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Palmetto

Fern Conservation in a Biodiversity Hotspot ● Saving the Endangered Florida Key Tree Cactus

Saving the Endangered Florida Key Tree Cactus (*Pilosocereus robinii*) Using New Genetic Tools

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Above: *Pilosocereus robinii* stand in the Florida Keys. Photo: Jennifer Possley, Center for Tropical Conservation/Fairchild Tropical Botanic Garden.

Biological systems around the globe are being threatened by human-induced landscape changes, habitat degradation and climate change (Barnosky et al. 2011; Lindenmayer & Fischer 2006; Thomas et al. 2004; Tilman et al. 1994). Although there is a considerable threat across the globe, numerically the threat is highest in the biodiversity hotspots of the world. Of those hotspots, South Florida and the Caribbean are considered in the top five areas for conservation action because of the high level of endemism and threat (Myers et al. 2000). South Florida contains roughly 125 endemic species and is the northernmost limit of the distribution of many tropical species (Abrahamson 1984; Gann et al. 2002). The threat to these species comes predominantly from sea level rise, which could be >1 m by the end of the century (Maschinski et al. 2011).

Above: *Pilosocereus robinii* in bloom at the Center for Tropical Conservation. Photo: Devon Powell, Center for Tropical Conservation/Fairchild Tropical Botanic Garden.

Tonya Fotinos removing epidermal tissue of *Pilosocereus robinii* for analysis from the population on Big Pine Key. Photo: T. Fotinos.

Restoration of imperiled populations is a priority for mitigating the looming species extinctions (Barnosky et al. 2011). Populating new or previously occupied areas or supplementing a local population of existing individuals are strategies that improve the odds that a population or species will survive. Undertaking conservation actions while taking genetics into consideration increases the chance of success and can bolster overall population genetic diversity, thus further improving a species' chance of persistence (Godefroid et al. 2011). The increase in success occurs because genetic factors affect population viability at the same rate or faster than demographic or ecological factors (Frankham & Ralls 1998; Saccheri et al. 1998), making their consideration just as vital as increasing overall population numbers.

Plants, by their nature, are subject to particular genetic trends that can be detrimental to the success of restoration efforts. Plant populations with low numbers of individuals are affected by increased rates of genetic drift and inbreeding. Also, plants are sedentary so they have other genetic considerations such as outbreeding depression and clonality. Supplementing a population with individuals can increase local genetic diversity if done appropriately in conjunction with genetic testing and drastically improve the odds of successful restoration. Conversely, unknowingly introducing individuals without critical, locally adapted genes or other deleterious genetic issues can doom a restoration project.

The Key tree cactus, *Pilosocereus robinii*, is a federally endangered columnar cactus native to the tropical hardwood hammocks in the Florida Keys (Figure 1). *Pilosocereus robinii* is part of a larger *Pilosocereus* complex of species found in the Caribbean but is the only representative of the genus that occurs in North America. The phylogenetic relationships of the various *Pilosocereus* populations in the Keys have been disputed since their discovery in 1838. Botanists John Torrey and Asa Gray first officially documented the cactus in 1838 as *Cereus peruvianus* (Torrey & Gray 1838). The cactus was renamed six more times including genus and species names (see full history USFWS 1999). The Key Largo population has historically been treated as *P. bahamensis* (Britton) Byles & G.D.Rowley (USFWS 2010). Despite this classification, both *P. robinii* and *P. bahamensis* have at times been grouped into the more widespread Caribbean-based species *Pilosocereus polygonus* (Lem.) Byles & G.D.Rowley (Anderson 2001; Zappi 1994), but inclusion into *P. polygonus* has not been upheld elsewhere (ITIS 2013). Therefore, the relationship between the Key Largo population and the rest of the Keys population has to be resolved for appropriate management and reintroduction action for this species.

As early as 1917, botanist John Small noted in his description that the cactus was rare in the Keys and in danger of extirpation as a consequence of colonization in the area (Small 1917). An extensive survey done in 1984 also noted declines in previously occupied areas (Adams & Lima 1994). Although historically low, the number of remaining individuals has declined by more than 80% in the past decade because of continued habitat loss and environmental change, particularly

