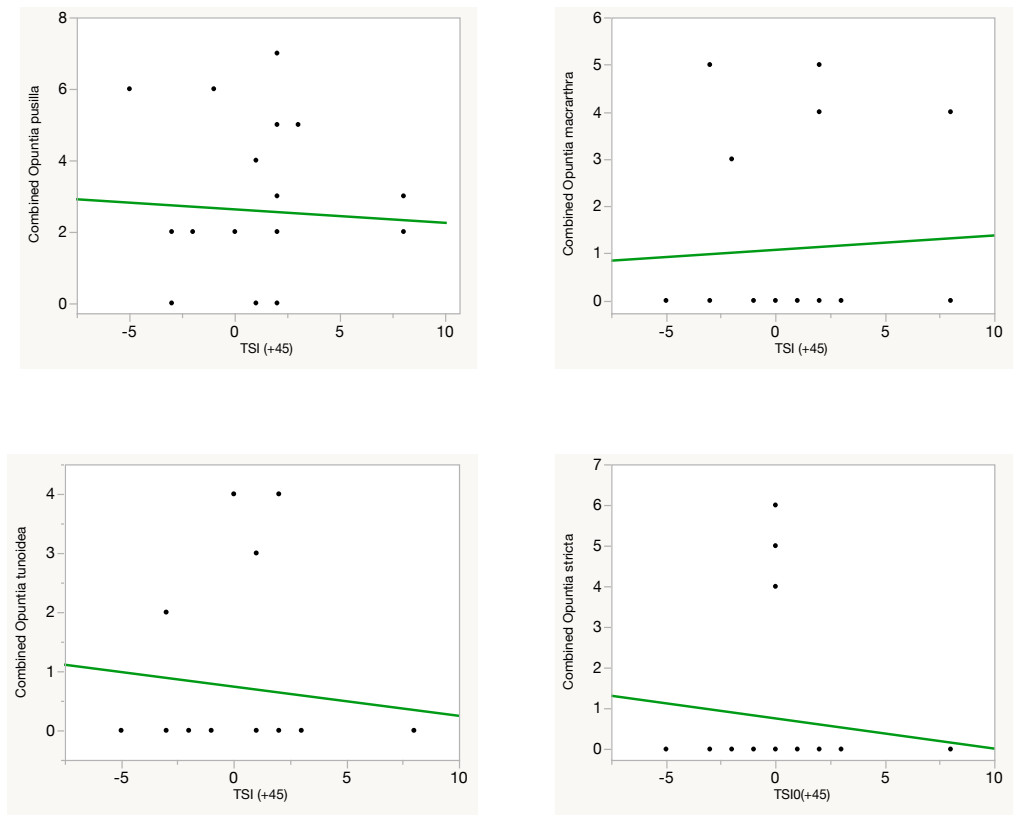


Figure 3.3 Correlation graphs between amount of *Opuntia* species cover and Terrain Shape Index (slope of the ground) taken at +45 degrees from the center of each plot for 22 sampled plots on the coast of South Carolina. Each graph indicates the amount of a particular *Opuntia* species and how many plots contained that species at a particular Terrain Shape Index.

Figure 3.4 shows what is referred to as the direct lines to *Opuntia pusilla*. There are three different direct lines that had the greatest significance in the analysis. Line a. shows that when pH is less than 7.7 and phosphorous is less than 23 lbs per acre, there is a 60% chance that there will be a presence of *Opuntia pusilla* at a cover value of 5.5(7.5%). Line b. shows that when the acidity is greater than or equal to 0.4 meq/100g of soil, there is a 63.4% chance that there will be a presence of *Opuntia pusilla* at a cover value of 3.76(2%). Line c. shows that when *Uniola paniculata* (Sea Oats) has a cover value less than 2(0-1%) and *Ilex vomitoria* (Yaupon Holly) has a cover value greater than 5(5-10%), there is a 64% chance that there will be a presence of *Opuntia pusilla* at a cover value of 3.76(2%).

