GRUSONIA PULCHELLA CLASSIFICATION AND ITS IMPACTS ON THE GENUS *GRUSONIA*: MORPHOLOGICAL AND MOLECULAR EVIDENCE

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Summary. This report is an investigation into the relationships among members of the genus *Grusonia* sensu Anderson (2001) with an emphasis on examining the relationship of *Grusonia pulchella* within Opuntioideae (Cactaceae). *G. pulchella* is morphologically and geographically distinct from other *Grusonia* species, and nrITS DNA sequence data suggest that it may represent an independent evolutionary lineage from other grusonioid cacti. With the morphological, geographical, and molecular evidence considered, I propose to resurrect the generic concepts *Corynopuntia* Knuth (1935), *Grusonia* Reichenbach ex Schumann (sensu Britton and Rose, 1919), and *Micropuntia* Daston (1946).

Introduction

The current circumscription of the *Grusonia pulchella* (sand cholla) complex includes several described taxa. The first was *Opuntia pulchella* (Engelmann, 1863), whose type locality is on the Walker River in Nevada. Several specimens from Utah and bordering Nevada were described as new species under the new genus *Micropuntia* (Daston, 1946), i.e., *Micropuntia barkleyana*, *M. brachyrhopalica*, and *M. spectatissima*. Benson's (1957) circumscription of *O. pulchella* included these three morphotypes (as "aberrant forms") and notes that the species' affinities are difficult to ascertain. Robinson (1973) combined sand cholla along with seven other species into the genus *Grusonia,* previously described by Reichenbach (1896). The most recent treatments of *Grusonia* also include the genera *Corynopuntia* Knuth, *Marenopuntia* Backeberg, and *Micropuntia* Daston (Anderson, 2001; Stuppy, 2002; Wallace and Dickie, 2002; Gibson, ined.)

The *G. pulchella* (Engelm.) H. Rob. complex is a morphologically variable taxon consisting of scattered populations of small, terete-stemmed opuntioid cacti found in the Great Basin of western North America (Benson, 1982; Kartesz, 1987) (Fig. 1). Although plants of the *G. pulchella* complex have long been considered to possess affinities with other grusonioid cacti (Engelmann, 1863; Britton and Rose, 1919; Benson, 1957, 1982; Anderson, 2001; Stuppy, 2002), aspects of the morphology, distribution, and habit of *G. pulchella* suggest that it may represent an evolutionary lineage distinct from other opuntioids, as first suggested by Daston (1946).

Figure 1. Habitat of *Grusonia pulchella*: view south across the Columbus salt marsh from the ghost town/alluvial fan of Columbus, Esmeralda County, Nevada, USA.

Figure 2. *Grusonia pulchella* in an alluvial fan above the Columbus salt marsh.

