



North American *Opuntias* (Cactaceae) in Argentina? Remarks on the phylogenetic position of *Opuntia penicilligera* and a closer look at *O. ventanensis*

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Abstract

Opuntia, the most widespread genus of cacti, occurs throughout the Americas from Patagonia to Canada. Various species have very wide distributions in the Americas, and thus may be considered as both native and aliens. We reexamined data based on recent work on the phylogenetics and taxonomy of *Opuntia* from southern S-America and showed that two presumed endemic species to Argentina—*O. penicilligera* and *O. ventanensis*—are likely derived from, or may be conspecific, with North American species. In particular, *O. penicilligera* is most closely related to members of the *O. macrorhiza* species complex and is morphologically similar to both *O. macrorhiza* and *O. cymochila*. *O. ventanensis* shares nearly all vegetative and reproductive morphological characters with *O. fragilis* and likely is conspecific with that taxon. Owing to the wide distribution of *Opuntia* species and the movement of many taxa by people, extra care must be exercised when describing new species or for carrying out taxonomic treatments. A phylogenetic perspective, as well as a careful study of species across the distribution of the genus, is recommended.

Keywords: nopales, *Opuntia*, Opuntioideae, phylogenetics, prickly pears, South America

Introduction

Members of the prickly pear cacti, tuna or nopales, *Opuntia* Miller (1754: without page) (Cactaceae Juss.) are naturally widely distributed throughout and are endemic to the Americas (see e.g., Anderson 2001). The group most likely originated in southern South America with subsequent dispersals and further diversification in North America (Majure *et al.* 2012a). The genus *Opuntia* (including *Nopalea* Salm-Dyck (1850: 233) is composed of two South American clades and one large and very diverse North American clade composed of six subclades (Majure *et al.* 2012a, Majure & Puente 2014). Many taxa endemic to northern Colombia and Venezuela [e.g., *O. boldinghii* Britton & Rose (1919: 155), *O. schumannii* F.A.C. Weber ex A. Berger (1904: 34)] were actually derived from the North American clade, and were shown to have originated via the dispersal of North American clade members back to South America (Majure *et al.* 2012a).

Species of *Opuntia* have been introduced throughout the world for use as ornamentals, forage for livestock, as well as for the production of agricultural products for human consumption, such as nopales and tunas, i.e., stem segments and prickly pear fruit, respectively (Casas & Barbera 2002, Nefzaoui & Ben Salem 2002). The range of many species within the Americas also has been substantially modified, as a result of the long-term use by humans for the above-stated reasons. Likewise, some taxa that do not appear to be widely used by humans also have been broadly dispersed, presumably by natural forces, such as large herbivores (Janzen 1986).

Opuntia ficus-indica (Linnaeus 1753: 468) Miller (1768: without pagination), a domesticated polyploid derivative of Mexican prickly pear species, is found throughout most of tropical America where it was presumably dispersed by humans (Griffith 2004), and its arrival in South America is assumed to have been as early as 8,000 years ago (Kiesling 1998, Ervin 2012). *O. pubescens* Wendland ex Pfeiffer (1837: 149), another native species of Mexico, also occurs in South America (Bolivia, Colombia, Ecuador, Peru, Venezuela), although there are no known human uses for this

species, at least outside of cultivation as an ornamental. Moreover, *O. fragilis* (Nuttall 1818: 296) Haworth (1819: 82), one of the most widespread species in North America, has long been presumed to have been dispersed via migrating buffalo, where the easily disarticulating stem segments (cladodes) easily stick into the fur of passing animals by way of their strongly retrorsely-barbed spines (see e.g., Ribbens 2008). Thus, these cladodes act as vegetative propagules for this hexaploid species, which very seldomly reproduces sexually (Ribbens *et al.* 2011). Perhaps the same method of dispersal (via migrating animals) could have been involved in the production of the wider distribution of *O. pubescens* outside of its original range in Mexico, and could potentially explain other known disjunctions of some species of *Opuntia*.

