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DOI: 10.1007/s12228-013-9307-z

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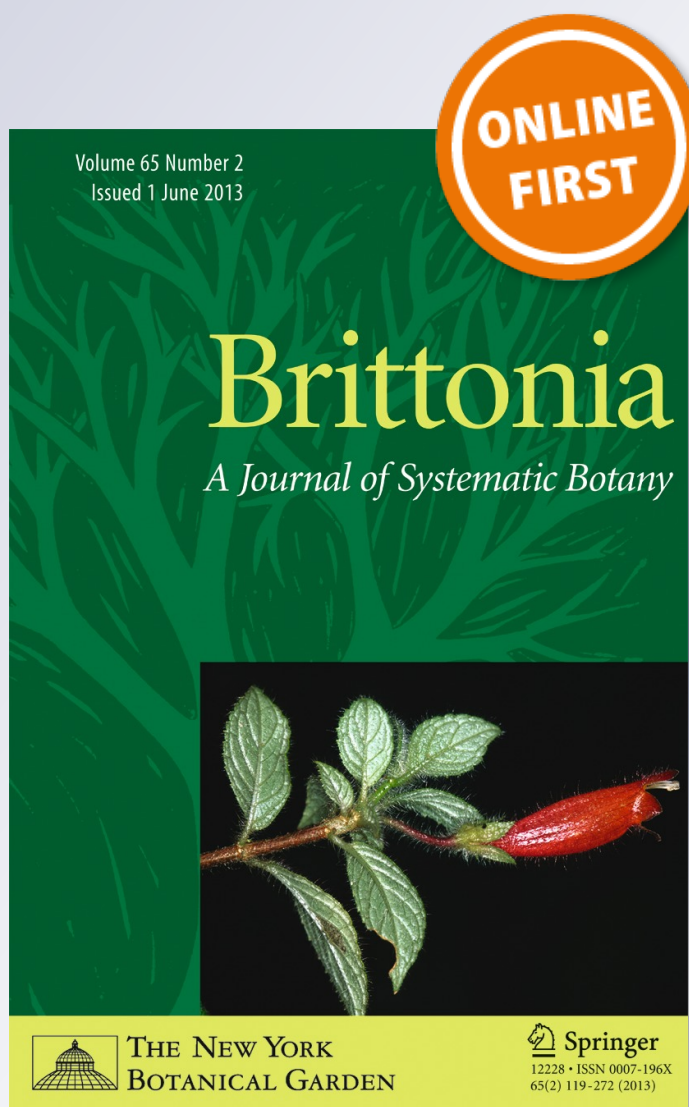
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**Brittonia**

ISSN 0007-196X

Brittonia

DOI 10.1007/s12228-013-9307-z



 Springer

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# A case of mistaken identity, *Opuntia abjecta*, long-lost in synonymy under the Caribbean species, *O. triacantha*, and a reassessment of the enigmatic *O. cubensis*

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**Abstract.** *Opuntia abjecta* and *O. militaris* have been placed in synonymy under the Caribbean species *O. triacantha* for the past 30 years. Recent molecular phylogenetic evidence has shown, however, that *O. abjecta* and *O. triacantha* are actually in two very different clades suggesting that the Floridian endemic *O. abjecta* should be recognized as a distinct species. Here, we summarize major morphological differences between *O. abjecta* and *O. triacantha*. We also include new sequence data from the rare Cuban taxon, *O. militaris*, in a molecular phylogenetic analysis to determine its relationship to *O. triacantha* and *O. abjecta*. We discuss the putative hybrid taxa *O. cubensis* and *O. ochrocentra*, which currently are treated as synonyms. We also show through analysis of morphological and molecular characters that these two taxa were derived from two independent origins from divergent maternal progenitors, confirming that *O. ochrocentra* should not be treated as synonymous with *O. cubensis*. A key is provided for identifying these taxonomically confusing taxa and their close relatives. This study emphasizes the distinctions among *O. abjecta*, *O. militaris*, and *O. triacantha* and illustrates that extreme caution must be employed when using herbarium specimens for identifying species of *Opuntia*. It also indicates that broad phylogeographic assumptions regarding species' relationships in *Opuntia* may sometimes be misleading. Hybridization and polyploidy are common in *Opuntia* and have played a role in the formation of new species in this group as well. A neotype is here designated for **O. triacantha**.

**Key Words:** Cactaceae, Caribbean, Cuba, hybridization, *Opuntia*, phytogeography.

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John Kunkel Small, Curator of The New York Botanical Garden from 1898–1934, wrote a flora for the Southeastern United States for his Ph.D. dissertation (NYBG, 1999). He produced three editions of his treatment of the southeastern flora from 1903–1933 (Small, 1903, 1913, 1933) in which he paid special attention to the cacti of the southeastern United States. Small described 16 species of *Opuntia* from Florida alone and even grew most of these taxa in Buena Vista, Florida, in a common

garden to monitor their growth habits in a “controlled” environment (Small, 1919). Two species, *Opuntia abjecta* Small and *O. ochrocentra* Small, were described from Big Pine Key, Florida (in Britton & Rose, 1923). The population of *O. abjecta* at Big Pine Key was the only population that Small mentioned in the description of the species (Britton & Rose, 1923) and was the only population known until recently (K. Bradley, Institute for Regional Conservation, pers. comm.). *Opuntia ochrocentra* was known from the

type locality and apparently further north (135 km) at Cape Romano (Small, 1933), although only specimens from Big Pine Key and Big Munson Island have ever been seen (Benson, 1982; Majure et al., 2012a).

Benson (1982) produced the beautifully illustrated and detailed, “The Cacti of the United States and Canada,” in which he placed *O. abjecta* and a Cuban species, *O. militaris* Britton & Rose, in synonymy with the Greater and Lesser Antillean species, *O. triacantha* (Willd.) Sweet. Since that publication, the name *O. triacantha* has been used, mostly without question, for material from the Florida Keys (Doyle, 1990; Pinkava, 2003; Wunderlin & Hansen, 2003, 2011; Hunt et al., 2006; Ward, 2009) and Cuba (Anderson, 2001; Hunt et al., 2006). However, some Cuban researchers still recognize *O. militaris* at the species level and consider the use of *O. triacantha* in Cuba to be erroneous (Berazain et al., 2005). Interestingly, Anderson (2001) treated *O. abjecta* as a synonym of *O. triacantha*, but did not include the Florida Keys within the geographic distribution of that species. *Opuntia abjecta* (under *O. triacantha*) is considered an endangered species in Florida (Coile & Garland, 2003) and thought to represent the northernmost population of *O. triacantha* in North America, occurring as a northern disjunct from the nearest population of *O. triacantha* in southeastern Cuba (i.e., *O. militaris*; Benson, 1982; Fig. 1).

