LUCAS C MAJURE

Towards an evolutionary understanding of the *Opuntia humifusa* complex of North America

The genus *Opuntia*, also known as prickly pear cactus or nopal, has long intrigued botanists and plant enthusiasts alike for their resistance to drought, ease of propagation, amazing growth forms and beautiful flowers, as well as their wide distribution (growing virtually throughout the Americas, naturally). The genus *Opuntia* also is found farther north than any other cactus (e.g., *O. fragilis*). In certain areas prickly pear cacti form immense stands and virtually dominate the landscape. Owed to their usage and abundance, the opuntias have become iconic in certain countries, such as Mexico, where they are even represented on the national flag. Prickly pear cacti historically have been used widely as foodstuffs by native peoples, hedgerows along property lines, for rearing insects (such as *Dactylopius*: scale insects) used in making cochineal dye for textiles, and for a variety of home remedies. They are also commonly used as ornamentals. Tunas (fruit of prickly pear) and nopales (their stem segments or cladodes) can be found being sold in most local grocery stores for consumption in the home, so the economic value of prickly pears is increasing even within the United States. As well, there is current research emphasizing the importance of prickly pears for their use in the medical field and for ameliorating other common problems associated with anthropogenic disturbance such as pollution and erosion. For example, extracts from *Opuntia* spp. could reduce blood-glucose levels in diabetics[1,2], buffer effects of organic pesticides on the liver[3], hinder brain damage from glucose and oxygen deprivation[4], and even be used for heavy metal removal in contaminated areas[5]. So other than just for their ornamental, traditional and other socioeconomic roles, species of *Opuntia* may be incredibly useful in modern medicine and even for bioremediation!

Delineation of the genus *Opuntia* has been drastically modified in recent years. The subgenus *Cylindropuntia*, for example, has been raised to generic status as have other genera such as *Consolea* and *Nopalea* in some treatments[6]. Phylogenetic analyses (i.e., analyses used to determine evolutionary history) of the genus (sensu lato) have shown in some cases that these genera are evolutionarily cohesive (for instance, *Cylindropuntia*) and so are more closely related to one another than to other taxa. However, other genera may be better recognized as part of *Opuntia*, since they are more or less derived from within the genus (e.g., *Nopalea*)[7]. Recognizing genera such as *Nopalea* may be reflecting an artificial human construct instead of true evolutionary history and relationships among taxa. Needless to say, generic issues within this group are not yet fully resolved.

BELOW *Euphorbia davyi, Ornithogalum* species nova, and *Rabiea albipuncta* photographed growing together on a dolomite outcrop west of Malopo Oog, Northwest Province.

I am interested in the genus primarily from an evolutionary and systematic point of view, especially at the level of species. Species limits within *Opuntia* can be problematic, since the group as a whole is depauperate in good morphologically defining characters. There apparently is frequent hybridization among taxa leading to a wide variety of phenotypic combinations intermediate between better defined taxa. They are highly morphologically plastic in response to environmental constraints, and on top of it all they make poor herbarium

specimens and are only occasionally collected in the first place. These problems lead to the over description of species that may not actually exist in nature, but this also leads to ignoring many taxa that may need to be recognized, which are hard to tell apart from true intraspecific, morphological polymorphism. It could be considered a somewhat daunting task to figure out the differences in true species, their hybrids and potentially just a lot of environmentally induced morphological variation.