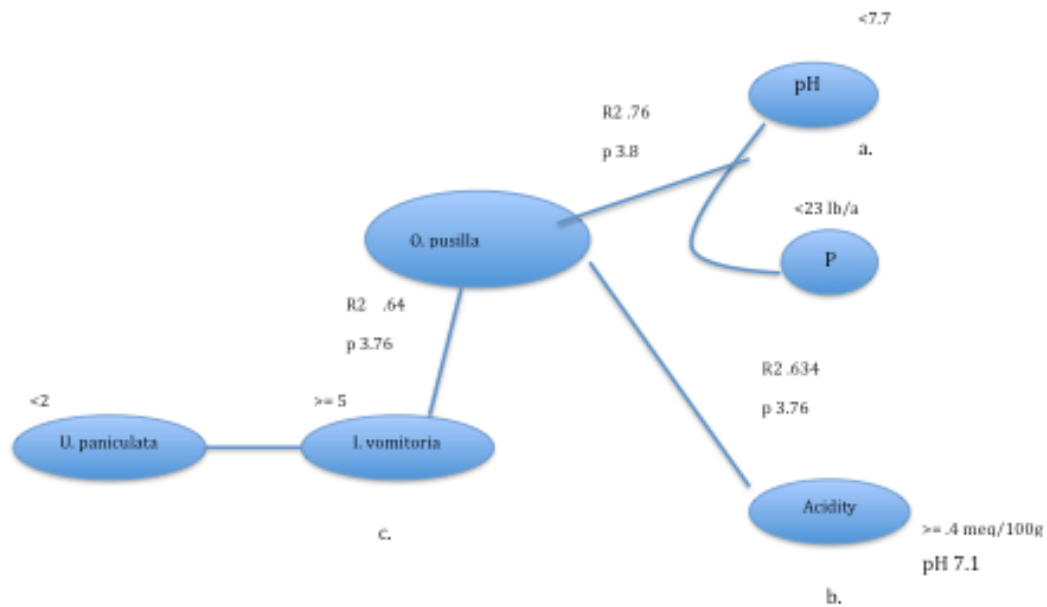


Figure 3.4 Direct line Path Map for *Opuntia pusilla*. Lines (3) represent the ecological variable or variables that predict the presence of *O. pusilla*. The R^2 for each line is the percent variability reduction in the prediction of *O. pusilla* cover density (p) due to the variable or variables indicated.

The path map in figure 3.5 represents predicting lines found by the partition analysis for *Opuntia tunoidea* presence. Line a. depicts that when the terrain shape index slope at 225 degrees is greater than or equal to 2 and is less than 1 at the aspect, there is a 25% chance of *O. tunoidea* presence at a cover value of 3.25(2%). Significant associated species are exhibited by line b. It indicates when the *Spartina patens* (Saltmeadow Cord Grass) has a cover greater than or equal to 6(10-25%) and *Uniola paniculata* (Sea oats)

has a cover value less than 2 (0-1%), there is a 45% chance of a presence of *O. tunoidea* at a cover value of 3.25(2%). Soil parameters revealed by partitioning are displayed in line c. It signifies that when the acidity is greater than or equal to 2 millequivalents per 100 grams of soil, and when the Cation Exchange Capacity is greater than 33 millequivalents per 100 grams of soil, there is a 40% chance that there will be a presence of *O. tunoidea* at a cover of 3.25(2%).

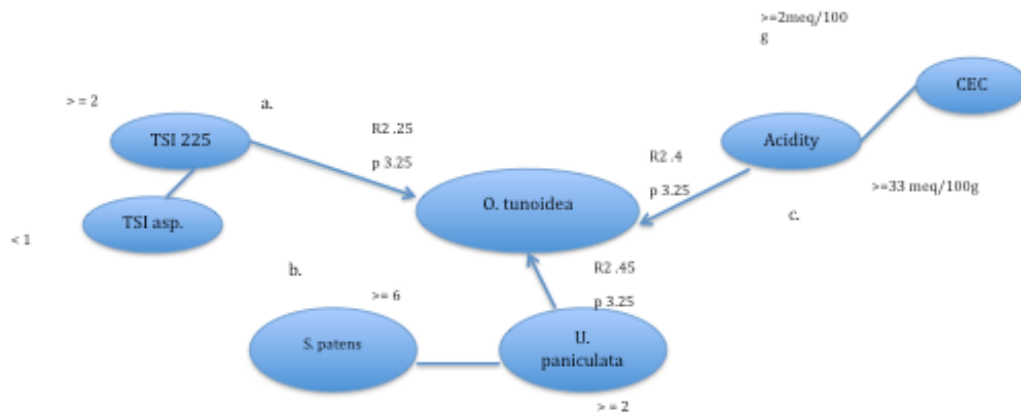


Figure 3.5 Direct line Path Map for *Opuntia tunoidea*. Lines (3) represent the ecological variable or variables that predict the presence of *O. tunoidea*. The R^2 for each line is the percent variability reduction in the prediction of *O. tunoidea* cover density (p) due to the variable or variables indicated.

The path map in Figure 3.6 demonstrates indicating variables of *Opuntia macrarthra* presence. Line a. shows the relationship between the associated vegetation species *Cenchrus tribuloides* (Sand Spur) and *O. macrarthra*. When *C. tribuloides* has a cover greater than or equal to 2(1%), there is a 74% chance that there is a presence of *O. macrarthra* at a cover value of 4.2(5%). Line b. indicates that when Phosphorous (P) in

the soil is greater than or equal to 690 pounds per acre, there is a 74% chance of *O. macrarthra* presence at a cover of 4.2(5%). Line c. describes a combination of a soil component and terrain shape. Base saturation relates to the percentage of exchange sites on soil particles occupied by the basic cations Ca, Mg, K, and Na. If a soil has a base saturation of 100%, there is no exchangeable acidity (Clemson Public Service Activities, 2015). When the base saturation is greater than or equal to 98%, and the slope at 45 degrees is greater than or equal to 2, there is a 30% chance of *O. macrarthra* presence at a cover of 4.2(5%). A better description of line c. would be a soil with very alkaline pH and a slightly above even terrain.