Opuntia penicilligera Spegazzini (1902: 291) was described from the southern part of Argentina, and it is treated as an endemic taxon by various authors (e.g., Cabrera & Fabris 1965, Kiesling 1988, Kiesling 1999, Zuloaga *et al.* 1999), although its morphological similarity with the other southern South American (sSA) species has never been clear (Spegazzini 1905, Spegazzini 1925, Britton & Rose 1919). However, a recent taxonomic revision of the *Opuntia* ser. *Elatae* Britton & Rose (1919: 156) based on morphological data, tentatively included the species in the sSA species group (Font 2014). Likewise, *O. ventanensis* Long (2012: 79) is a recently described taxon also from the southern Argentina region, and it has been treated as an endemic species. Although the morphological affinities of *O. ventanensis* with other sSA species have been discussed (Long 2012), the affinities with non-sympatric species in a broad context of the genus have not yet been considered.

Recent phylogenetic and cytogenetic analyses of sSA taxa of *Opuntia* aimed at determining the relationships among species occurring in Argentina and neighboring areas (Realini *et al.* 2014a, 2014b). Unfortunately, their work did not obtain the level of topological resolution using only the plastid *trnL-trnF* and *psbJ-petA* intergenic spacers, as well as ISSR markers, to fully understand relationships of the sSA taxa or test hypotheses regarding the putative relationships of some taxa with North American species due to their lack of taxon sampling.

Here, we propose a re-evaluation of the phylogenetic relationship of *Opuntia penicilligera* using previous molecular data generated for a broad scale phylogeny of the prickly pears (Majure *et al.* 2012a), as well as for southern South American species (Realini *et al.* 2014a). We also used data from the literature and herbarium materials to compare morphological characters of *O. ventanensis* and *O. fragilis*, a potentially closely related and phenetically very similar species from North America. Our primary goal was to test the hypothesis that those two presumably endemic species from Argentina could have putative origins from North American *Opuntia* species.

Material and methods

DNA sampling, sequence alignment and phylogenetic analyses

Newly generated plastid sequences (*psbJ-petA*, *trnL-trnF*) from Realini *et al.* (2014a) were downloaded from GenBank and incorporated into a plastid dataset composed of the six plastid loci (*atpB-rbcL*, *matK*, *ndhF-rpl32*, *psbJ-petA*, *trnL-trnF*, *ycf1*) used in Majure *et al.* (2012a). These included both diploids and some polyploids for all major clades (two South American and six North American clades). Some species names used in Majure *et al.* (2012a) have been updated or corrected (e.g., Majure *et al.* 2013, Majure *et al.* 2014, Majure *et al.* 2017), and those corrections are given in Appendix 1. Sequences were aligned using the MAFFT (Katoh & Standley 2016) plugin in Geneious (v. 11.1.5, Biomatters Ltd.) and then corrected manually. Maximum likelihood (ML) in RAxML (Stamatakis 2014) was employed for phylogeny reconstruction using the GTR+G model of molecular evolution and undertaking 1000 bootstrap pseudoreplicates.

Morphological data

Morphological comparisons between *Opuntia fragilis* and *O. ventanensis* were based on observations in the field (Majure 6762, 6781, 6791; DES), examination of herbarium specimens (BAF, DES, FLAS, acronym according to Thiers 2019+), and analysis of relevant literature (Parfitt 1991, Pinkava 2003, Ribbens 2008, Long 2012). The following characters were analyzed: growth form, morphology of the stem, number and presence of the spines, and floral and fruit characters (see Table 1).

with which the taxon is morphologically similar (Fig. 2). Only a partial *trnL-trnF* sequence was available for *O. ventanensis*, which did not provide any useful phylogenetic information to place that species in a phylogenetic context. So, we only explored the morphological characters of that taxon, as compared to the proposed closely related species *O. fragilis*.