As if things were not complicated enough within the genus, polyploidy (genome duplication) also is common in the genus[8]. These are cases where taxa may have three times or more chromosomes than a diploid taxon and may be derived from genome duplication within the same species (autopolyploid) or from a hybridization event between two different species that have undergone subsequent genome duplication (allopolyploid). Polyploidy in *Opuntia* is most commonly inferred to be the result of unreduced gametes produced through meiosis[8], so gametes may be diploid or triploid instead of haploid for instance and so would produce polyploid offspring, if fertilization is successful. Polyploidy can lead to direct speciation, since oftentimes polyploids and their diploid progenitors are reproductively isolated or incompatible. In fact, polyploidy is often referred to as an undisputed means by which species may arise sympatrically (growing together). This is a very exciting area of research and may have had an enormous effect on the evolution and diversification of the genus *Opuntia*

At the moment I am working on species delimitation within a small group of the overall genus, known as the *Opuntia humifusa* (Raf.) Raf. complex, but specifically am focusing on eastern North America effectively covering the range of what is typically considered to be one species, *O. humifusa* Within this range there are numerous taxa that have been separated as species by other taxonomists in the past. This is especially evident in the southeastern United States (see Snow[9] for a wonderfully detailed history of the Cactaceae of the southeastern United States). To determine species boundaries within this group I am using a variety of methods from cytogenetics (study of chromosomes) to molecular phylogenetics (use of DNA characters to determine evolutionary history). It is difficult to understand how to delimit species without understanding their evolutionary history. Taking this into consideration I will produce a phylogeny of the group based on DNA data and then use morphological, cytological, biogeographical, and ecological information to understand further differences among taxa, which will provide evidence for delimiting species boundaries. From there I will be able to decide whether or not previously recognized taxa of the *O. humifusa* complex actually deserve recognition.

So far I have collected all of the taxa within the *O. humifusa* complex currently recognized or previously recognized by taxonomists and have counted chromosomes of all of those. Since the current distribution of the *O. humifusa* complex covers most of the United States, there has been a considerable amount of traveling involved in this project. I have made collections through parts of the Midwest, the Northeast and most of the southeastern United States. I also have made collections in the Caribbean and the southwestern United States to include comparative taxa, which may also be closely related to or even be nested within the *O. humifusa* complex. In the following paragraphs, I will outline some of those taxa seen and/or collected on my recent trips.

TOP LEFT *Rabiea albipuncta* in flower in the late afternoon, photographed at about 16:30. **TOP RIGHT** The deciduous leaves of *Euphorbia davyi* turn a rich pinkish brown just before they are shed in the late winter. This photograph was taken west of Malopo Oog on 9 July 2009. **BOTTOM LEFT** The leaf blade of the undescribed *Ornithogalum* pressed onto a flat dolomite rock. The tip has started to wither, a typical habit of this species in early July. **BOTTOM RIGHT** Two *Ornithogalum* nova species photographed on an outcrop of exposed dolomite, west of Malopo Oog. The leaves are cryptic, a paler version of the darker gray dolomite, in the cold light of the late afternoon, once the sunlight has slanted away.

United States, East of the Mississippi River

In June of 2008 a friend and I made the long trip from Gainesville, Florida, through Ohio, Michigan, and Wisconsin, and back down through Indiana, Kentucky, and Tennessee. We found numerous populations of *Opuntia. Opuntia humifusa* and *O. macrorhiza* Engelm. are normally considered to occur in this area. Benson[10] identified *O. macrorhiza* from Illinois, Michigan, and Wisconsin along

with *O. humifusa.* Voss[11] stated that one specimen (on different sheets) from Michigan was identified by Benson as both *O. humifusa.* and *O. macrorhiza*. This further exemplifies errors that can be made when relying on herbarium specimens of *Opuntia,* but also illustrates the confusion among this group of taxa and species delimitation. In Michigan populations exist that demonstrate characters of *O. macrorhiza* and what is traditionally called *O. humifusa.* Populations of what mainly look like *O. macrorhiza* typically are found in Wisconsin, but populations with different combinations of characters can be found. In Ohio, Kentucky, Indiana and Tennessee most populations consist of what I have been recognizing as *O. cespitosa* Raf.[12]. These plants have dark green to glaucous pads when young, long white spines (up to 3 per areole, but mostly 1-2), which are castaneous-colored at the base when immature, and yellow flowers with red to orange-red centers. Flowers can be almost completely yellow with only slightly red centers. *Opuntia cespitosa* generally is considered synonymous with *O. humifusa.*, but it has numerous morphological characters that separate it from that taxon. Part of my PhD project will be to determine whether or not *O. cespitosa* should be treated as a different species from *O. humifusa.*