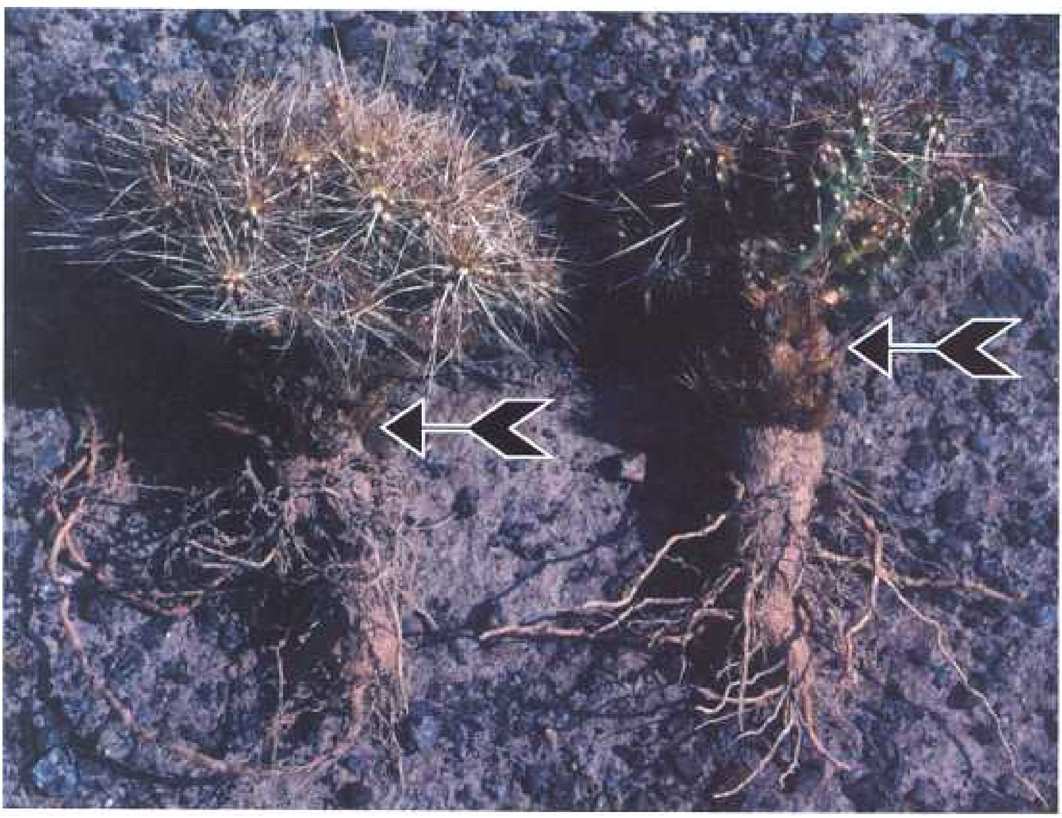

Figure 3. Two specimens of *Grusonia pulchella.* Note enlarged fleshy underground areole- and glochid-bearing structure.

Grusonia pulchella (Fig. 2) differs morphologically from other members of the genus in a number of significant ways. These plants are marked by an enlarged fleshy underground structure featuring areoles with prominent yellow glochids (Fig. 3). Some authors term this morphological feature a "tuberous root" (Benson, 1982; Anderson, 2001), but the areoles and glochids present upon the upper portion of the structure provide evidence that it may be stem-derived (Britton and Rose, 1919) or perhaps a true tuber (Jackson, 1928). The presence of an underground tuber would make this species unique among the grusonioids. Other *Grusonia* species possess somewhat thickened roots (Engelmann, 1859; Benson, 1982; Ralston and Hilsenbeck, 1989), but these do not approach the proportions of the storage tuber of *G. pulchella*. They also are derived entirely from root tissue. Terminal stems of *G. pulchella* are often cylindrical rather than fully clavate (Britton and Rose, 1919) and are rather flexible, much like those of *Pereskiopsis* Britton & Rose, although much reduced in size. Unlike most *Grusonia* species, *G. pulchella* has tubercles that are often inconspicuous (Benson, 1982), but this varies (Britton and Rose, 1919; Daston, 1946; Anderson, 2001). The areoles of *G. pulchella* bear persistent wool similar to the areolar wool of *Pereskiopsis*, whereas the areoles of other *Grusonia* species have early-deciduous wool or lack wool entirely (Anderson, 2001). Although displaying these subtle morphological affinities with *Pereskiopsis*, sand cholla obviously lacks the overall habit and persistent leaves of that genus. Spines on the fruit of *G. pulchella* differ from those of other *Grusonia* species by being antrorsely barbed rather than retrorsely barbed (Parfitt, 1988; Pinkava, 1999). Seeds of *G. pulchella* are sufficiently distinct morphologically from other *Grusonia* seeds as to place them in the monotypic subgenus Micropuntia sensu Stuppy (2002). One unique opinion even considers possible affinities of *G. pulchella* with *Maihuenia* (Philippi ex Weber) Schumann and *Pterocactus* Schumann (Daston, 1946).

Grusonia pulchella is disjunct from other members of the genus *Grusonia* (Fig. 4), which occur further to the southeast, through southernmost Nevada, Arizona, New Mexico, and Texas, and south into Mexico (Benson, 1982; Pinkava, 2002; Gibson, ined.). The geographically nearest congener of sand cholla is *G. parishii* (Orcutt) Pinkava. Both species have been collected in Nye County, Nevada. Based on locality data from specimens cited in Benson (1982), the ranges of these two taxa do not appear to overlap. Furthermore, *G. parishii* occurs at elevations below 900-1200 m, and *G. pulchella* is found at 1200-1500 m and higher (Benson, 1969, 1982; Pinkava, 1999; Morefield, 2001). All chromosome counts of *G. pulchella* show it to be diploid, $2n = 22$ (Pinkava, 2002).

The habit of *G. pulchella* differs from that of congeneric plants. The majority of *Grusonia* species are easily identifiable to genus at a distance, as they form dense, spreading mats of branching, clavate cladodes (Benson, 1982; Ralston and Hilsenbeck, 1989, 1992; Powell, 1998; de la Cerda-Lemus, 1999; Anderson, 2001), which sometimes root adventitiously (Engelmann, 1859). *Grusonia pulchella* is not mat-forming but is better described as a cushion or a dense, low-growing single-trunked shrub (Benson, 1982; Pinkava, 1999). The distal cladodes of *G. pulchella* do not root adventitiously while attached, nor do they detach easily (pers. obs.).