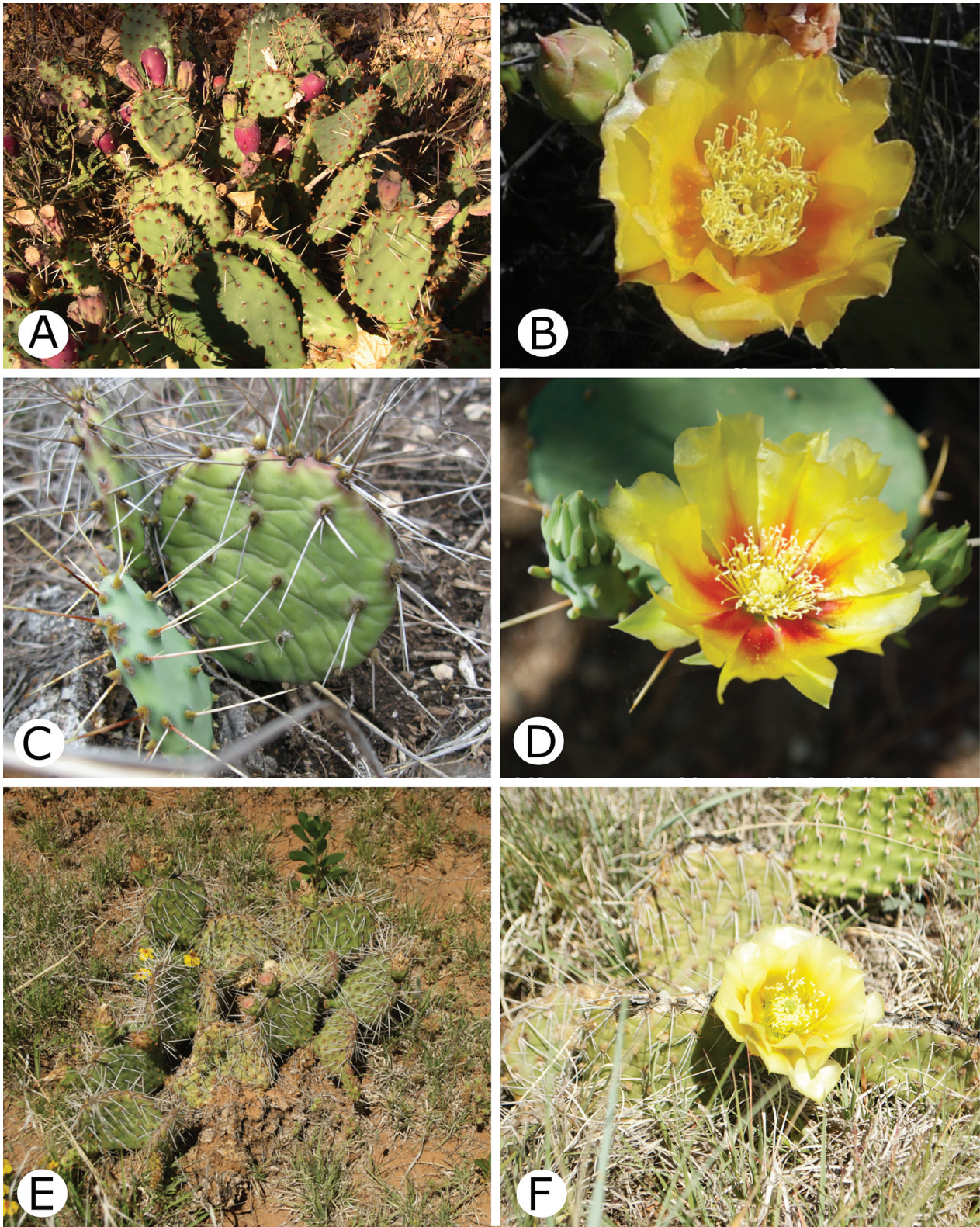


FIGURE 2. Members of the *Opuntia macrorhiza* complex compared with *O. penicilligera*. A) *O. penicilligera* from Mendoza, Tunuyán (Argentina) (Logarzo547-BAF), B) flowers of *O. penicilligera* from Mendoza, Tunuyán (Argentina) (photo only), C) *O. macrorhiza* from Kerr Co., Texas, USA, in habitat, D) *O. macrorhiza* in flower (Majure 3510-FLAS), E) *O. cymochila* (spiny form) from Quay Co., New Mexico, USA, in habitat and F) in flower (Majure 6854-DES, FLAS). (photo A by L. Varone, B by C. Inchauspe, and C-D by L.C. Majure).