*Opuntia cubensis* Britton & Rose was originally described from the Guantánamo Bay area of Cuba (Britton & Rose, 1912; Fig. 1) and was thought to be a hybrid between *O. militaris* and *O. dillenii* (Ker-Gawl.) Haw. (Britton & Rose, 1920). Benson (1982) later determined that the species from the Florida Keys, *O. ochrocentra*, was synonymous with *O. cubensis*, although Britton and Rose (1920) had considered *O. ochrocentra* to be a close relative of *O. dillenii*. Most authors have followed Benson’s work and also included *O. ochrocentra* within *O. cubensis* (Anderson, 2001; Pinkava, 2003; Wunderlin & Hansen, 2003, 2011; Hunt et al., 2006; Ward, 2009).

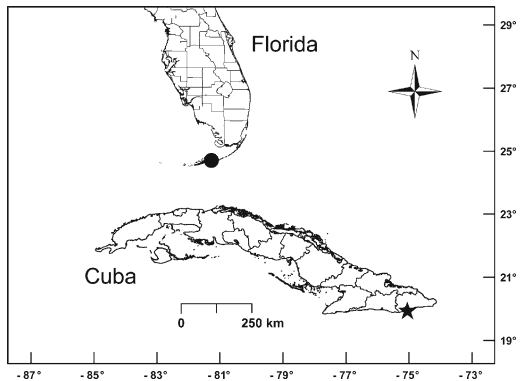


FIG. 1. Distribution of diploid *O. abjecta* and *O. ochrocentra* (circle) in the Florida Keys, and *O. militaris* and *O. cubensis* in Guantánamo, Cuba (star).

Phylogenetic analyses of *Opuntia* (Majure et al., 2012b) and morphological studies of *Opuntia* for a monograph of the *Humifusa* clade (Majure unpubl. data) suggest that *O. abjecta* is a different species and evolutionary divergent from *O. triacantha* and another of its synonyms, *O. militaris*. Furthermore, Majure et al. (2012b) determined that material of “*O. cubensis*” from the Florida Keys was likely derived from hybridization between *O. abjecta* and most probably *O. dillenii*, instead of *O. militaris* from Cuba. We expand upon those previous analyses here with the inclusion in our phylogeny of *O. cubensis* and *O. militaris* from Cuba. We also present a detailed morphological examination of *O. triacantha* s.l. (i.e., *O. abjecta*, *O. militaris*, *O. triacantha*) and *O. cubensis* s.l. (i.e., *O. cubensis*, *O. ochrocentra*) to provide a clear understanding of why *O. abjecta*, *O. triacantha*, *O. cubensis*, and *O. ochrocentra* should not be considered conspecific. We then discuss the relationship of *O. militaris* to *O. triacantha* from a morphological and phylogenetic perspective and neotypify *O. triacantha*.

## Materials and methods

Previously gathered data from the plastid intergenic spacers, *atpB-rbcL*, *ndhF-rpl32*, *psbJ-petA*, *trnL-F*, the plastid genes, *ycf1* and *matK*, the nuclear ribosomal internal transcribed spacers (ITS; White et al., 1990) and the nuclear gene *ppc* (Majure et al., 2012b) were used for our phylogenetic

analyses. The PCR specifications for each DNA region used are covered in Majure et al. (2012b). We increased our sampling to include *O. cubensis* and *O. militaris* from Cuba. Live material of *O. cubensis* s.str. was obtained from field-collected (Cuba; *Areces s.n.*) material cultivated at Gemini Botanical Garden, Florida. Although to our knowledge, no recent specimens of *O. militaris* exist, we were able to extract (using a modified CTAB method; see Majure et al., 2012b) and amplify DNA from an herbarium specimen collected in 1951 (*R.N. Jervis 1033*; MICH) from the Guantánamo Bay area, the type locality for the taxon (see Appendix 1). Both tepal and epidermal tissue produced amplifiable DNA, although tepal tissue yielded DNA that was less degraded than that from epidermal tissue. *Opuntia triacantha* also was sampled from an herbarium specimen (*Mori et al. 22693*; NY), as we did not have live material of that species. We cloned ITS and *ppc* PCR products of polyploid *O. cubensis* ( $2n=4x=44$ ; Majure et al., 2012c) and *O. ochrocentra* ( $2n=5x=55$ ; Majure et al., 2012a) using the Stratagene cloning kit (Stratagene, La Jolla, CA) and sequenced eight clones of each using bacterial primers (T3–T7) from the kit. We sampled one individual from the type population of *O. abjecta* (diploid;  $2n=2x=22$ ; Majure et al., 2012a) and *O. ochrocentra* (pentaploid;  $2n=5x=55$ ) from Big Pine Key, as well as available herbarium material for morphological work, including the type specimens of *O. abjecta*, *O. cubensis*, *O. militaris*, and *O. ochrocentra*.

We also included diploid members of the *Humifusa* clade (i.e.,  $2n=2x=22$ ), the closely related *Macrocentra* and *Scheerianae* clades, and members of the *Nopalea* clade (sensu Majure et al., 2012b), to which *O. triacantha* is morphologically most similar (e.g., *O. caraccasana* Salm-Dyck, *O. guatemalensis* Britton & Rose, *O. jamaicensis* Britton & Harris). *Opuntia retrorsa* Speg. and *O. macbridei* Britton & Rose from South America were used as outgroups based on results from Majure et al. (2012b).

GenBank accession numbers for new sequences generated for *O. cubensis* and *O. militaris* are provided in Appendix 2. Sequences were edited in either Sequencher

4.2.2™ (Gene Codes, Inc., Ann Arbor, MI) or Geneious Pro™ 5.1 (Biomatters Ltd., Auckland, NZ). Sequence alignment was carried out in Muscle (Edgar, 2004), and the alignment adjusted manually in Se-AL v2.0 (Rambaut, 2007). All gaps introduced during alignment were coded as missing data.

Combined nuclear and plastid regions were analyzed for all putative diploid taxa (see Majure et al., 2012b), including *O. militaris*, using maximum likelihood (ML) in RAxML (Stamatakis, 2006) conducting 10,000 nonparametric bootstrap pseudoreplicates under 25 rate categories and implementing the GTR+ $\Gamma$  model of molecular evolution. Data were not partitioned, as preliminary trials in RAxML suggested the use of the GTR+ $\Gamma$  model of molecular evolution for all loci. As in Majure et al. (2012b) analyses of diploid taxa only were conducted initially, as polyploid taxa may have been derived from reticulate evolution and thus may not be appropriate for inclusion in combined analyses of nuclear and plastid data sets. Therefore, separate plastid, ITS, and *ppc* data sets containing polyploid *O. cubensis* and *O. ochrocentra* were analyzed using the same methods.

Morphological characters (e.g., cladode shape, flower color, glochid color, growth form, spine color/development pattern) were scored, and measurements were taken from herbarium specimens of *O. abjecta*, *O. cubensis*, *O. militaris*, *O. ochrocentra*, and *O. triacantha*, and from live material of *O. abjecta*, *O. cubensis*, and *O. ochrocentra*. As mentioned above, no live material of *O. militaris* or *O. triacantha* was available for study. We also compared *O. militaris* and *O. triacantha* to herbarium specimens of a closely related and morphologically similar Caribbean species, *O. repens* Bello (see Majure et al., 2012b).