Recent molecular analyses have provided much insight into the relationships among the genera of Opuntioideae (Wallace and Dickie, 2002; Wallace and Gibson, 2002). Chloroplast DNA sequences demonstrate a sister group relationship of four species of *Grusonia* sensu

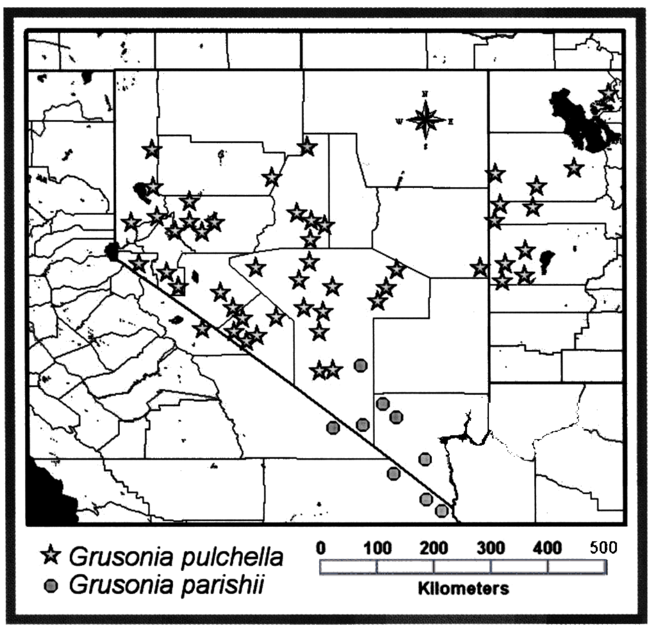

Figure 4. Map showing the distribution of *Grusonia pulchella* and its most geographically proximate congener, *G. parishii*. The range of *G. parishii* extends further south into California. Other *Grusonia* species occur further south and east, through the southwestern United States and into Mexico. Adapted from Benson, 1982; Albee et al., 1988; Morefield, 2001; Pinkava, 2002; and Gibson, ined.

Anderson (2001) with a limited sampling of *Cylindropuntia* (Engelm.) Knuth. Sampled *Grusonia* in these studies include the type species *G. bradtiana*, along with *G. clavata*, *G. marenae*, and *G. stanleyi*. These two genera form a well-supported monophyletic group with *Pereskiopsis* and *Quiabentia* Britton & Rose. The relationship of *G. pulchella* within the Opuntioideae has not yet been elucidated. Given the conflicting evidence of morphological data towards the relationship of *G. pulchella* within the Opuntioideae, there is a potential for molecular data to help resolve this species' taxonomic position. Toward this goal, I have gathered DNA sequence data from the nrITS region for specimens of *G. pulchella* and putative related taxa.

Methods and Materials

Live material was obtained through fieldwork or from live vouchered plantings at Rancho Santa Ana Botanic Garden, Claremont, CA; Desert Botanical Garden, Phoenix, AZ; and Sul Ross State University Cactus Garden, Alpine, TX. This study sampled 34 specimens, including 2 specimens of *G. pulchella*, 7 specimens of other *Grusonia* species, including representatives from all other described genera currently circumscribed within *Grusonia* sensu Anderson (2001), and 25 specimens representing all other genera currently recognized in Opuntioideae (Table 1). DNA was extracted using 2X CTAB, followed by precipitation in cold isopropanol (Griffith and Porter, in press). All extractions used approximately 0.5 g of epidermal shoot tissue. Polymerase Chain Reaction (PCR) amplification of templates of the nuclear ribosomal internal transcribed spacer region (nrITS) follows the methods outlined by Columbus et al. (1998) using the primers ITS4 and ITS5 (White et al., 1990). Polyethylene glycol precipitation (Morgan and Soltis, 1993) purified all templates. Purified template amplifications were sequenced directly with four primers, ITS2, ITS3, ITS4i, and ITS5i (White et al., 1990; Porter, 1997), using "big dye" chemistry from Applied Biosystems Incorporated, according to the manufacturer's specifications. An Applied Biosystems Incorporated 3100 automated DNA sequencer gathered all sequences from PCR products. Genbank accessions from a previous study (Hershkovitz and Zimmer, 1997) provided 3 additional sequences representing outgroup taxa.