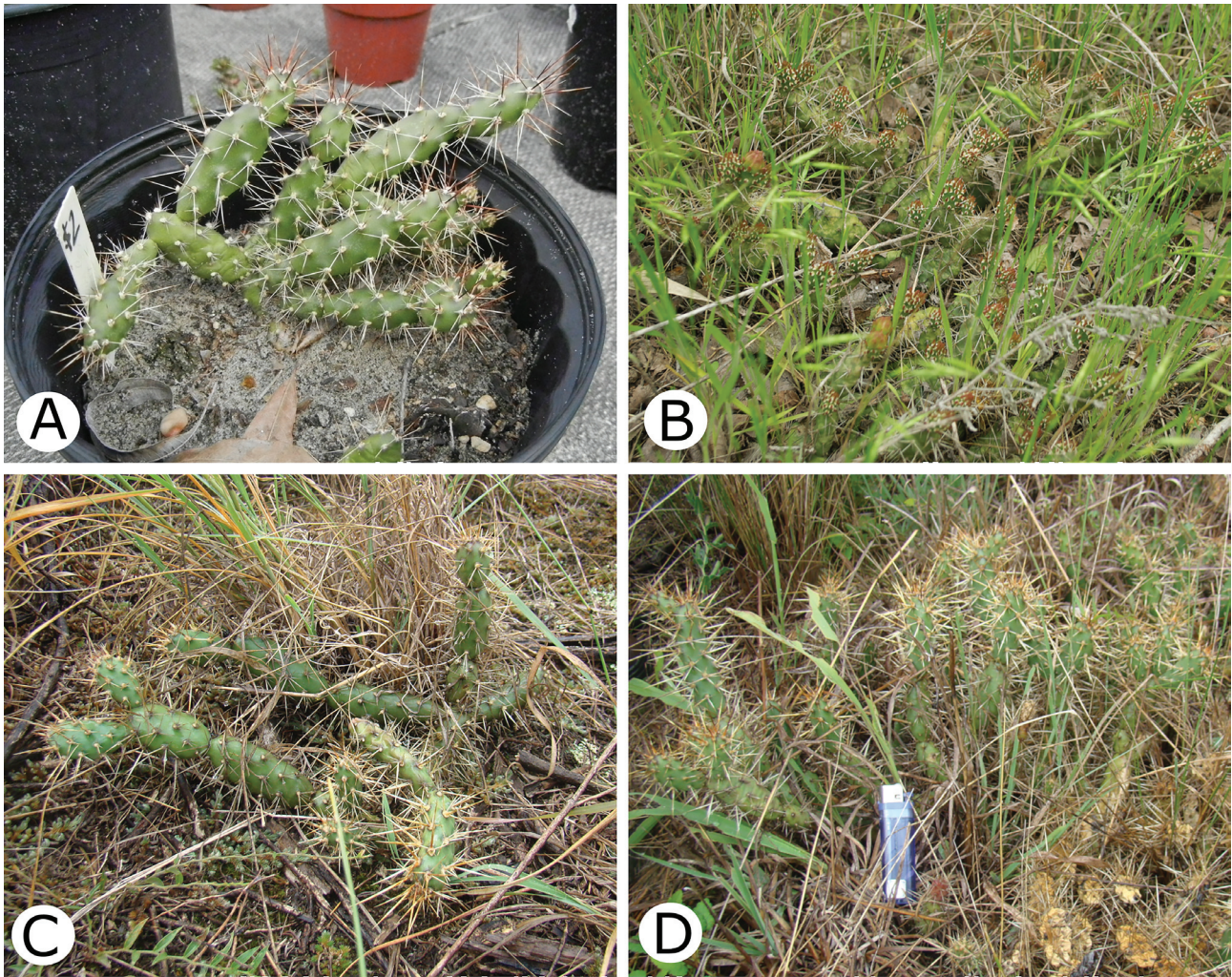


FIGURE 3. Morphological characters of *Opuntia fragilis* compared with *O. ventanensis*. A) in habitat showing A) clump-forming habit of *O. fragilis* (Ribbens s.n.-FLAS), from Marquette Co., Michigan, USA, B) *O. fragilis* in habitat from Buffalo Co., Nebraska, USA (Majure 6762-DES, FLAS), C-D) *O. ventanensis* in habitat from Córdoba (Font 634-BAF) (photos A–B by L.C. Majure and C–D by F. Font).