From late May to early June 2009 my father and I made the journey from Gainesville, Florida, to Cape Cod, Massachusetts, on the hunt for "typical" *O. humifusa.* in order to nail down the distribution of this species and determine whether or not there was any overlap with populations of *O. cespitosa*. Since the typification of *O. humifusa.* was made by Leuenberger[13] from a specimen by Wherry collected in Pennsylvania, this species would then be restricted to the eastern United States with populations ranging from Massachusetts, south along the Appalachian Mountain chain and into the southeastern United States. This of course would be the case only if *O. cespitosa* is recognized as a distinct species. The flowers of *O. humifusa.* are completely yellow, and plants typically are nearly spineless, unlike most *O. cespitosa*. Spine, pad and glochid color also differ between these two taxa. *Opuntia humifusa.* is abundant throughout the area we surveyed and can be found growing on limestone, granite, slate, or just purely sandy soils.

We found many populations of *O. pusilla* (Haw.) Haw. mostly along the coast, although some populations were found inland inhabiting granite outcrops and oftentimes growing sympatrically with *O. humifusa.* There were no signs of hybridization at these sites, although hybridization has been suggested to occur between *O. humifusa.* and *O. pusilla*[9,10]. *Opuntia pusilla* is a relatively common component of stabilized dune systems along coastal areas in the Southeast, but in some instances may be found much farther inland from the coast[12]. This species easily disarticulates at the nodes and

TOP *Opuntia cespitosa* growing over a limestone outcrop in Kentucky.
BOTTOM *Opuntia* cf. *macrorhiza* plant from Michigan growing in sand.
Both are shown in flower having yellow tepals, which are basally tinged red, although modestly so on the *O.* cf. *macrorhiza* material.

TOP LEFT *Opuntia humifusa.* in Virginia growing over slate.
TOP RIGHT *Opuntia pusilla* growing on granite outcrops in South Carolina and sandy soils along the North Carolina coast (**BOTTOM**).

has strongly retrorsely-barbed spines, so it commonly propagates itself asexually.

We should be on high alert!

Cactoblastis cactorum Berg., the cactus moth as it is called, has been known from the southeastern United States since 1989[14] and now has been found as far east as Louisiana[15]. This moth, originally from Argentina, is considered by many to be a poster child for biological control. It was released in the early 20th century into Australia and subsequently wiped out vast populations of introduced, invasive prickly pear. Later this moth was released into Hawaii, South Africa, the Mediterranean region, and eventually into the Greater and Lesser Antilles to control native prickly pear that were considered nuisances by the local governments and people. This led to another case of biological control gone wrong, as the moth now was destroying native populations of prickly pear and eventually ended up in the Florida Keys. There

ABOVE Rabiea albipuncta clings tenaciously to life with the upper portion of its root fully exposed in a vertical fissure running down a large dolomite rock.
BELOW The stubby branches of Euphorbia davyi are typical of this species west of Malopo Oog.