## Results

### PHYLOGENY

**Analysis of diploids using combined plastid and nuclear data.** *Opuntia triacantha* s.l. as currently circumscribed (i.e., including *O. abjecta* and *O. militaris*) was resolved in

three places in our analysis of diploid taxa. *Opuntia abjecta* was nested in the southeastern United States subclade of the *Humifusa* clade (bs=72), and *O. triacantha* s.str. was closely related to the Caribbean and Central American taxa, *O. caracassana*, *O. jamaicensis*, and *O. guatemalensis* of the *Nopalea* clade (Fig. 2; bs=92). *Opuntia militaris* was recovered within the *Nopalea* clade as well, although it was sister to *O. caracassana* (bs=70) and not closely related to *O. triacantha* s.str. (Fig. 2).

**Analysis of polyploids using separate plastid and nuclear data sets.** *Opuntia cubensis* s.l. was resolved in three places in our phylogenies. *Opuntia ochrocentra* (from the Florida Keys) was nested within the *Humifusa* clade using plastid data. In contrast, *Opuntia cubensis* s.str. (from Cuba) was nested in the *Nopalea* clade using plastid data (Fig. 3A). Two ITS copy types were discovered for both *O. cubensis* s.str. and *O. ochrocentra* after excluding putative recombinants. One ITS haplotype of *O. ochrocentra* was resolved in

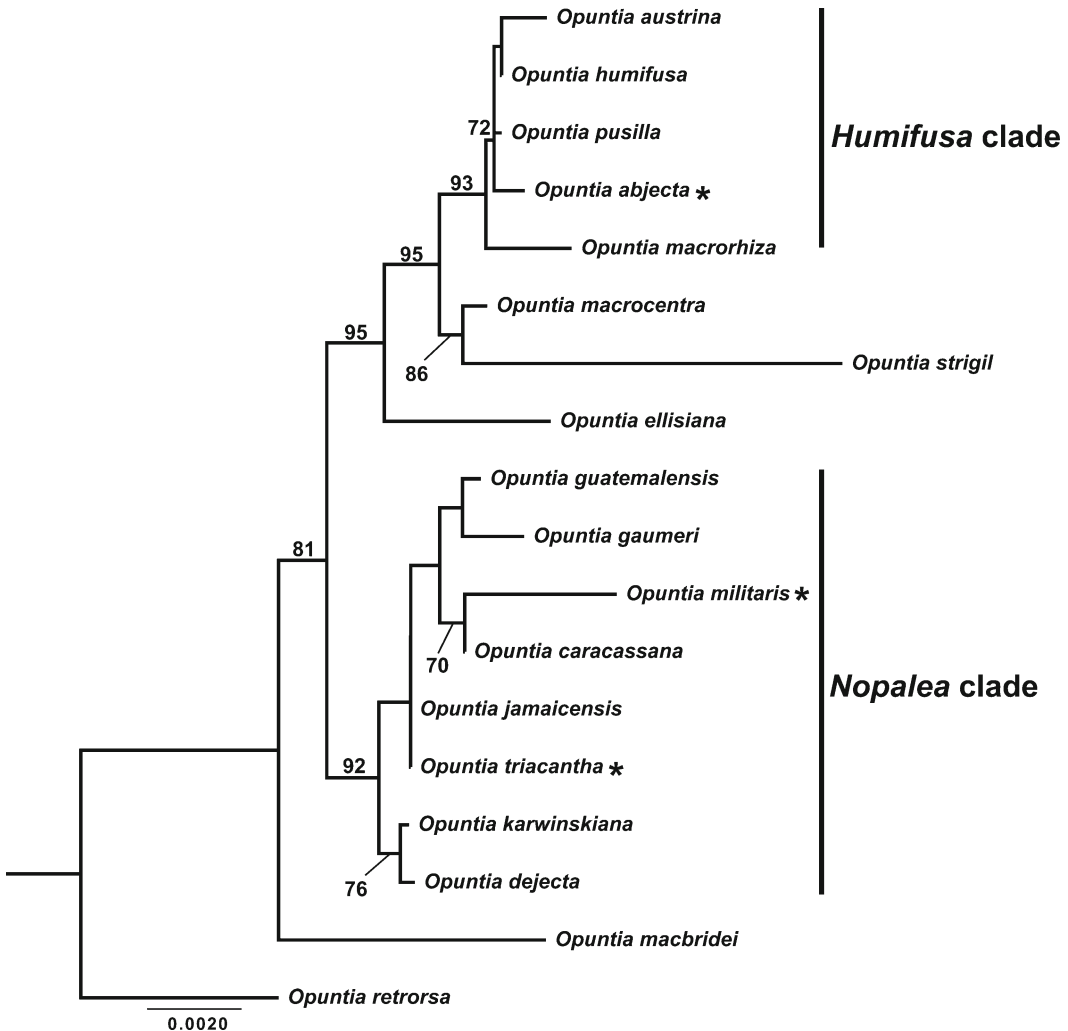
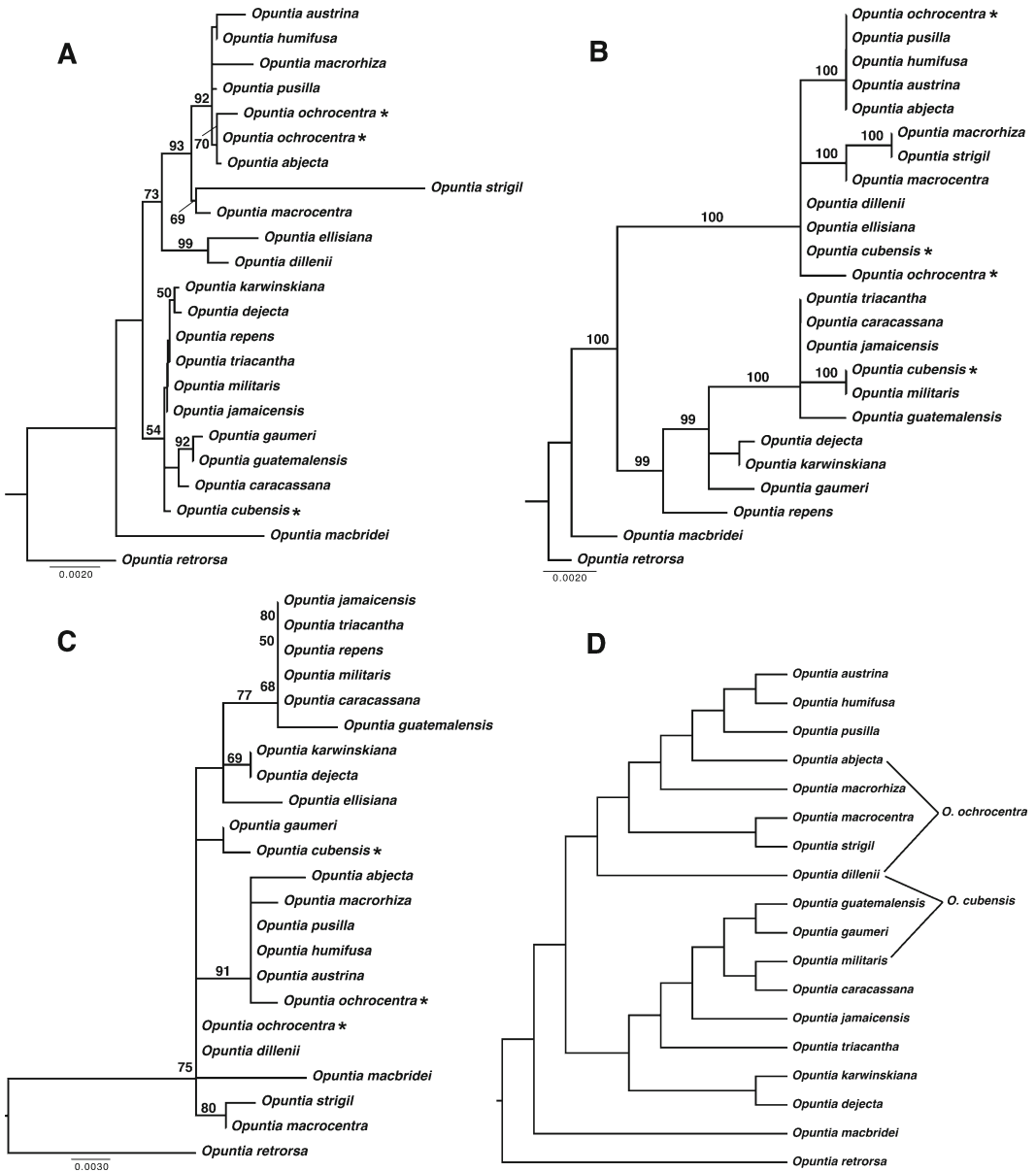