Opuntia ventanensis shares nearly all morphological characters, including vegetative and reproductive features, with the North American species *O. fragilis* (Tab. 1). Both *O. ventanensis* and *O. fragilis* are low, sprawling shrubs (Fig. 3) and have small, easily disarticulating cladodes and strongly, retrorsely-barbed spines. Both have yellow inner tepals and pinkish or reddish staminal filaments, as well as green stigma lobes, and both taxa exhibit spiny pericarpels. Chromosome number is apparently $2n=55$ in *O. ventanensis*, and $2n=66$ in *O. fragilis*.

Discussion

Opuntia penicilligera has long been a puzzling name for the Argentinian flora. Although it was described in 1902 by C. Spegazzini, its identity remained unresolved for a long time, since no original material had been designated and the type never located (see Spegazzini 1902, Kiesling 1984, Katinas 2004, Leuenberger & Arroyo-Leuenberger 2014). During this time, the taxon has been reported as a native and endemic to the north Patagonian Argentinian region, occurring in the hills and dry plains south of Buenos Aires, north of Río Negro, south of La Pampa, northwest of Neuquén and west of Mendoza provinces (Cabrera & Fabris 1965, Kiesling 1988, Kiesling 1999, Zuloaga *et al.* 1999, Kiesling *et al.* 2008, Font 2014).

Recently, a first step was made to elucidate the correct application of the name *Opuntia penicilligera*, with the designation of a neotype (see postscript of Leuenberger & Arroyo-Leuenberger 2014). Although the selected specimen is incomplete lacking information regarding flowers, it is assumed to correspond with the original description

(Spegazzini 1902) exhibiting the conspicuous penicillate rusty brown glochids on orbicular to obovate stems with one principal spine up to 5 cm long, and none or 3–4 much shorter spines, with the receptacles cylindrical to conical and the fruits reddish. However, as previously reported by Leuenberger & Arroyo-Leuenberger (2014), plants identified by collectors and botanists hitherto as *O. penicilligera* may belong to one, two or up to three morphotypes potentially related to North American species.

Phylogenetic analysis of those data generated by Realini *et al.* (2014a) and Majure *et al.* (2012a) placed *Opuntia penicilligera* in the North American *Humifusa* clade composed of *O. macrorhiza* and relatives, in accordance with both morphology and ploidy. *O. penicilligera* is morphologically very similar to *O. macrorhiza* and close relatives based on observation of photos of live plants, as published in Font (2014) and Leuenberger & Arroyo-Leuenberger (2014), and on examination of herbarium specimens (B, BAF, LP, SI, acronym according to Thiers 2019+). So, the placement with members of a clade containing *O. macrorhiza* is not surprising. However, it also appears that what is being called *O. penicilligera* may be a range of taxa in the *Macrorhiza* complex and possibly even the *Macrocentra* clade (*sensu* Majure *et al.* 2012a). Likewise, the material sequenced by Realini *et al.* (2014a, Font 531 at BAF), is one of numerous morphotypes (Font, pers. obsv.) that may or may not represent typical material of *O. penicilligera* as described by Spegazzini (1902). As revealed in our phylogenetic analysis here, based on the specimens sequenced by Realini *et al.* (2014), it is very clear from the phylogenetic placement that *O. penicilligera* is not at all closely related to other members of the *Elatae* clade, as tentatively proposed by Font (2014).

Although *Opuntia macrorhiza* has been treated by several authors (e.g., Britton & Rose 1919, Benson 1982, Pinkava 2003), it has not been taxonomically revised and, on the basis of personal observations (Majure, unpubl. data), this taxon forms a species complex consisting of numerous morphotypes (both diploid and tetraploid) that are currently under study. However, the majority of the taxa belonging to the *O. macrorhiza* complex is composed of tetraploids ($2n=44$; see Majure & Ribbens 2012, Majure *et al.* 2012b), the same ploidy as that reported for *O. penicilligera* by Realini *et al.* (2014b). Likewise, another close relative in the *Macrorhiza* complex, *O. cymochila* Engelman & Bigelow (1856: 295), which shows some morphological affinities to *O. penicilligera*, has been reported as both tetraploid and hexaploid (Majure *et al.* 2012b). Thus, we cannot rule out the possibility that what is referred to as *O. penicilligera* could be represented in Argentina by both *O. cymochila* and *O. macrorhiza*.