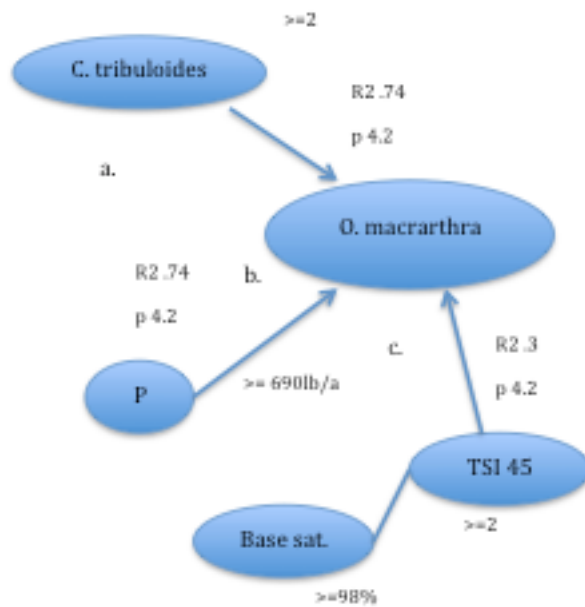


Figure 3.6 Direct line Path Map for *Opuntia macrarthra*. Lines (3) represent the ecological variable or variables that predict the presence of *O. macrarthra*. The R^2 for each line is the percent variability reduction in the prediction of *O. macrarthra* cover density (p) due to the variable or variables indicated.

Figure 3.7 displays the direct lines of variables that predict the presence of *Opuntia stricta*. Line a. indicates that when the TSI (slope) at the aspect is less than one and the TSI at 135 degrees is greater than or equal to 1 there is a 52% chance of *O. stricta* presence at a cover of 5(5-10%). Line b. implies that if there is a presence of *Ilex vomitoria* (Yaupon Holly) at a cover value greater than or equal to 3(2%), then there is a 22% chance of *O. stricta* presence at a cover of 5(5-10%). The soil variables that show the greatest significance for *O. stricta* appear in line c. When sodium is less than 120

lbs/acre and Ca is greater than or equal to 2329 lbs/acre, there is a 30% chance of *O. stricta* presence with a cover of 5(5-10%).

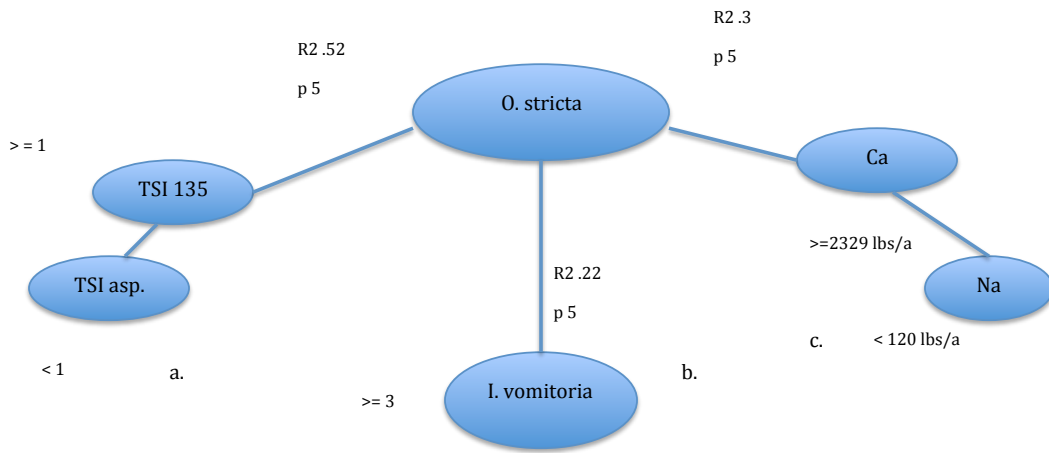


Figure 3.7 Direct line Path Map for *Opuntia stricta*. Lines (3) represent the ecological variable or variables that predict the presence of *O. stricta*. The R^2 for each line is the percent variability reduction in the prediction of *O. stricta* cover density (p) due to the variable or variables indicated.

Discussion

The results begin to tell the story of the ecological classification of the 4 species in this study. The goal of this study was to identify variables that help predict presence and infer speciation based on ecological factors. The correlation graphs help provide evidence of sites where *Opuntia* may occur. The graphs describe sites that are open, less protected, relatively flat or convex, and void of many woody stems that may outcompete the *Opuntia* for light and other resources. Although these conditions may describe fore dunes, it is strongly inferred that these sites are much more stable compared to the highly dynamic active dunes. *Opuntia* cannot survive on an active fore dune because the fore dunes are constantly shifting. *Opuntia* does not grow fast enough, doesn't have the root architecture or growth habit to keep up with the dynamics of the fore dune. From an ecological succession standpoint, the *Opuntia* are found in an area somewhat between primary and secondary succession. The species shown to be the most significantly correlated with each species are not only an indicator of presence, but also location within the dune system. The path maps help us visualize this with several species of associated vegetation.

Uniola paniculata is commonly known as Sea Oats. Its name comes from the shape of the seed heads. The grass that can reach up to 2 m tall and have leaf blades up to .75 m long. It is recognized as a pioneer species or stabilizing species of the fore dune. The root system and production of rhizomes contributes to the survival of the plant and dune stabilization. As a result of its ability to survive on active dunes, it has been used in many dune restoration projects.

Uniola paniculata helps to trap sand on the active dune. It responds to burial by accretion with increased shoot growth (Walsh, 1994). *Opuntia* and other species do not grow fast enough to handle this. *Uniola paniculata* is described in correlation with both *Opuntia tunoidea* and *Opuntia pusilla*. Each species has differences in relation to the cover value of *U. paniculata*. *Opuntia tunoidea* presence is more predictable when the presence of *U. paniculata* is greater than or equal to 2. The greatest density of *O. pusilla* is when the cover of *U. paniculata* is less than 2. *Uniola paniculata* occurs predominantly on the active fore dunes. The cover value of its existence in correlation with the *Opuntia* species implies location. With this information alone, the implication is that *O. pusilla* is found further behind the active dune and *O. tunoidea* can be found closer to it. A justification could be plant habit.