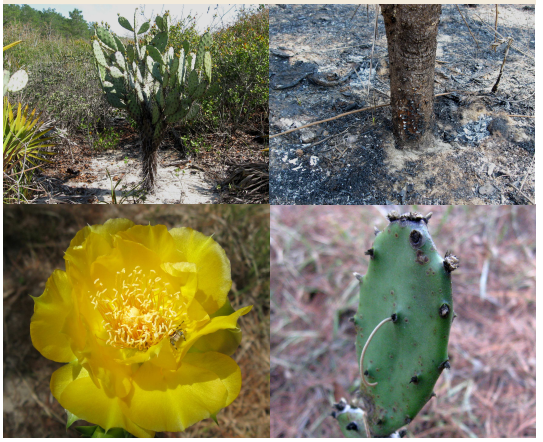
it began feeding on the extremely endangered Consolea *corallicola* (syn. *Opuntia corallicola* Small) and several other native species (e.g., *O. cubensis* Britton & Rose, *O. stricta* (Haw.) Haw., *O. triacantha* (Willd.) Sweet). From the Keys this moth has spread northward and now feeds on all species of *Opuntia* found mainly along the coast in most states through Louisiana, but can be found all throughout the state of Florida, which has several potentially endemic species of *Opuntia* (see below). This places many of our native prickly pear species at risk, as *C. cactorum* can greatly diminish population size and potentially species' reproductive output from year to year by continuously breaking down sexually mature plants leaving them unable to reproduce sexually.

Southeast

For the past few years my collecting efforts have been focused on the southeastern United States. This area has been the cause for much confusion in the taxonomy of the genus *Opuntia* for the past 180 years (since CS Rafinesque), and the issues underlying these problems are not as yet settled. Numerous species were described from Florida alone, mostly by JK Small in the early 20th century. He obviously saw enormous variation in the morphology and distribution of taxa in this area and attributed this variation as proof of different species. Later authors, although recognizing this extreme variation, have not considered that so many different species should be recognized. Recent work based on molecular, morphological and cytological data (Majure unpub. data) is suggesting that some of the species recognized by JK Small may actually be good species. Ward[16] has recently produced a key for Floridian species of *Opuntia* and recognizes several of Small's species, but his work is merely based on anecdotal observations and no analyses of any kind. More work is absolutely necessary in order to properly delimit species and provide up to date nomenclatural changes of taxa in Florida and the southeastern US in general. The following are a series of several taxa found in Florida, which currently may or may not be recognized. Some of these taxa are part of the *O. humifusa* complex and others are distantly related such as *O. stricta*.

Opuntia ammophila Small is commonly found in central Florida down to Fort Pierce, the type locality. It forms large shrubs to small trees up to 1.5 m tall with large cylindrical trunks. If transplanted to slightly cooler climates such as central Mississippi and subjected to more frequent freezing temperatures, the growth form is maintained. Growth form appears to be genetically constrained and not merely an environmentally induced phenomenon. Seedlings of this species automatically produce upright growth, as opposed to the sprawling, decumbent growth produced by *O. humifusa* and other members of the complex. One major question, "Why the upright growth form"? These plants typically grow in long-leaf pine savannas that are frequently subjected to burning, or Florida scrubland habitat, so woody trunks may reduce effects of fire damage and the upright growth would place the plants above harmful flames, or the upright growth could make them more competitive in a dense scrubland habitat. *Cactoblastis cactorum* is found frequently on *O. ammophila* virtually throughout its range and can rapidly reduce large plants to a pile of rotting cladodes. They often sprout back from roots or the few cladodes that are not attacked just to be attacked again during the next cycle of oviposition, of which there may be two to three cycles in Florida.

BELOW *Opuntia ammophila* showing the commonly, heavily armed, cylindrical trunk and upright growth common to the species. The next photos show a trunk burned by a prescribed fire, and the species in flower. Also illustrated is an egg stick of *C. cactorum*, which has been laid on the glochids of *O. ammophila*.



Opuntia austrina Small is found mostly in south Florida and probably is the least well known of the Floridian segregates of *O. humifusa. Opuntia austrina* was described by JK Small in 1903 from Miami. Subsequently most of those populations have since been destroyed from development in the area. Some ambiguity surrounds this taxon, since Small considered it to be one of the most common in Florida in later publications[17], but initially considered its range to be more restricted[18,19]. He also changed his ideas about the growth form of the taxon, later on stating that the species was not just low-growing and spreading along the ground, but that it could also form small trunks and grow erect. This has led some taxonomists to subsequently lump other taxa into *O. austrina* such as *O. pollardii, O. polycarpa*, and of course that taxon into the broad concept of *O. humifusa.* This taxon can grow up to 1 m tall and has cladodes, which easily disarticulate upon applying any amount of pressure, much like *O. pusilla*.