Opuntia cymochila is a morphologically variable species, likely partly a result of its putative hybrid origin between *O. macrorhiza* and *O. polyacantha* Haworth (1819: 82) (see Majure 2012), and it is probable that what we refer to as *O. cymochila* has been derived numerous times through repeated hybridization events (Majure 2012), as is common for most hybrid derived species (Soltis and Soltis 2009). *O. cymochila* displays characters that are mosaics of both putative parents, with fruit that may be fleshy or mostly dry and spiny or mostly spineless. Cladodes of *O. cymochila* may be extremely spiny, as in *O. polyacantha*, or with much fewer spines, as in *O. macrorhiza*. The number of areoles per diagonal row in *O. cymochila* (6–8) normally exceeds that of *O. macrorhiza* (4–6) and is fewer than that of *O. polyacantha* (8–10). The inner tepals of *O. cymochila* may be entirely yellow as in *O. polyacantha* or with a reddish to reddish-brown base, as in *O. macrorhiza*.

Opuntia ventanensis is morphologically very similar to *O. fragilis* based on several characters, i.e. growth habit (low, sprawling shrubs forming mats or cushions; Fig. 3), disarticulating cladodes (vegetative propagules) with strongly retrorsely-barbed spines (these vegetative propagules are easily dispersed by animals in both taxa; Ribbens 2008, Long 2012, Majure & Ribbens 2012 & refs. therein), numbers of spines per areole which overlap [3–8 in *O. fragilis* vs. 1–3(–5) in *O. ventanensis*]. Although Long (2012) stated that *O. ventanensis* only has up to 3 spines per areole, the photos of cladodes in her paper show up to six spines per areole including both central and radial spines, and it should be noted that both central and radial spines are present, as in *O. fragilis* (a common character in the *O. polyacantha* complex; Parfitt 1991). Both species rarely flower (see Bennett *et al.* 2003, Pinkava 2003, Ribbens 2008, Long 2012). Both taxa have yellow inner tepals, these may have a pink midrib in *O. ventanensis* (Long 2012) or be reddish or greenish at the base in *O. fragilis* (Ribbens 2008). Flowers of both exhibit green stigma lobes, as well as small fruit (2×1.2 cm in *O. ventanensis* vs. $1-3 \times 0.8-1.5$ cm in *O. fragilis*; Pinkava 2003, Long 2012). The staminal filaments in *O. fragilis* are red to reddish-brown, and those in *O. ventanensis* were described as pink (Long 2012). Figure 2B and 3A-B in Long (2012) show a spiny pericarpel in *O. ventanensis*, yet another character shared with *O. fragilis*.

Opuntia fragilis has only been reported as a hexaploid ($2n=66$) from throughout parts of its range (Parfitt 1991, Majure & Ribbens 2012). Interestingly, Realini *et al.* (2014b) reported a pentaploid chromosome number for *O. ventanensis* ($2n=55$). So, if indeed *O. ventanensis* proves to be closely related or even synonymous with *O. fragilis*, then either the chromosome number is variable in the species, or perhaps chromosome numbers should be re-analyzed for the Argentinian populations, since just one count has been reported. On the other hand, that *O. fragilis* has not been analyzed for ploidy across its native range leaves open the possibility of pentaploid individuals in North American

populations as well. Likewise, although it is clear that *O. fragilis* is part of the *Polyacantha* clade (Parfitt 1991, Majure *et al.* 2012a), it is still unclear as to the origin of the hexaploid species.

Numerous non-native species of *Opuntia* and other cacti have been introduced into Argentina for the purpose of producing agricultural products, as well as for use as ornamentals (Castellanos & Lelong 1934). When and where *O. macrorhiza* (and potentially *O. cymochila* and *O. fragilis*) could have been introduced into the country is unknown, but they likely could have been introduced as ornamentals as well. The colonization of Argentina, especially the Buenos Aires region, goes back to the 16th century, and the alien flora has been relatively well documented and studied (Hauman 1925, 1927, Molfino 1926, Rapoport & Brion 1991, Söyrinki 1991, Zuloaga & Morrone 1996, 1999, Zuloaga *et al.* 1999). Thus, it is known that the northern Patagonia floras include a high number of exotic plants (Speziale & Ezcurra 2011). Along those lines, the description of presumably new taxa from that region should include comparisons, not only with South American species, but also with potential alien relatives. This should also be the standard for taxa being described from other parts of the distribution of *Opuntia*, since so many species have been so widely cultivated and introduced outside of their natural range.