Opuntia tunoidea is a taller species and may be able to handle some sand accretion, which would occur closer to the fore dune. *Opuntia pusilla* is prostrate in habit and sand burial may be detrimental. The cover values are, however, not extreme enough to infer that there is a large separation in distance between the two *Opuntia* species, just that there is some. Not every associated species paints a clear picture of the location of each individual *Opuntia* species. However, their presence suggests that the *Opuntia* species all grow further away from the active part of the dunes.

Ilex vomitoria is indicated to have a significant correlation to the presence of *O. pusilla* and *O. stricta*. *Ilex vomitoria* is an evergreen shrub to small tree. It has small leaves, simple alternate, finely serrated, dark green leaves usually attached by purple petioles. The plant produces red drupe fruits in September and October. Compared to *U. paniculata*, it is a slow growing plant. It responds well to salt spray and the hot sun. It is

not a plant that can keep up in the dynamic environment of the active dune (Coladonato, 1992). This provides some clarification that *O. pusilla* and *O. stricta* occur in somewhat stable areas. The combination of *I. vomitoria* and *U. paniculata* suggest that *O. pusilla* occurs in areas that are less dynamic. This could be an area that has a more developed fore dune with lots of grassy species. It could also describe an area further back in the dune system.

Opuntia tunoidea may be more tolerant of more dynamic areas due to its size. *Opuntia tunoidea* also has a significant correlation with *Spartina patens*. *Spartina patens* is a thin, round blade grass that can be found year-round in the dune system. It is described as the dominant species in a saltmeadow marsh. This is considered the 3rd stage in the succession of a salt marsh (Walkup, 1991). The correlation with *O. tunoidea* implies that it too is a later successional species. However, it is far from a species found in a climax community.

Cenchrus tribuloides appears to be an excellent indicator species of *Opuntia macrarthra*. None of the other *Opuntia* species have a significant correlation with it. *Cenchrus tribuloides* is a prostrate stoloniferous-type grass that has individual leaf blades that feather out from a brownish sheath. It, however, is most recognizable by its painful spiked fruit/seeds. The seeds are an adaptation to dispersal. This is known as hitchhiker dispersal. Plants that exhibit hitchhiker dispersal generally inhabit areas of early ecological succession (Porcher and Rayner, 2001). It does not, however, respond well to dynamic environment. It does not tolerate sand burial or storm surge action. This is due to its low growing habit and shallow root system. The density of *C. tribuloides* was recorded in plots at 23% on Miller Field Beach, Staten Island, NY, before Super Storm

Sandy. A year later in September of 2013, it was no longer present in the same plots (Cheplick, 2016). Based on this information, it can be assumed that *C. tribuloides* occurs further back from the fore into the mid-dune.

The partition analysis revealed seven variables related to soil composition. These included: Phosphorous, pH, acidity, cation exchange capacity, base saturation, calcium, and sodium. Soil composition changes as the dunes age. Tackett and Craft (2010) describe coastal dunes on Sapelo Island, Georgia as having three different zones. Classified from the shore inland they are the fore dune, mid dune, and forested dune. The plants that inhabit them characterize the zones. The plants are able to inhabit the zones due to reduced environmental stress further from the ocean. Accretion and salt spray decrease further inland, increasing stability (Maun and Perumal, 1999). Organic matter starts to accumulate, moves into the mid-dune, and increases into the forested dune. The increase in organic matter is due to things such as leaf litter and decomposing buried plants. Increasing organic matter causes an increase in CEC, decrease in pH, decrease in base saturation, and change in nutrient status. In the study on Sapelo Island, nutrient cycling was analyzed for Carbon, Nitrogen, and Phosphorous. Compared to the fore dune, plant-available Phosphorous was shown to increase in the older mid-dune and forested dune (Tackett and Craft, 2010). Sand has low CEC and water holding capacity. Phosphorus or Phosphate (the plant-available form) has a negative charge. The low CEC and water holding capacity of the sand in the fore dune causes Phosphorus to be leached out of the soil profile.

The association of decrease in Phosphorous with *O. pusilla* is believed to be a function of *I. vomitoria*. Due to the biology of *Opuntia pusilla*, its nutrient requirements

are much lower. *Opuntia pusilla* is a CAM plant whereas *Ilex vomitoria* is a C₃ plant. C₃ plants will need more Phosphorus for water scavenging, energy (ATP) for active nutrient uptake, carbon sequestration, and several other processes. Therefore, the available Phosphorus has been severely utilized by *I. vomitoria* and other woody species that may be present. In relation to zonation, it may be inferred that *O. pusilla* occurs at the greatest density in the back of the mid-dune closer to the forested dune. *Opuntia pusilla* may be more tolerant of woody species due to its small size, as well as its ability to escape close competition by disarticulating pads.

Opuntia macrarthra also appears to have a significant correlation with Phosphorus according to its path map. Several things may be implied about *O. macrarthra*. It occurs in the mid-dune due to the amount of Phosphorus. However, it may be far enough away from larger wood species or there are not enough present to deplete the pool of Phosphorus. The parent material where *O. macrarthra* is found may also have a higher Phosphorus content.