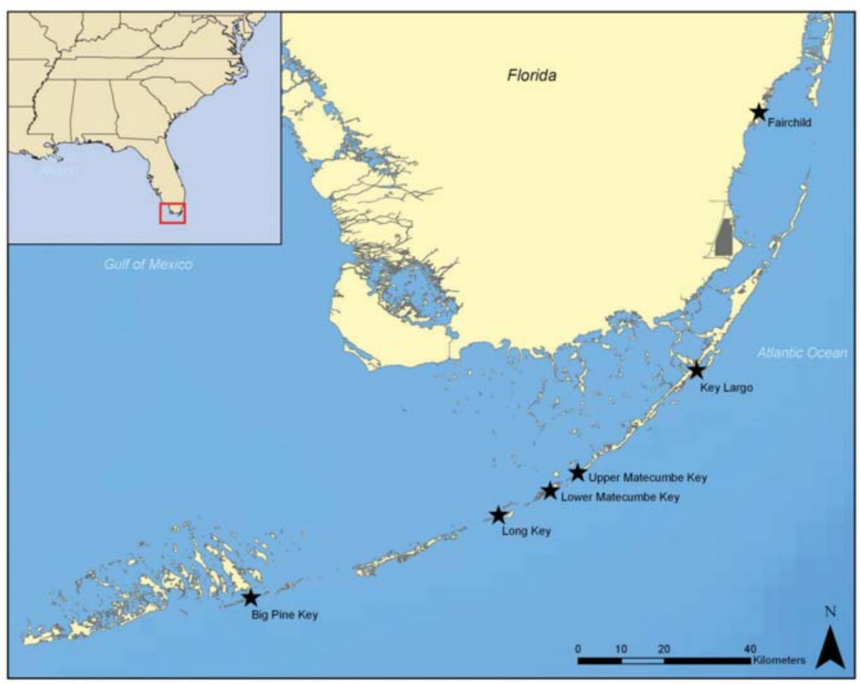

Figure 1: Map showing the populations of *Pilosocereus robinii* in Florida including the ex situ collection held at Fairchild Tropical Botanic Garden in Coral Gables, Florida.

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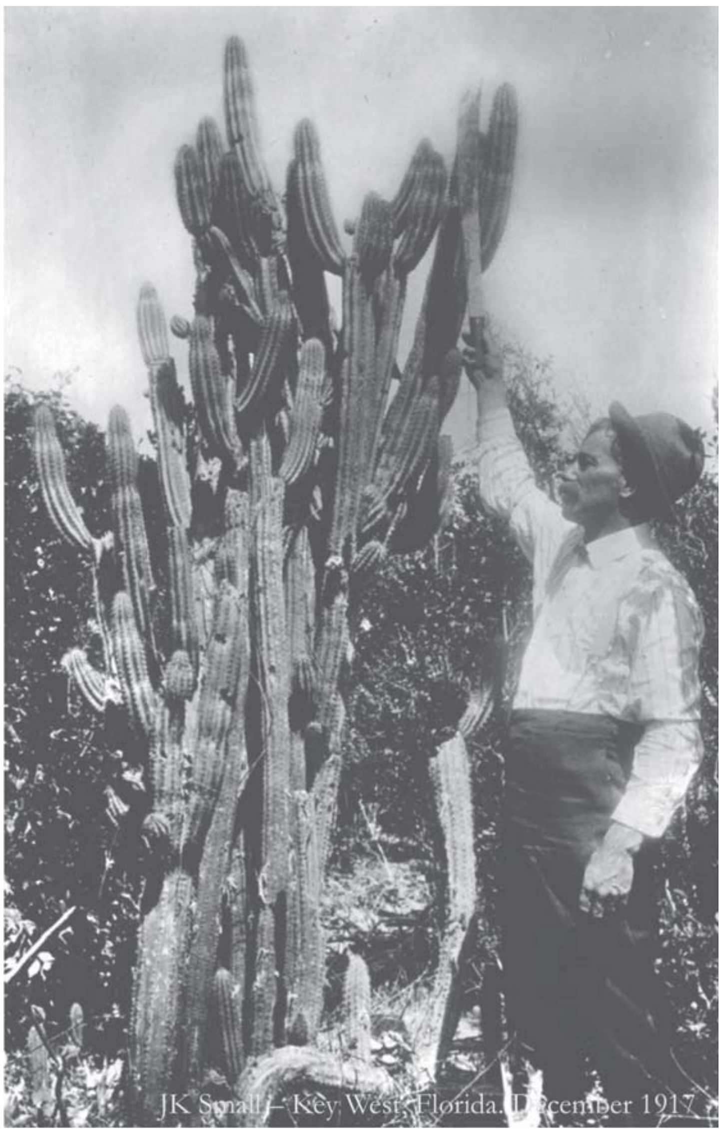
soil water salinization (Goodman et al. 2012; USFWS 2010). The tropical hardwood hammocks of the Florida Keys are found on limestone outcroppings that represent the areas of highest elevation on the islands. These forests harbor a large number of endemic populations from the Caribbean region. Tropical hardwood hammocks are threatened currently and historically by urbanization, anthropogenically-induced change in fire frequency, conversion to agriculture, and climate change (Harveson et al. 2007; Ross et al. 2001; Ross et al. 2009; USFWS 1999). Rising sea level is of particular concern and, coupled with a recent increase in storm frequency and intensity, is predicted to have a potentially devastating impact on the small remaining populations in the Florida Keys (Maschinski & Haskins 2012; Maschinski et al. 2011).