FIG. 2. ML phylogeny of diploid species from combined analysis. *Opuntia triacantha* is resolved in a different clade (i.e., the *Nopalea* clade) from *O. abjecta* (*Humifusa* clade). *Opuntia militaris*, though nested within the *Nopalea* clade, is not resolved as sister to *O. triacantha*, with which it is currently placed in synonymy. *Opuntia abjecta*, *O. militaris*, and *O. triacantha* are denoted by asterisks. Bootstrap values are indicated above branches.



**FIG. 3.** A–C. Most likely topologies from ML analyses of **A**) plastid, **B**) ITS, and **C**) *ppc* data including the putative hybrid taxa *O. cubensis* and *O. ochrocentra* (indicated with asterisks). **D**) The separate origins of *O. cubensis* and *O. ochrocentra*. *Opuntia ochrocentra* was derived maternally from *O. abjecta*, while *O. cubensis* was derived maternally from *O. militaris*. *Opuntia dillenii* represents the putative paternal lineage for both species. Bootstrap values are given above branches in A–C.

the *Humifusa* clade and another was unresolved in a grade containing members of the *Scheerianae* clade, which contains one of the putative parents of *O. ochrocentra* (based on morphology and sympatry), i.e., *O. dillenii*. One ITS haplotype of *O. cubensis* s.str. was

resolved within the *Nopalea* clade, as closely related to *O. militaris* (i.e., one haplotype was nearly identical to *O. militaris*), and the position of the other haplotype was unresolved, as was *O. ochrocentra*, within a grade containing members of the *Scheerianae* clade (Fig. 3B).



Two copy types also were found in *ppc* clones of *O. ochrocentra*, which were placed in the *Humifusa* clade and in a grade of other taxa (*ppc* data provide very poor resolution at the clade level); however, only one copy type of *ppc* was recovered from *O. cubensis*, which shared synapomorphies only with members of the *Nopalea* clade (Fig. 3C). Thus, based on DNA sequence data, *O. cubensis* s.l. was derived from two separate origins. *Opuntia ochrocentra* was most likely derived from hybridization between *O. abjecta* and *O. dillenii*, while *O. cubensis* s.str. was derived from hybridization between *O. militaris* and *O. dillenii* (Fig. 3D).