The acidity and CEC variable indicate that *O. tunoidea* occurs in the mid-dune as well. The associated species have already suggested that it occurs closer to the fore dune within the mid-dune. Base saturation affects CEC as well as acidity. *Opuntia macrarthra* seems to have significant presence when the base saturation is greater than or equal to 98 percent. This may imply that it favors areas with no acidity.

A high amount of Calcium was associated with *Opuntia stricta*. This is because areas where it was sampled with the most cover contained lots of oyster shell. These included the oyster shell rake that is Ashe Island, and old cotton fields on Fenwick Island. Spreading oyster shell was an old farming practice, so Fenwick Island soil has lots of

Calcium. Shells are made up primarily of Calcium Carbonate. As the shell degrades, it releases Calcium into the soil. Higher Calcium causes the soil to be more alkaline. The soil that *O. stricta* was growing in on Ashe Island was almost pure oyster shell. With the excessive amounts of Calcium, there is no room for acidity in the soil and the soil is very coarse. Sodium (Na) in the soil less than 120 lbs/acre in addition to the high amounts of calcium seems to be indicative of its location on both Fenwick Island and Ashe Island. The Fenwick Island sites are protected from salt spray and salt water flooding. Soil Na from plots on Fenwick were 15 and 38 lbs/acre. Ashe Island is a high marsh island susceptible to salt spray and flooding from the tides. All of the *Opuntia* species appear to be tolerant of substantial amounts of Na in the soil. The Soil Na in the 22 plots ranged from 13 lbs/acre to 249 lbs/acre. The plots with the lowest Sodium were where *O. pusilla* occurred in an open area in the woods of the Hobcaw Barony beach property on Debordieu Island, SC. They were well protected from normal tides and salt spray. The highest soil Na was found on a shell mound marsh island in Georgetown also owned by Hobcaw Barony.

The terrain shape index is important for each species. They all grow in a level to slightly raised terrain. The path maps imply that three of the four species show significance of this type of terrain. Just because TSI didn't show up in the *O. pusilla* path map doesn't mean it doesn't follow the same pattern. Other variables were just stronger at indicating its presence.

(Tackett and Craft, 2010) used historical aerial photos to estimate the age of the dunes by the location of cabanas 50 years prior to the project. The cabanas at the time of the study in 2010 were 50 meters behind the fore dunes. They were essentially 50 meters

from their location 50 years prior. That infers that they moved exactly 1 meter per year. Based on this they estimated the fore dunes of Sapelo Island to be 10 years old because they were between 0 and 20 meters from ocean. The mid dunes were estimated to be about 90 years old, and the Forested dunes 500 years old. That is quite a significant change in age between the three zones. This implies that a location of one *Opuntia* could be in an area of the dune that took 50 years to develop and another in a location that only took 30 years. The changes that occur during the development of different zones within the dune could create a “niche”.

It is easy to infer that *Opuntia pusilla* and *Opuntia tunoidea* seem to be tolerant or find themselves in different ecological situations within a dune system. However, it could be argued that there is not enough spatial variation since they can be found in close proximity of one another within the dune. *Opuntia macrarthra* can be found near *O. pusilla* or *O. tunoidea*, but a combination of all three has yet to be found. Maybe *O. tunoidea* occurs in mid-dunes where there is more organic matter built up and an active fore dune, whereas *O. macrarthra* may occur where there are less “woody” species and less organic matter.

Morphological characteristics imply that *Opuntia stricta* seems to occur in places where the other four species do not (i.e. Fenwick Island and Hutchinson Island). Hutchinson Island is an old shell mound, which is known for having high amounts of Calcium. Fenwick Island, on the other hand, is a collection of old cotton fields, which also have large amounts of Calcium. All plots with *Opuntia* had significant amounts of Calcium. However, the shell mound in Georgetown and on Ashe Island had the greatest amounts. All individuals that matched the morphology of *O. stricta* were found in areas

different than a dune system. The one exception was a questionable morphological individual with similar characteristics to *O. stricta* in the dunes of Otter Island. It could have been a mistake in identification. It could be also be a hybrid or a cryptic taxon.

Some may considered each of the called species as following the Ecological species concept and others may not. A lot of the conditions where each species occur were very similar and some were slightly different. There is a fine line here that may be better defined by Phylogenetic research.

Geographic Range

Clues from past descriptions of *Opuntia* in (Small, 1933) helped to better understand and find the habitat typification. Also these descriptions were made pre-anthropogenic development. Therefore, areas not disturbed by anthropogenic factors (tourists, etc.) were sought out. *Opuntia pusilla*, called *O. drummondii* at the time, was described to occur in sand dunes and outer coastal plain habitats. *Opuntia macrarthra* was described to occur on the SC coast, and *Opuntia tunoidea* in coastwise sand dunes from Georgia and northeast Florida to North Carolina. Sites were documented during the collection of individuals for both the morphological and ecological studies. In order to visualize a range of each species, the results were summed up in five distribution maps. The maps are shown in figure 3.8.