Although phylogenetic data are available regarding the relationships of major clades within *Opuntia* s.str. (Majure *et al.* 2012a, Majure & Puente 2014), those results are not always taken into consideration for revisions of closely related species or for nomenclatural changes (Guiggi 2015). On the contrary, the outdated system of using traditional groupings based on phenetic similarity and oftentimes geography (Britton & Rose 1919, Backeberg & Knuth 1935) is still being utilized. Likewise, certain characters, for example, associated with hummingbird pollinated flowers (reddish tepals, nectar chambers, lack of staminal thigmonasty), when not placed in a phylogenetic context are overemphasized as being unique (e.g., Oakley & Kiesling 2016; *O. quimilo*), when on the contrary they are common throughout tribe Opuntieae (e.g., *Tacinga*, *Macbridei* clade, *O. stenopetala*, *Nopalea* clades), and likewise *O. quimilo* is deeply nested within the *Elatae* clade (Fig. 1). It is clear that traditional taxonomic species groups in specific instances, such as *Opuntia* series *Aurantiacae* and *Curassavicae sensu* Britton & Rose (1919), each composed of members from two different clades, are not natural groupings in the broad sense. Although the phylogenetic relationships of the sSA species are still not fully understood, and are currently under study (Köhler *et al.* 2018; Köhler *et al.*, unpubl. data), *Opuntia* series *Elatae* Britton and Rose (1919: 156) or *Opuntia* series *Armatae* Schumann (1899: 743) *sensu* Leuenberger (2002: 413), for example, clearly excludes many of the close relatives of that clade, such as *O. quimilo* Schumann (1898: 746), *O. retrorsa* Spegazzini (1905: 571) and potentially the Galapagos' Island species (Griffith & Porter 2009, Majure *et al.* 2012a). Thus, it is most appropriate where relationships are known that taxa traditionally circumscribed under such names be re-evaluated when carrying out taxonomic revisions. The combination of morphological and cytological work, coupled with phylogenetic work have the potential to yield more robust hypotheses of species relationships, as well as species limits.

Considering the morphological characters outlined above, it seems likely that *Opuntia ventanensis* is very closely related or may even belong within the species concept of *O. fragilis*. However, further morphological and phylogenetic study, including samples from the type locality, are necessary to clarify the relationship of *O. ventanensis* with other species. The possible origin of these North American taxa in Argentina is of much interest, and it would be fascinating to understand when and where they were originally introduced, as well as the intention of the introduction. It seems likely that cattle introduced from the United States could have been one source of prickly pears in the country, as well as introduction for ornamental purposes or even long-distance dispersal via migrating birds.

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APPENDIX 1. Identifications needing taxonomic updates or corrections from Majure *et al.* (2012a). Here, we first give the corrected name and in parentheses the name as listed in Majure *et al.* (2012a).

Brasiliopuntia schickendantzii 2010 (*O. schickendantzii*_2010), *Opuntia anacantha*_2010 (*O. assumptionis*_2010), *Opuntia austrina* Majure 2753, Majure 3450 (*O. ammophila* Majure 2753, 3450), *Opuntia drummondii* Majure 753 (*O. pusilla* Majure 753), *Opuntia rioplatense* (*O. quitensis*_0262), *Opuntia engelmannii* var. *engelmannii*_0579 (*O. martiniana* 0579), *Opuntia mesacantha* Majure 3785 (*O. humifusa* Majure 3785), *Opuntia phaeacantha* Snow (*O. atrispina* Snow), *Opuntia setispina*_RP (*O. gosseliniana*_RP), *Opuntia sulphurea*_2010 (*O. cochabambensis*), *Tacinga lilae*_0369 (*O. lilae*_0369).