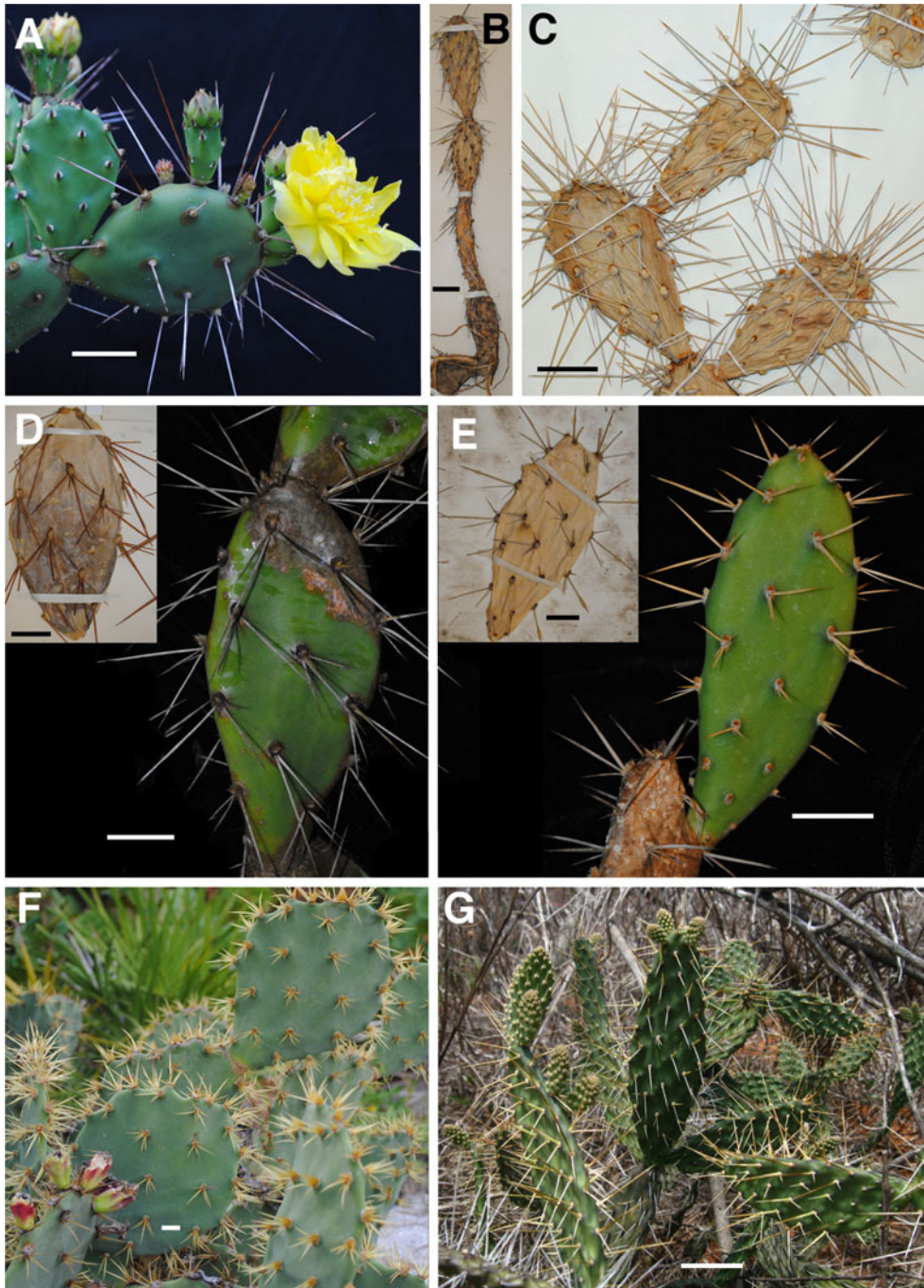
#### MORPHOLOGY

***O. abjecta* vs. *O. triacantha*.** *Opuntia abjecta* is strikingly different from *O. triacantha* in growth form, spine color and arrangement of spines, flower bud shape, flower color, and color of areolar trichomes and glochids (Fig. 4A–C). *Opuntia abjecta* is a small spreading-ascending shrub with basally disposed, radiating branches that reach up to 30 cm in height. *Opuntia triacantha* is a small, erect to semi-erect shrub generally with a central, semi-cylindrical trunk (Fig. 4B), much like that of other close relatives (i.e., *O. caracassana*, *O. jamaicensis*, *O. repens*), and reaches heights of up to 40 cm or more. The spines of *O. abjecta* are strongly retrorsely barbed like those of *O. triacantha*, but they are a lustrous, dark reddish-brown during development, instead of dull yellow as in *O. triacantha* (Fig. 4A, C). Spines of *O. abjecta* mature to bright white instead of a pale white color. The spines of both taxa become dark gray in age. Up to three spines are produced from the areoles of terminal cladodes of *O. abjecta*, and these are usually all in the same plane of symmetry (e.g., all spreading, all reflexed, etc.). Up to six spines can be produced from the areoles of terminal cladodes of *O. triacantha*, and they are in two planes of symmetry with the central spine typically divergent (porrect at  $\geq 70^\circ$  angle) from the lower spines produced (Fig. 4C), as in the closely related species, *O. repens* and *O. caracassana*. The spines of *O. triacantha* are also shorter on average than those of *O. abjecta* (3.7 cm vs. 4.4 cm). *Opuntia abjecta* has a rounded flower bud apex, while *O. triacantha*

has an acute flower bud. *Opuntia abjecta* has entirely lemon-yellow inner tepals, while *O. triacantha* has sulfur-yellow inner tepals that are often tinged pink along the midrib. Tepals are obovate in *O. abjecta* with a rounded to emarginate apex and mucronate tip, and oblong to obovate in *O. triacantha* with a rounded apex, most commonly without a mucro. The areolar trichomes of *O. triacantha* are yellowish, while the areolar trichomes of *O. abjecta* are white. *Opuntia abjecta* has stramineous glochids on younger cladodes, while *O. triacantha* has bright yellow to yellow-orange glochids on younger cladodes. In general, *O. abjecta* may be differentiated from *O. militaris* by the same features used to distinguish it from the morphologically similar *O. triacantha*; however, as indicated in the next section, *O. militaris* and *O. triacantha* are also morphologically distinct.

***O. militaris* vs. *O. triacantha*.** *Opuntia militaris* is morphologically similar to *O. triacantha*, although in general *O. militaris* is smaller and more delicate than *O. triacantha*. Like *O. triacantha*, *O. militaris* grows erect with one central trunk eventually producing a small, branching shrub to 30 cm high (Britton & Rose, 1920). The flower color of *O. militaris* and *O. triacantha* is similar, with both having sulfur-yellow inner tepals that may be tinged pink along the midvein and lacking a strong mucronate tip. Flower buds in both species are acute, as in other species of the *Nopalea* clade. The average cladode length and width of *O. militaris* contrasts with *O. triacantha* (6.2 × 2.8 cm for *O. militaris* and 7.8 × 3.9 cm for *O. triacantha*). Spine lengths and diameters also are smaller in *O. militaris*, as compared to *O. triacantha* (2.5 cm × 0.5 mm vs. 3.7 cm × 0.76 mm). *Opuntia triacantha* may have up to six spines per areole, and *O. militaris* may have up to four spines per areole, although difference in spine number needs to be explored further in the field, as it can be a highly variable character in *Opuntia*. *Opuntia militaris* also exhibits the porrect spines, yellow areolar trichomes, and yellow glochids of *O. caracassana*, *O. repens*, and *O. triacantha*.

***O. cubensis* vs. *O. ochrocentra*.** *Opuntia ochrocentra* from the Florida Keys and *O.*



**FIG. 4.** Morphological characters of *O. abjecta*, *O. triacantha*, *O. cubensis*, *O. ochrocentra*, *O. dillenii*, and *O. repens*. **A.** *O. abjecta* morphological features (Majure 3908). **B.** *O. triacantha* erect trunk (Duss 3071). **C.** *O. triacantha* morphological features (Smith 10442). **D.** *O. ochrocentra* cladodes, first year (from type: Small s.n.) and living second year growth (Majure 3907). The first year cladodes show yellowish spines as in *O. dillenii*, and the second year cladodes show spines turning white-gray and deflexing in age. **E.** *O. cubensis* type collection (Britton 2064) and living, young cladode and spines (Areces s.n.). **F.** *O. dillenii* spines (Buckaneer State Park, FL). **G.** *O. repens* spines (Majure 3839). Scale bars=2.5 cm. Photos taken by Lucas C. Majure.

*cubensis* from Cuba share morphological features suggesting that *O. dillenii* could be one of the parents of both. *Opuntia dillenii* occurs throughout the Caribbean region and in parts of the southeastern United States and is sympatric with both *O. ochrocentra* (Majure pers. obsv.) and *O. cubensis* (Britton & Rose, 1920). This similarity likely led Britton and Rose (1920) to include these taxa in the same series as *O. dillenii*, i.e., *Opuntia* series *Dillenianae*. In both taxa the spines are produced in a stellate to fasciculate pattern from the areoles (Fig. 4D, E); they also produce radial spines that are basally flattened and often spreading, as in *O. dillenii* (Fig. 4F). Most developing radial spines of *O. ochrocentra* are lustrous yellow to yellow-orange as in *O. dillenii*, but central spines are produced that are lustrous red, to mottled or banded red-brown, as in the developing spines of *O. abjecta*. Although the spines of *O. cubensis* are produced from the areole as in *O. dillenii*, the young developing spines are dull yellow to creamy white as in *O. militaris* and *O. triacantha* and the central spines may be porrect as well (like *O. militaris*, *O. triacantha*, *O. repens*, etc.; Fig. 4C, E, G). Cladodes of *O.*

*ochrocentra* are on average larger than those of *O. cubensis* (15.6×7.5 cm vs. 12.3×4.8 cm) and produce longer central spines (5.3 vs. 3.1 cm long). Average central spine diameters are nearly the same for both taxa (1.05 vs. 1.01 mm). The central spines of both *O. ochrocentra* and *O. cubensis* are generally round in cross section and may or may not be twisted at the base, as in both *O. abjecta* and *O. militaris*. Mature spines of *O. ochrocentra* turn white and then dark gray in age and become strongly deflexed, while mature spines of *O. cubensis* turn white and then light brown in age and do not deflex. Both species also have moderately easily disarticulating, terminal cladodes, as those of their putative maternal species, *O. abjecta* and *O. militaris*.