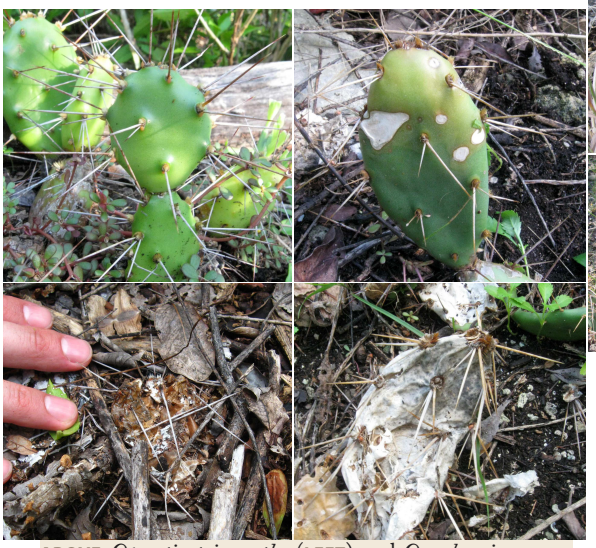
LEFT *Opuntia austrina* growing in southwest Florida. Note the smaller clones that have been produced by the continual disarticulation of joints from the larger plant.

Opuntia stricta is a common component of the coastal dune and marshland vegetation in the southeastern United States. JK Small even discovered the species growing in mangroves in the Everglades of Florida[20]. It is never found very far from the coast except for those areas where it has been planted. JK Small recognized numerous species, which since have been placed in synonymy with *O. stricta* (e.g., *O. keyensis* Small, *O. magnifica* Small, *O. zebrina* Small[10]). David Griffiths described a new species in Florida, *O. bentonii*[21], which also is now recognized as *O. stricta*. Benson divided the species into two varieties, *O. stricta* var. *stricta* and *O. stricta* var. *dillenii*. *O. stricta* var. *dillenii* is the common morphotype found throughout the Caribbean and parts of coastal, eastern Mexico such as the Yucatán Peninsula, as well as along the Atlantic coast and the Keys of Florida. *Opuntia stricta* var. *stricta* is more common along the gulf coast of the southeastern United States, although it virtually occurs throughout Florida as well. Pinkava[22] does not recognize these two varieties and from personal observation, they both are often found within the same population (see below), and the characters used to separate them (spininess) do not seem to be very stable. Benson[10] mentioned that character combinations of both varieties were commonly found in Floridian populations.

ABOVE *Opuntia stricta* and what is traditionally known as *O. dillenii* or *O. stricta* var. *dillenii*. These plants were growing side by side in beach vegetation in St. Johns County, FL.

Opuntia triacantha is a state listed endangered species in Florida, however, *O. cubensis* probably is the rarest and in most danger of extirpation from the US only occurring at one location in the Keys. This location has been affected heavily by human disturbance, recent hurricanes, and of course attack from *C. cactorum.* The site is also being invaded by a *Kalanchöe* sp., which could easily take over the Key Largo limestone outcrops where the two

species grow. Until my recent trip to the Keys with fellow scientist Mariela Pajuelo, *Opuntia cubensis* had not been seen in the Keys since Benson[10] last studied it[23]. *Opuntia cubensis* is considered by many to be of hybrid origin based on morphology, although this has never been tested, and no reliable chromosome counts exist for the species.



ABOVE *Opuntia triacantha* (**LEFT**) and *O. cubensis* (**RIGHT**) in the Florida Keys. Below each photo of the live plants are photos taken of plants that have been attacked and killed by *Cactoblastis cactorum. Opuntia cubensis* is only found in one location in the Keys and is apparently heavily affected by *C. cactorum.* Urgent work is needed to save this species from being extirpated from the US.