Chromatograms from sequencing reactions were assembled into contigs and edited using Sequencher v4.1 (Gene Codes Corporation, Inc.). Consensus sequences were aligned manually with Se-Al v2.0a72 (Rambaut, 1996). Gaps were treated as missing data. Informative indels were coded. Phylogenetic relationships among these taxa were estimated using Fitch parsimony, in PAUP* v4.0ß8 (Swofford, 1998). Estimations of confidence in the clades obtained by these analyses were gathered through bootstrap analysis (Felsenstein, 1985) with 10,000 pseudoreplicates, and through jackknifing (Farris et al., 1996), also with 10,000 pseudoreplicates (63% deletion) as performed by PAUP*.

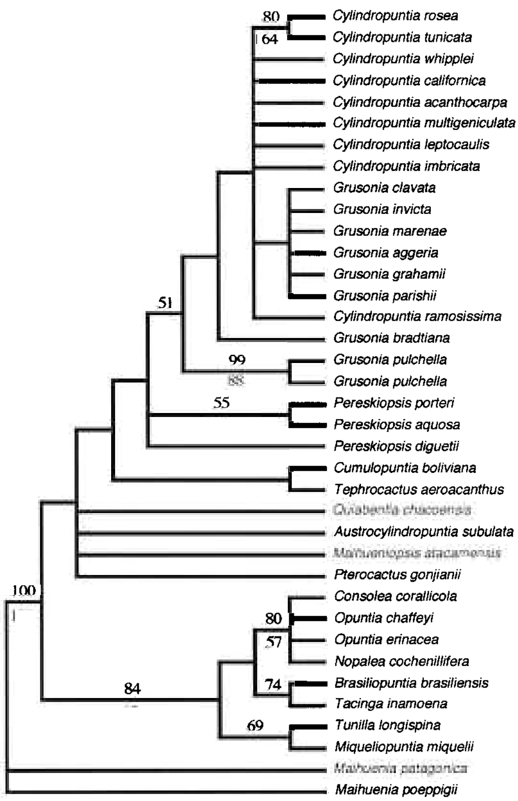

Figure 5. A phylogenetic analysis of *Grusonia pulchella* and related taxa. Strict consensus of 809,300 most-parsimonious trees found by the heuristic search of the nrITS DNA data. With uninformative characters excluded, length = 208; CI = 0.6667; RI = 0.8427; rescaled consistency = 0.6847. Bootstrap percentages above 50% are indicated above the branches, and jackknife support above 50% is indicated below the branches.

Results and Discussion

As a whole, *Grusonia* sensu Anderson (2001) is not supported as a monophyletic group, rather, it is apparently paraphyletic. The two specimens of *G. pulchella* form a well-supported monophyletic group (99% bootstrap, 88% jackknife) that is sister to the remaining samples of *Grusonia* (*Corynopuntia*, *Grusonia*, and *Marenopuntia*) and all sampled members of the genus *Cylindropuntia* (Fig. 5). Although this sister group relationship lacks strong statistical support, this topology was recovered in all 809,300 most-parsimonious