Below we provide a key to distinguish *O. abjecta*, *O. militaris*, *O. triacantha*, *O. cubensis*, and *O. ochrocentra*. We also include the widespread species *O. repens*, a close relative of and morphologically similar to *O. triacantha*, as shared morphological characters and sympatry of those two taxa often lead to misidentifications. This artificial key is based on both live material and herbarium specimens.

**Key to *O. abjecta*, *O. ochrocentra*, and selected Caribbean taxa**

1. Spines disposed from areoles in stellate pattern, radiating in all directions, or fasciculate, radial spines prominently flattened dorsiventrally at base, central spines round or twisted in cross section.
  2. Immature spines dull yellow to cream or dull light brown; mature spines stout, 2.2–4.2 cm long; central spine 1, round to flattened in cross section; Cuba . . . . . *O. cubensis*
  2. Immature spines bright, lustrous yellow to orange-red or mottled yellow and reddish-brown banded; mature spines delicate, 4.7–5.8 cm long; central spines ≥1, round or twisted in cross section; Florida Keys . . . . . *O. ochrocentra*
1. Spines disposed from areoles in same plane, or with some porrect, radial spines round in cross section to merely twisted at base, central spines (when present) as radials.
  3. Mature plants caespitose; stems numerous; inner tepals entirely yellow; immature spines dark red-brown to mottled red-brown and white; spines mostly spreading from areoles in 1 plane (at ≤45°); flower bud apices round; cladodes rotund to obovate; glochids stramineous; Florida Keys . . . . . *O. abjecta*
  3. Mature plants not caespitose (occasionally forming dense patches from disarticulation of terminal cladodes); stems solitary; inner tepals yellow to yellow-green, often tinged with pink along the tepal midvein; immature spines dull to lustrous-yellow or creamy-white; spines usually spreading with 1–2 large porrect and 1–numerous, deflexed spines (at ≥70°); flower bud apices acute; cladodes narrowly elliptic, oblong, to obovate; glochids yellow; Greater and Lesser Antilles.
    4. Cladodes sub-cylindrical to flat, narrow. 3.3–8.3 cm long, 1.1–2.9 cm wide; immature spines lustrous-yellow, flexible (delicate); 0.32–0.55 mm in diameter, 2.3–3.9 cm long; plants delicate; Greater and Lesser Antilles . . . . . *O. repens*
    4. Cladodes flat, wide, 4.7–10.9 cm long, 2.3–5.8 cm wide; immature spines dull yellow, stout, 0.38–0.84 mm in diameter, 1.4–6.2 cm long; plants robust; Greater and Lesser Antilles.
      5. Cladodes 5–10.9 cm long, 2.4–5.8 cm wide; spines 2.3–6.2 cm long and 0.64–0.88 mm wide; Greater and Lesser Antilles . . . . . *O. triacantha*
      5. Cladodes 4.7–8.5 cm long, 2.3–3.5 cm wide; spines 1.4–3.1 cm long and 0.38–0.62 mm wide; Cuba *O. militaris*

### Discussion

#### *OPUNTIA ABJECTA* VS. *O. TRIACANTHA*

Chromosome counts reveal that the type population of *O. abjecta* is diploid, while other material from the Florida Keys is tetraploid (Majure et al., 2012a). Also, material cultivated at Big Pine Key in a resident's yard obtained from Montgomery Botanical Center was tetraploid (*LCM 3318*; Majure et al., 2012a), suggesting that another population of *O. abjecta* may exist somewhere in the lower keys. The population of *O. abjecta* on Long Key is morphologically identical to that of other tetraploid material and thus is most likely tetraploid as well (cladodes of tetraploid *O. abjecta* are typically more elliptical and larger than those of the diploid population). Spencer (1955) reported a diploid count for *O. triacantha* from Puerto Rico; however, we have not been able to confirm this count. No chromosome counts have been reported for *O. militaris*, however, considering our results here and the ploidy of *O. cubensis* (tetraploid; Majure et al., 2012c) and *O. dillenii* (hexaploid; Majure et al., 2012a, c), the species is most likely diploid.

Benson (1982) likely placed *O. abjecta* in synonymy with *O. triacantha* because these taxa share several morphological features, such as disarticulating cladodes, and terminal cladodes that often exhibit 2–3 spines per areole. Spines of both species overlap in length and diameter, and cladode shapes and sizes slightly overlap, as does the height of both species. Benson (1982) also worked mostly from herbarium specimens, in which many taxonomically useful characters are lost in *Opuntia*. *Opuntia abjecta* is only found in the Florida Keys and was considered merely a northern extension of the Caribbean *O. triacantha* (Benson, 1982; Pinkava, 2003). This southern Florida/Caribbean disjunction is very common for numerous taxa (Wunderlin & Hansen, 2003). In Cactaceae alone, *Acanthocereus* (Engelm. ex A. Berger) Britton & Rose, *Consolea* Lem., *Harrisia* Britton, and *Pilosocereus* Byles & G.D. Rowley occur both in Florida and on neighboring Caribbean Islands (Acevedo-Rodriguez, 1996). *Opuntia triacantha*

is commonly found on “coastal rocks” (Britton & Rose, 1920); *Opuntia abjecta* likewise is found on limestone outcrops (Key Largo Limestone) within 0.5 km or less of the ocean (Benson, 1982; L.C. Majure, pers. obs.). Additionally, the misidentification of the interspecific hybrid presumably involving *O. triacantha*, *O. ochrocentra* (as *O. cubensis*), added further evidence for the northern disjunct distribution of *O. triacantha* in the Florida Keys (Benson, 1982).

Coincidentally, *O. austrina* Small, a species endemic to the peninsular Florida scrub is much more similar morphologically to actual *O. triacantha* than is *O. abjecta*. *Opuntia austrina* forms treelets to large shrubs and generally is characterized by a single, cylindrical stem, which may be copiously spiny as in *O. triacantha* (Fig. 3B). However, *O. austrina* also is a member of the *Humifusa* clade, so these morphologically similar characters are merely convergent between *O. austrina* and *O. triacantha*. *Opuntia austrina* is morphologically similar to *O. abjecta* as well, having similar spine characters, glochid and flower colors, and cladode shapes. Consequently, Benson (1982) also misidentified some material of *O. abjecta* from the Florida Keys as *O. austrina* (see Benson, 1982; Fig. 443). Although Anderson (2001) includes *O. abjecta* in synonymy with *O. triacantha*, as mentioned above, he does not include the Florida Keys within the distribution of the species. However, his photo of *O. triacantha* is actually of *O. abjecta* from the Florida Keys. Hunt et al. (2006) used the same photo in The New Cactus Lexicon.