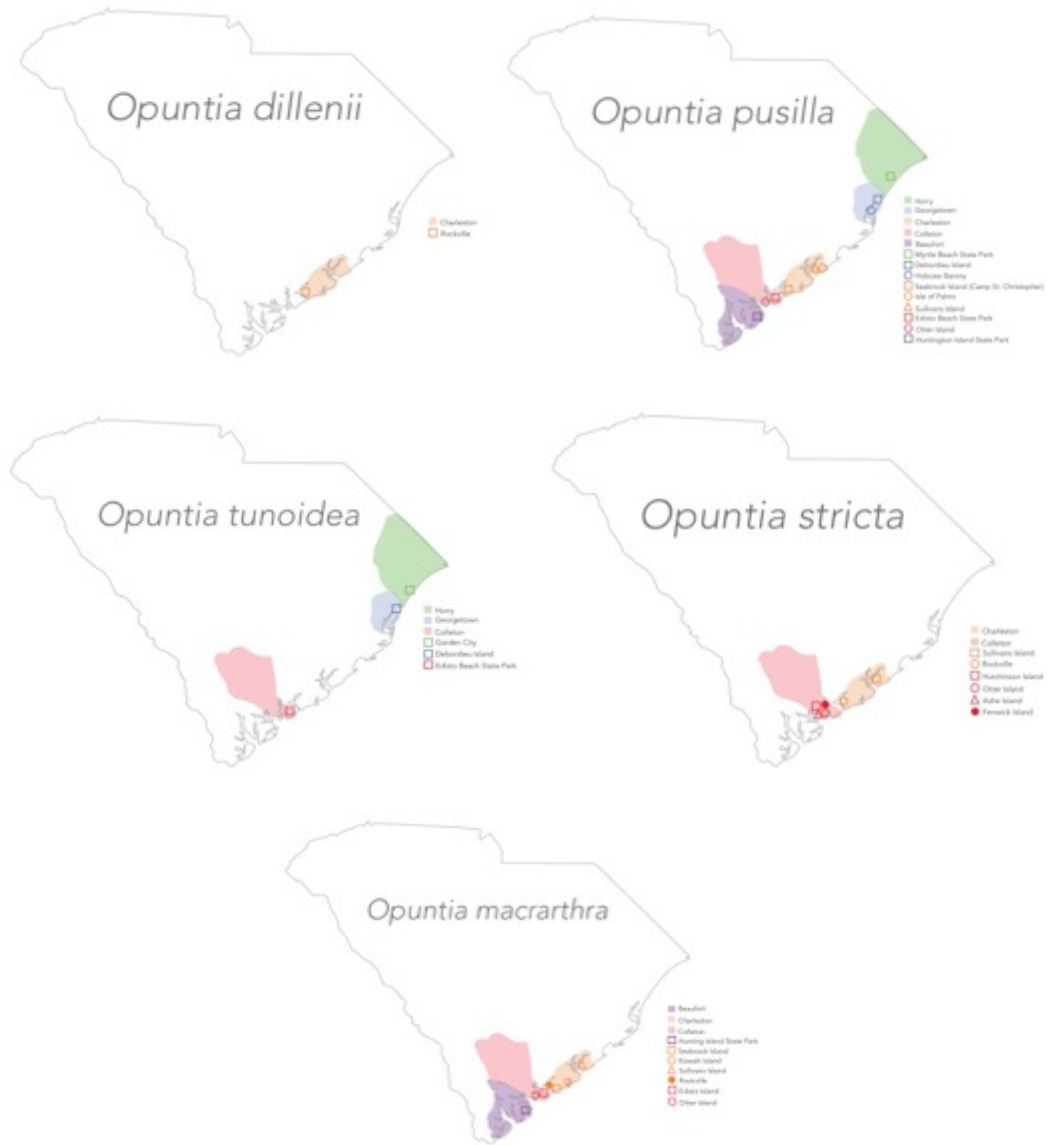


Figure 3.8 Distribution maps indicating the counties and locations within those counties that each *Opuntia* species was found during this study.

Conclusions

The purpose of this project was to describe and clarify five assumed species of *Opuntia* on the coast of South Carolina. Past descriptions were studied in order to get an idea of described morphology and general location. The five species studied were *O. dillenii*, *O. pusilla*, *O. stricta*, *O. macrarthra*, and *O. tunoidea*. The following questions were asked: Are there *Opuntia* species matching past descriptions of these on the coast of South Carolina? What are their morphological characteristics? Are there morphological characteristics that differ between the five species that can be used to tell them apart? Do the morphological characteristics differ from other *Opuntia* described from South Carolina and ones native to Texas? Do each of the five species follow the morphological species concept? Where do the species occur? What do they occur with? What are the conditions like where they occur? Do they follow the ecological species concept?

In the Introduction chapter, each of the five species is described morphologically. A brief synopsis of the past descriptions is included with the current morphological descriptions. Measurements of morphological characteristics described were used to create groups of like individuals in cluster analysis. The variables that were used to place individuals in the groups were identified. After identification, the raw data from each variable was compared using ANOVA and Tukey HSD tests. The purpose of the morphological study was to answer the main questions: The purpose of this study was to answer the following questions: Do the five species of *Opuntia* matching past descriptions occur on the coast of South Carolina? What are their morphological characteristics? Are there morphological characteristics that differ between the five study species that can be used to tell them apart? Do the morphological characteristics differ

from other *Opuntia* described from South Carolina and ones native to Texas? How can they be differentiated from one another? Does each behave on the level of morphological species? Can each of the five species study species be classified based on these traditional concepts with the data collected?