Southwestern United States

In August 2008 my father and I headed off to the southwest to collect numerous species for phylogenetic analyses, comparative morphological analyses and for chromosome counts. Numerous taxa were described by David Griffiths in the early 20th century but have since been placed in synonymy with *O. humifusa* or *O. macrorhiza*. These taxa were of particular interest, since they are likely to be allied to the *O. humifusa* complex (e.g., *O. allairei* Griffiths, *O. nemoralis* Griffiths). Numerous other more distantly related species of *Opuntia* were also seen during our travels.

Caribbean

In mid June my father and I traveled to the islands of St. Thomas (of the US Virgin Islands) and to Puerto Rico to look for *Opuntia repens* Bello, *O. stricta*, and *O. triacantha*. We were able to find not only *O. repens* in St.

ABOVE Several of the species of *Opuntia* seen during our travels: From left to right, top to bottom, *Opuntia macrocentra, O. polyacantha, O. macrorhiza, O. allairei, O. tortispina*, and *O. phaeacantha*.

Thomas, but also *Pilosocereus royenii* (L.) Byles & GD Rowley, *Melocactus intortus* (Mill.) Urb., *Mammillaria nivosa* Link ex Pfeiff., *Consolea rubescens* (Salm-Dyck ex DC.) Lem., and *Opuntia stricta* on the island.

With the help of local botanist, Frank Axelrod from the University of Puerto Rico, in San Juan, we surveyed a large portion of the southwestern part of the island and found numerous cacti including some incredible populations of *O. repens* in flower! We also encountered magnificent populations of Melocactus intortus and Pilosocereus royenii growing along the banks of the salt flats at Salinas del Cabo Rojo. However, we also observed the grave damage commonly seen as a result of attack by the infamous *C. cactorum*

LEFT *Pilosocereus royenii, Melocactus intortus, Consolea rubescens*, and *Mammillaria nivosa* (**RIGHT**) on the island of St. Thomas, VI. *Mammillaria nivosa*, although found in many islands of the Caribbean, apparently is rare on the island of St. John[24] and no record of it appears to exist from St. Thomas. The specimen seen could be a new record for the island.

ABOVE *Opuntia repens* is a common species found in the Caribbean. These photos were taken from Puerto Rico. In some areas this species forms large stands produced by vegetative reproduction from the disarticulation of the cladodes. Spines on the cladodes are extremely, retrorsely barbed, and so disarticulated stem segments easily attach themselves to the fur or skin of any passer-by, much like cholla species of the Southwest.

on many specimens of *O. stricta*. In several cases, entire plants were reduced to mere piles of rotting cladodes.

What next?

Taxonomically problematic species of *Opuntia* are in need of much systematic and evolutionary study throughout the range of the genus. Detailed studies of smaller subclades and species complexes within the genus *Opuntia* (inc, Parfitt[25]), will help us develop our understanding of the group at the level of species. Those species, which may be cryptic morphologically, but represent divergent evolutionary lineages, will be further illuminated through such studies and increase our knowledge of the biodiversity represented by this group. Developing a phylogenetic framework by which to infer evolutionary relationships among lineages within the genus is imperative and will allow us to diagnose species limits more effectively, which will aid in determining areas in dire need of conservation efforts to preserve this biodiversity on a finer scale. Considering current threats to natural populations of *Opuntia* such as *C. cactorum*, over collecting, and habitat loss, we need to do everything possible to secure the future of these widely beneficial and marvelous cacti.

ABOVE Notice the pile of rotting and dried cladodes of *O. stricta* and in one case a larvae of *C. cactorum* crawling out of a hole from where I removed a dead pad. Most *O. stricta* that were seen while in Puerto Rico were heavily affected by *C. cactorum* Other cactophagous larvae were seen on *O. repens*, but none of *C. cactorum*, so *O. stricta* seems to be a preferred host, at least in the areas where we saw it in Puerto Rico.

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