As mentioned above, *O. triacantha* is listed as endangered in Florida (Coile & Garland, 2003) taking into consideration that these populations merely represent a northern disjunct of a more widely distributed species. However, as these populations represent a different species altogether, *O. abjecta*, which is endemic to Florida and only known from three populations in the Florida Keys, the status of the species in the state may need to be reevaluated.

#### *O. MILITARIS* VS. *O. TRIACANTHA*

*Opuntia militaris* shares numerous morphological features with *O. triacantha*, although it is generally less robust and has

fewer spines, characters that may be influenced by differing environmental constraints across the distribution of the two taxa. However, even with the limited data presented here, it is clear that *O. triacantha* and *O. militaris* are not genetically identical (e.g., *O. militaris* is more closely related to *O. caracasana* in our diploid phylogeny; Fig. 1). Thus, our phylogenetic data suggest that *O. triacantha* and *O. militaris* represent distinct lineages. *Opuntia militaris* is also disjunct from the nearest population of *O. triacantha* on Desecheo Island, Puerto Rico, by ca. 765 km. It will be necessary to study morphological characters and ploidy levels of living material of *O. militaris*, *O. triacantha*, and other closely related species within the Greater and Lesser Antilles (e.g., *O. caracasana*, *O. jamaicensis*, *O. repens*, *O. taylori* Britton & Rose) to determine species limits within this group. However, *O. militaris* is tentatively considered specifically distinct here, and therefore is included in the above key.

#### THE *OPUNTIA CUBENSIS* ENIGMA

Benson (1982) considered *O. ochrocentra* described from Big Pine Key to be a synonym of *O. cubensis* described from Guantánamo, Cuba (Britton & Rose, 1912). *Opuntia cubensis* has generally been considered a hybrid between *O. dillenii* and *O. militaris* (Britton & Rose, 1920), and molecular data support the hybrid origin of *O. cubensis* from the Florida Keys (i.e., *O. ochrocentra*) between a member of the *Humifusa* clade and *O. dillenii* (Majure et al., 2012b; Fig. 3A–D in this study). However, this study confirms that *O. militaris* is not conspecific with *O. abjecta* and may be more closely related to, although likely not conspecific with, *O. triacantha*. *Opuntia cubensis* s.str. is shown here to be derived from *O. militaris* and likely *O. dillenii* (Fig. 3A, B). Therefore, based on plastid data, *O. cubensis* s.str. is derived from a different maternal progenitor, *O. militaris*, than that of *O. ochrocentra*, which is derived maternally from *O. abjecta* (i.e., plastids in *Opuntia* s.str. are generally considered to be maternally inherited; Corriveau & Coleman, 1988). Thus the interclade origin of “*O.*

*cubensis*” as identified by Majure et al. (2012b) should be corrected to *O. ochrocentra*, given that *O. cubensis* and *O. ochrocentra* were derived from separate origins. This is further exemplified by ploidal level; *O. cubensis* is tetraploid (Majure et al., 2012c) and *O. ochrocentra* is pentaploid (Majure et al., 2012a).

*Opuntia ochrocentra* as figured in Benson (1982: Fig. 429, p. 428, as *O. cubensis*) bears a striking resemblance to the South American species, *O. sulphurea* G. Don and not *O. ochrocentra* at all. The dark brownish-black, young central spines and strongly tuberculate cladode surface are not seen in *O. cubensis* s.str. or *O. ochrocentra* but are common features of *O. sulphurea*. Nothing resembling material figured by Benson (1982) has been found on Big Pine Key (Majure pers. obs.), and the type specimen of *O. ochrocentra* collected by J.K. Small (Fig. 4D) and other specimens of *O. ochrocentra* made by other collectors throughout the years do not resemble material from Fig. 429 in his text. It seems most likely that material he obtained of “*O. cubensis*” from Big Pine Key was actually derived from cultivated material of *O. sulphurea*. The material from this photo is grown at the Desert Botanical Garden and was received from the Fullerton Arboretum, which originally received it from Benson (identified as *O. cubensis*; R. Puente, pers. comm.). That material does not exhibit the characters mentioned above or the same growth form of either *O. cubensis* or *O. ochrocentra* (Majure, pers. obs.) and conforms to *O. sulphurea*.

Characters of *O. abjecta* and *O. militaris* exhibited by “*O. cubensis* s.l.” could easily be mistaken for any of those putative progenitors (*O. triacantha* s.l.), since those characters differentiating *O. cubensis* s.l. from *O. dillenii* are spine color and shape, the smaller growth form, and cladode disarticulation, all characters shared to some degree by the putative maternal progenitors (*O. abjecta* and *O. militaris*). Identifying these species is made more difficult when using only herbarium material, as most identifying characters of these stem succulents are lost during the drying process (see Reyes-Agüero et al., 2007). Benson’s (1982) general reliance on herbarium specimens instead of observations

based on living material likely led him to treat *O. ochrocentra* as a synonym of *O. cubensis*.

*Opuntia cubensis* and *O. ochrocentra* are morphologically separable. *Opuntia ochrocentra* shares the mottled yellow to reddish-brown and white young spines of *O. abjecta*, and *O. cubensis* has dull-yellow young spines, as do *O. triacantha* and *O. militaris*. The spine patterns of *O. ochrocentra* and *O. cubensis* are slightly different, with *O. cubensis* always having one strong, oftentimes porrect, cylindrical to basally flattened central spine and *O. ochrocentra* with several weaker, cylindrical or basally twisted central spines. The spines in *O. ochrocentra* deflex and become appressed along the stem in age, a character not exhibited in specimens of *O. cubensis*. *Opuntia cubensis* generally has shorter spines and smaller cladodes than *O. ochrocentra*, as mentioned above. The cladodes of *O. cubensis* are typically elliptical in outline, while cladodes of *O. ochrocentra* may be either elliptical or obovate.

Reticulate evolution and polyploidy are commonly reported in *Opuntia* (see Majure et al., 2012b, c). In addition, hybridization between *O. dillenii* has been frequently reported with other species of *Opuntia* and members of the *Consolea* clade, with which the species is sympatric (Majure et al., 2012b). The origin of *O. cubensis* s.str. likely represents yet another occurrence of hybridization involving the hexaploid (Majure et al., 2012a), *O. dillenii*, and provides one more example of the high frequency of hybridization and polyploidy in *Opuntia* in general. Factors driving hybridization among *O. dillenii* and other species needs further study, although the sympatry of *O. dillenii* with other members of tribe Opuntieae DC. generally leads to some degree of hybrid formation.