The results from the cluster analysis confirmed that the variables measured on individuals collected separated them cleanly into groups based on what they were identified as in the study. Each of the individuals were identified as one of the following: *Opuntia dillenii*, *Opuntia pusilla*, *Opuntia macrarthra*, *Opuntia stricta*, *Opuntia tunoidea*, *Opuntia lindheimeri*, *Opuntia lata*, and *Opuntia mesacantha ssp. mesacantha*. Discriminant analysis results from the clusters of individuals formed revealed a set of variables that were significant to group placement. Furthermore, ANOVA and Tukey tests helped construct a set of variables that could be used to differentiate the species from one another, as well as from other described species.

These variables have formed a tool that can be used to tell *Opuntia* species apart from one another on the coast of South Carolina. The ability to find discriminatory variables confirms gaps in the morphological characteristics between each one. Therefore, it is inferred that there are five different morphological species based on the morphological species concept that occur on the coast of South Carolina. The comparison of past and current descriptions indicates existence of individuals of the described species: *Opuntia dillenii*, *Opuntia pusilla*, *Opuntia stricta*, *Opuntia macrarthra*, and *Opuntia tunoidea*. Based on morphology, the data also infer morphological differences between species described as *Opuntia dillenii*, *Opuntia pusilla*, *Opuntia macrarthra*, *Opuntia stricta*,

Opuntia tunoidea, *Opuntia lindheimeri*, *Opuntia lata*, and *Opuntia mesacantha* ssp. *mesacantha*.

Due to limited habitat space, *Opuntia dillenii* had to be omitted from the ecological characterization study. The other four species were studied to get a better indication of the sites where they occur. Carolina Vegetation Protocol was used to record vegetation associated with the *Opuntia*, soil characteristics, site exposure, and shape of the terrain where they occur. The data collected were then used in partition analyses to determine significant variables associated with the presence of each species, and if these variables revealed an ecological niche.

When the constructed path maps were interpreted, the evidence may or may not imply that each obeys the ecological species concept. Within the dunes system, it depends on one's perception of space. The space between the dune zones has been described as being between 10 and 500 years old based on meters away from the ocean. Each meter away from the ocean is said to represent one year. That seems like a lot more separation than 10 and 500 meters (Tackett and Craft, 2010). The years it takes to form the zones, plant associated with each zone and the conditions that come with each zone could be considered a "Niche". There is a fine line in saying separation of *Opuntia* in dune zones is a "niche" or not.

Morphological evidence implies that each of the five study species behaves like a species as well as *O. lindheimeri*. This may back ecological inference. However, phylogenetic research is needed to form a clear answer. Regardless of opinion about the ecological species concept, the results of the ecological study have provided indicators of *Opuntia* presence. Correlation graphs have suggested that *Opuntia* mostly grows in areas

that are open, relatively flat (compared to raised fore dunes), and less dense with woody species. The path maps reveal indicator species for each species of *Opuntia*, soil conditions associated with each, as well as terrain shape conditions. This provides insight as to what conditions to look for, what other plant species to look for, and where, within a dune system, to look for a particular *Opuntia* species.

The geographic range was documented by a series of distribution maps. Each species is documented as to what county it occurs in and where in the county it occurs. These maps are based primarily on morphological characteristics. They provide information as to where individuals from each species were found at the time of the study. These maps are a tool to aid in additional research on *Opuntia* species and their habitat on the coast of South Carolina.

References

- Ballard, Robert 2015. Clemson University. Angiosperm Evolution: Microsoft Power Point file.
- Bohm, Harmut 2008. "Opuntia dillenii"- An Interesting and Promising Cactaceae Taxon: *Journal of the Professional Association for Cactus Development*.
- Candelario Mondragon, Jacobo and Salvador Perez-Gonzalez; 2001 *Cactus as a Forage*.
- Cheplick, Gregory P. 2016. Changes in Plant Abundance on a Coastal Beach Following Major Storm Surges: *The Journal of the Torrey Botanical Society* 143(2) 180-191.
- Clemson Public Service Activities: Agricultural Service Laboratory 2015. CEC, acidity, and percent base saturation.
- Coladonato, Milo 1992. *Ilex vomitoria*. In: Fire Effects Information System; U.S Department of Agriculture, Forest Service. Rocky Mountain Research Station, Fire Sciences Laboratory.
- Feugang, Jean M. , Konarski, Patricia, Zou, Daming, Stintzing, Florian C. and Changping Zou 2006: *Nutritional and medicinal use of Cactus Pear (Opuntia sp.) cladodes and fruits*.
- Global Invasive Species Database: *Cactoblastis cactorum*. Visited 10-24-14
<http://www.issg.org/database/species/ecology.asp?si=729&lang=FR>
- Hervé Abdi and Lynne Williams 2010. *Tukey's Honestly Significant Difference: Encyclopedia of Research Design*. Thousand Oaks, CA: Sage.
- Hight, Stiphen D. and Carpenter, James E. 2009: Flight Penology of Male *Cactoblastis cactorum* (Lepidoptera: Pyralidae) at Different Latitudes in the Southern United States.
- Johnson, Duncan S. 1918. *The Study of Opuntia Fulgida; A Study of Perennation and Proliferation of Certain Cactaceae*. Carnage Institute of Washington.
- Judd, Walter S. , Campbell, Christopher S., Kellogg, Elizabeth A., Stevens, Petter F., and Donoghue, Michael J. 2008. *Plant Systematics: A Phylogenetic Approach*. Third Edition. Sinauer Associates, pp. 145.
- Majure, L.C. & Ervin, Gary N. 2007. Department of Biological Sciences, Mississippi State University: *Opuntias of Mississippi. Haseltoinia* 14: 111-126.