Man standing by **Cephalocereus keyensis* 1917 (*later renamed *Pilosocereus robinii*). Photo: JK Small, State Archives of Florida, *Florida Memory*, http://floridamemory.com/items/show/49460

To assess the genetic relatedness of the remaining Key tree cactus populations, root material was collected from twenty individuals from the Fairchild Tropical Botanic Garden ex situ collection which included at least two individuals from each of the five populations, nine of which were from individuals currently extirpated from the wild (Figure 1). A new genetic technique called Restriction site Associated DNA mapping (RAD) was used to identify Single Nucleotide Polymorphisms (SNPs) in the twenty samples. RAD can create hundreds to thousands of genetic markers to compare the relatedness of individuals or populations without the costly development of a DNA primer system. This technique will allow conservation projects to forgo the lengthy genetic marker discovery time for non-model species which could make it a very powerful tool for future conservation efforts (Davey & Blaxter 2010; Rowe et al. 2011). SNPs generated by the RAD technique have less genotyping error and increased statistical power because of the number of markers generated (Allendorf et al. 2010; Hohenlohe et al. 2011). This new technique has the ability to resolve fine scale patterns of variation allowing for resolution of past genetic flow or introgression (Hohenlohe et al. 2013). These methods have the potential to offer new insight into genetic questions that previous marker sets have been unable to address.

The populations of *Pilosocereus robinii* in the Florida Keys display considerable amounts of inbreeding and low levels of genetic diversity. These results are consistent with the species' having a history of population bottlenecks and colonization. There was little genetic difference between the populations of cacti across the Florida Keys. Further analysis indicated that most of the genetic variation is shared among the group as a whole rather than between individual populations. Although populations were genetically similar overall, the greatest amount of differentiation occurred between the Big Pine Key and Lower Matecumbe Key populations. The Big Pine Key population had the most significant deviations in the pairwise comparisons and the greatest breadth of genetic differentiation. However, it was the Lower Matecumbe Key population that was identified as a unique population upon further analysis.

The Key Largo population has long been thought to be a large clonal stand of genetically identical individuals, but DNA sequence data did not support this. These data demonstrate that the population on Key Largo is made up of a number of unique individuals. This cactus population is known to be a prolific reproducer in the wild and has one of the few individuals that has successfully set fruit in the wild since monitoring began. The low amount of differentiation between Key Largo and the other populations suggests that the Key Largo population is closely related to the settlement of *Pilosocereus* in the lower Keys.

The lack of differentiation from the stand of cacti on Key Largo compared to the rest of the populations could indicate that the Key Largo population was established by an initial colonizing event and could be the mother plant to the rest of the *P. robinii* in the Florida Keys. Further testing and comparisons between the *Pilosocereus* genus in the Caribbean can elucidate this issue. The Key Largo stand of cacti appears more similar to all of the rest of the populations than they do to each other. The lack of significant deviations from Key Largo to the rest of the populations lends credibility to the argument that *P. bahamensis* is in fact *P. robinii* since this particular cactus is more similar to the rest of the cacti in the Keys.

The reintroduction of *Pilosocereus robinii* into the Florida Keys is ongoing. The first transplant population was planted August 2012. Most of the transplants are thriving, although some mortality has occurred. The genetic analysis suggests that the reintroduced individuals have not interfered with population structure across the Keys, and that lower Keys material can be transplanted to higher elevation sites in the upper Keys because the Key Largo population is not a unique population or a different species. There is little genetic evidence to suggest that plantings need to remain within the population of origin. Lower Matecumbe and Long Key are priorities for collection since they contain distinct genetic variation and Big Pine Key for its overall greater genetic diversity.

Given the limited knowledge and frequent changes concerning the taxonomic relationship of this genus in the Caribbean, further genetic work must be completed to reveal the relationship of this North American-based species to the rest of *Pilosocereus*. *P. polygonus* samples from the Bahamas and Dominican Republic are being added to this dataset to answer these remaining questions.

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