The true identities of the Floridian taxa, *O. abjecta* and *O. ochrocentra*, were long obscured as a result of misidentifications based on incorrect assumptions regarding phytogeographic relationships of *Opuntia* from the Florida Keys and the Caribbean region and through the misidentification of herbarium specimens (Benson, 1982). *Opuntia abjecta* and *O. triacantha* are distinct species morphologically and phylogenetically. Thus, material from Florida should not be referred to as *O. triacantha*, but rather represents a species endemic to the state,

which should be recognized as *O. abjecta*. *Opuntia cubensis* is a Cuban taxon that does not occur in Florida and which most likely originated via hybridization between *O. militaris* of the *Nopalea* clade and *O. dillenii* of the *Scheerianae* clade, as suggested by Britton and Rose (1920). Material of "*O. cubensis* s.l." from the Florida Keys should be treated as *O. ochrocentra*, which is of hybrid origin most likely between *O. abjecta* of the *Humifusa* clade and *O. dillenii*. More research is warranted to determine whether or not *O. militaris* is distinct from *O. triacantha*, but given the morphological and phylogenetic data presented here, we suggest that *O. militaris* should be regarded as a separate species.

The type specimen of *O. triacantha* deposited at the Willdenow Herbarium at Berlin-Dahlem (B-W) was not relocated and thus presumably destroyed in the 1940s (Leuenberger, 2004). Other herbaria where Willdenow deposited type specimens (see Stafleu & Cowan, 1988) were reviewed and no type material of *O. triacantha* was located, thus we designate a neotype below. No type locality was cited by Willdenow (1814) in the protologue, however, identification of the species upon which the description was based support Greater Antillean material as being that of *O. triacantha* (Britton & Rose, 1920).

**Opuntia triacantha** (Willd.) Sweet, Hort. Brit. 172. 1826. *Cactus triacanthos* Willd., Enum. Pl. 34. 1814. Type. No definite locality cited (B-W, destroyed). Neotype, **here designated**: Antigua, English Harbour, A. C. Smith 10442, 4 Apr 1956 (US-2114819; isoneotypes: NY, K-000035303).

#### Acknowledgments

We thank the United States National Herbarium (US), University of Michigan (MICH) herbarium, and The New York Botanical Garden's Steere Herbarium (NY) for specimen loans; F. Axelrod and T. Majure for help with fieldwork in Puerto Rico; I. Marino and M. Pajuelo for help with fieldwork in the Florida Keys; the Boy Scouts of America and the Key Deer National Wildlife Refuge for providing access to populations of *O. abjecta* and *O. ochrocentra*; the Gemini Botanical Garden (G. Gann) for material of *O. cubensis* s.str.;

Montgomery Botanical Center (M.P. Griffith) for material of *O. repens* from Mona Island; and Desert Botanical Garden (R. Puente) for specimens used in our phylogenetic analyses and for information regarding specimens grown at DBG. The University of Florida Herbarium (FLAS) provided repository space for specimens, and we thank K. Perkins for help with specimen processing. We also thank O. Sida (PR), S. Mamer (OXF), S. Lajos, Z. Barina (BP), and H. Väre (H) for information regarding collections of *Opuntia* and A.M. Powell (SRSU) and A. Salywon (DBG) for helpful comments on an earlier version of the manuscript. Financial support for this work was provided by the Cactus and Succulent Society of America, the American Society of Plant Taxonomists, the Botanical Society of America, the Florida Division of Forestry, and the National Science Foundation (DEB-1011270).

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## Appendix 1. Specimens examined

***Opuntia abjecta*** Small. **UNITED STATES.** Florida: Monroe Co., Big Pine Key, 12 May 1919, *Barrisch s.n.* (US), 4 May 1951, *Killip 41332* (US), 10 Jan 1952, *Killip 41708* (US), 6 Mar 2010, *Majure 3908* (FLAS), 22 Feb 1935, *Miller, Jr. 1710* (US), 12 April 1921, *Small s.n.* (NY), 17 May 1922, *Small s.n.* (NY, US); Long Key, 23 April 1966, *Byrd s.n.* (FLAS); Crawl Key, July 2008, *Sauby s.n.* (FLAS). ***Opuntia cubensis*** Britton & Rose. **CUBA.** Guantánamo: Guantánamo Bay, Oriente, dry sand, valley near coast, 17–30 March 1909, *Britton 2064* (NY); cultivated at Gemini Botanical Gardens, s.d., *Areces-Mallea s.n.* (FLAS). ***Opuntia militaris*** Britton & Rose. **CUBA.** Guantánamo: Guantánamo Bay, Oriente, coastal hills, 17–30 March 1909, *Britton 1957* (NY), 4 Sept 1950, *Jervis 246* (MICH), 7 Jan 1951, *Jervis 1033* (MICH). ***Opuntia ochrocentra*** Small. **UNITED STATES.** Florida: Monroe Co., Big Pine Key, 12–18 Feb 1935, *Killip 31423*, (US), 2 Mar 1936, *Killip 31712* (US), 19 Mar 1952, *Killip 42026* (US), 6 Mar 2010, *Majure 3907* (FLAS), 11 Dec 1921, *Small s.n.* (NY), 17 May 1922, *Small s.n.* (US); Big Munson Island, 8 Mar 2010, *Majure 3968* (FLAS), 8 Mar 2010, *Majure 3969*. ***Opuntia repens*** Bello. **UNITED STATES.** Puerto Rico: Punta Melones, 26 Feb 1915, *Britton s.n.* (NY); Mona Island, *Griffith 369*, 22 Jan 2012 (FTG); Lajas, ca. 5 km NW of La Parguera, off of Hwy. 116, 15 June 2009, *Majure 3838* (FLAS); Cabo Rojo, Refugio de vida Silvestre, Salinas de Cabo Rojo, 15 June 2009, *Majure 3839* (FLAS). US Virgin Islands: St. Thomas, off of Hwy. 32E at Red Hook, ca. 1 km NE of interisland ferry, 13 June 2009, *Majure 3837* (FLAS). ***Opuntia triacantha*** (Willd.) Sweet. **UNITED STATES.** Puerto Rico: Desecheo Island, 18–19 Feb 1914, *Britton 1565* (NY). US Virgin Islands: St. Thomas, Buck Island, 25 Feb 1913, *Britton 1388* (NY). **FRANCE.** Saint-Martin: 1901, *Britton s.n.* (US). St. Barthelemy: 1939, *Anested 924* (US). Guadeloupe: Isle les Saintes, Désirade, Dec 1893, *Duss 979* (P); Désirade (Leprosérie), 1904, *Duss 3071* (NY, US), *Duss 3459* (NY, US); Mornes Basaltiques secs., Isle les Saintes; Terre de Haut, Morne Charreau, 15 May 1937, *Stehlé 1726* (NY, P). **NETHERLANDS.** Saba: SW corner of island, Giles Quarter Trail, 17° 36' 55"N 63° 14' 45"W, 4 Mar 2007, *Mori 22693* (NY). **FEDERATION OF ST. KITTS AND NEVIS.** St. Kitts, Basseterre, 2 Feb 1913, *Rose 3241* (NY). **ANTIGUA,** Galley Bay, 21 May 1938, *Box 1455* (US), 4–16 Feb 1913, *Rose 3304* (NY, US). **UNITED KINGDOM.** Montserrat: Roelir's Cliff and Surrurys Wall, 13 Feb 1907, *Schafer 543* (NY, US).