- Majure, L.C. R. Puente, M.P. Griffith, W.S. Judd, P.S. Soltis and D.S. Soltis. 2012a. Phylogeny of *Opuntia* s.s. (Cactaceae): clade delineation, geographic origins, and reticulate evolution. *American Journal of Botany* 99: 847-864.
- Majure, L.C., W.S. Judd, P.S. Soltis, and D.E. Soltis. 2012b. Cytogeography of the *Humifusa* clade of *Opuntia* s.s. Mill. (Cactaceae: Opuntioideae): Correlations with geographic distributions and morphological differentiation of a polyploid complex. *Comparative Cytogenetics* 6: 53-77.
- Mallet, James 2007. *Species, Concepts of. Encyclopedia of Biodiversity*. Vol. 5. Academic Press. pp. 427-440.
- Maun, M.A. and Perumal, J., 1999. Zonation of Vegetation on Lacustrine Coastal Dunes: Effects of Burial by Sand. *Ecology Letters*, 2, 14-18.
- Mayer, Ernst 1942. *Systematics and the Origin of Species*. Columbia University Press, New York.
- Peet, R.K., T.R. Wentworth and P.S. White. 1998. A flexible, multipurpose method for recording vegetation composition and structure. *Castanea* 63: 262-274.
- Porcher, Richard D. and Rayner, Douglas A.. 2001. *A Guide to the Wildflowers of South Carolina*. University of South Carolina Press. Columbia, SC.
- Powell, A.M. and J.F. Weedin. 2004. *Cacti of the Trans-Pecos and Adjacent Areas*. Texas Tech University Press. Lubbock, Texas. 509 pp.
- Powell, A.M. and J.F. Weedin. 2008. *Cacti of Texas: A Field Guide*. Texas Tech University Press. Lubbock, Texas. 365 pp.
- Radford, A.E., H.E. Ahles and C.R. Bell. 1968. *Manual of the Vascular Flora of the Carolinas*. University of North Carolina Press. Chapel Hill, NC. 1245 pp.
- Ramakrisna, Akula and Ravishankar, Gokare A. 2011. Influence of Abiotic Stress Signals on Secondary Metabolites in *Plants: Journal of Plant Signaling and Behavior*. Nov. 1: 6(11) 1720-1731.
- Russill & Jones, 1859 : Proceedings of the Elliott Society of Natural History of Charleston, South Carolina: Biodiversity Heritage Library.
- Sexton, Jason 2014. Species Range Limits: The Encyclopedia of The Earth.
- Small, J.K. 1933. *Manual of the Southeastern Flora*. University of North Carolina Press. Chapel Hill, NC. 1233 pp.

- Species Concepts 1998. *Encyclopedia of Ecology and Environmental Management*. Blackwell Press: pp. 709 – 711.
- Spira, Timothy P. 2011. *A Southern Gateways Guide: Wildflowers & Plant Communities of the Southern Appalachian Mountains & Piedmont*. UNC Press pp. 412.
- Stockburger, David W. 1996. *Multivariate statistics: Concepts, Models, and Applications*. Missouri State University.
- Tackett, N.W. and Craft, C.B., 2010. Ecosystem Development on a Coastal Barrier Island Dune Chronosequence. *Journal of Coastal Research*, 26(4), 736-742. West Palm Beach (Florida), ISSN 0749-0208.
- Taiz, Lincoln and Zeiger, Eduardo . 2010. *Plant Physiology – Fifth Edition*.
- Tripp, Drew: *The Press & Standard* September 3, 2014 Rare Lowcountry plant found on Edisto facing extinction.
- Ueckert, Darrell N. 2015. Texas Agricultural Experiment Station, Texas A&M University Agricultural Research & Extension Center, San Angelo, TX.
- University of Florida Entomology & Nematology: Common name: Catus Moth, Scientific name: *Cactoblastis cactorum* Berg. (Insecta: Lepidoptera: Pyralidae).
- Walkup, Crystal J. 1991. *Spartina patens*. In: Fire Effects Information System; U.S Department of Agriculture, Forest Service. Rocky Mountain Research Station, Fire Sciences Laboratory. (Producer).
- Walsh, A. Roberta 1994. *Uniola paniculata*. In: Fire Effects Information System; U.S Department of Agriculture, Forest Service. Rocky Mountain Research Station, Fire Sciences Laboratory.
- Ward, D.B. 2009. Keys to the Flora of Florida: 23, *Opuntia* (Cactaceae). *Phytologia* 91: 383-393.
- Weakley, Alan S. 2015. *Flora of The Southern and Mid- Atlantic States - Working Draft*: University of North Carolina Herbarium. University of North Carolina Botanical Garden. University of North Carolina at Chapel Hill. Copyright Alan S. Weakley.
- Whitaker, J. David , McCord John W., Maier, Philip P., Segars, Albert, Rekow, Megan L., Shea, Norm, Ayers, Jason and Browder, Rocky. Marine Resources Division South Carolina Department of Natural Resources Charleston, SC.2004: *An Ecological Characterization of Coastal Hammock Islands in South Carolina*.
- Whiley,, E.O. 1978. The Evolutionary Species Concept Reconsidered. *Systematic Biology*. 27:17-26.