Table 1. Sources of nrITS DNA sequences for specimens used in molecular analysis

Species	Source*	Voucher
Austrocylindropuntia subulata (Muehlenpfordt) Backeberg	D B G 1990 0692	
Brasiliopuntia brasiliensis (Schumann) Berger	D B G 1990 0559	
Consolea corallicola Small	D B G 1997 0397	
Cumulopuntia boliviana (Salm-Dyck) F. Ritter	D B G 1970 9884	
Cylindropuntia acanthocarpa (Engelm. & Bigelow)	San Bernardino County, CA, USA	*Griffith 211* (RSA)
Cylindropuntia echinocarpa (Engelm. & Bigelow) Knuth	Clark County, NV, USA	*Griffith 200* (RSA)
Cylindropuntia imbricata (Haworth) Knuth	Nuevo León, Mexico	*Griffith 250* (RSA)
Cylindropuntia leptocaulis (de Candolle) Knuth	Coahuila, Mexico	*Griffith 244* (RSA)
Cylindropuntia californica (J. Torrey & Gray) Knuth	Los Angeles County, CA, USA	*Columbus s.n.* (RSA)
Cylindropuntia ramosissima (Engelm.) Knuth	Clark County, NV, USA	*Griffith 202* (RSA)
Cylindropuntia rosea (de Candolle) Backeberg	Puebla, Mexico	*Griffith 187* (RSA)
Cylindropuntia tunicata (Lehmann) Knuth	Coahuila, Mexico	*Griffith 256* (RSA)
Cylindropuntia whipplei (Engelm. & Bigelow) Knuth	San Juan County, NM, USA	*Porter s.n.* (RSA)
Grusonia aggeria (Ralston and Hilsenbeck) Anderson	S R S U	*Powell 6006* (SRSC)
Grusonia bradtiana (Coulter) Britton & Rose	D B G 1985 0345	
Grusonia invicta (Brandegee) Anderson	R S A	*Griffith 218* (RSA)
Grusonia grahamii (Engelm.) H. Rob.	S R S U	*Hardy 634* (SRSC)
Grusonia marenae (Parsons) Anderson	D B G 1954 4980	
Grusonia parishii (Orcutt) Pinkava	R S A	*Wisura s.n.* (RSA)
Grusonia pulchella (Engelm.) H. Rob.	Churchill County, NV, USA	*Griffith 210* (RSA)
	Esmeralda County, NV, USA	*Griffith 353* (RSA)
Grusonia villis (Rose) H. Rob.	Coahuila, Mexico	*Griffith 246* (RSA)
Maihuenia patagonica (Philippi) Britt. & Rose	Hershkovitz and Zimmer, 1997	
Maihuenia poeppigii (Otto ex Pfeiffer) Philippi ex Schumann	Hershkovitz and Zimmer, 1997	
Maihueniopsis atacamensis (Philippi) Ritter	D B G 2001 0112	
Miqueliopuntia miquelii (Monville) F. Ritter	D B G 1997 0129	
Nopalea cochenillifera (L.) Salm-Dyck	D B G 1997 0395	
Opuntia chaffeyi Britt. & Rose	D B G 1990 0238	
Opuntia erinacea Engelm. & Bigelow	Mono County, CA, USA	*Honer 658* (RSA)
Pereskiopsis aquosa (Weber) Britt. & Rose	D B G 1997 0001	
Pereskiopsis porteri (Brande. ex Weber) Britt. & Rose	Hershkovitz and Zimmer, 1997	
Pereskiopsis diguettii (Weber) Britt. & Rose	Michoacan, Mexico	*Griffith 169* (RSA)
Pterocactus gonjianii Kiesling	D B G s.n.	
Quiabentia chacoensis Backeberg	D B G 1985 0046	
Tacinga inamoena (Schumann) Stuppy & Taylor	D B G 1999 0017	
Tephrocactus aeroacanthus (Lemaire) Lemaire	D B G 2001 0115	
Tunilla corrugata (Salm-Dyck) Hunt & Iliff	D B G Hunt 66371	

*D B G = Desert Botanical Garden, 1201 North Galvan Parkway, Phoenix, Arizona, USA; R S A = Rancho Santa Ana Botanic Garden, 1500 North College Avenue, Claremont, California, USA; S R S U = Sul Ross State University Cactus Garden, Alpine, Texas, USA.

trees. In addition, the type species of *Grusonia* (*G. bradtiana*) is sister to all sampled species of *Cylindropuntia* and *Grusonia*, with the exception of *G. pulchella*. The remaining *Grusonia* species sampled form a monophyletic group, although this clade also lacks strong statistical support. Since the paraphyly of *Grusonia* sensu Anderson (2001) lacks such support, hypotheses that propose an affinity between *G. pulchella* and other grusonioid cacti (Engelmann, 1863; Britton and Rose, 1919; Benson, 1957, 1982; Anderson, 2001; Stuppy, 2002) cannot be rejected by these data. Although the relationships between *Cylindropuntia* and *Grusonia* also lack strong statistical support, these data provide evidence that *Cylindropuntia* is nested within *Grusonia* sensu Anderson (2001). The current circumscription of *Grusonia* (Anderson, 2001; Stuppy, 2002) therefore taxonomically recognizes a non-monophyletic group.

Although the ITS data do resolve differences between *Cylindropuntia* and *Grusonia*, the sister group relationship of *G. pulchella* to all other non-tropical North American terete-stemmed opuntioids may indicate a distinct evolutionary lineage for the sand cholla. In the context of recent tendencies to split the large genus *Opuntia* sensu Benson (1982) into smaller genera reflecting natural groups (Robinson, 1973; Anderson, 1999, 2001; Stuppy, 2002; Wallace and Dickie, 2002), I propose a solution to improve the taxonomy of the grusonioid cacti. In the context of the morphological evidence, geographical separation, and the monophyly of the *G. pulchella* lineage, the recognition of these plants under the genus *Micropuntia* is supported. The generic concept of *Grusonia* may revert to that of Britton and Rose (1919), circumscribing only the type species *G. bradtiana*. Support for this narrow concept can also be found in that species' morphological distinctness from other *Grusonia* species (Britton and Rose, 1919; Anderson, 2001; Stuppy, 2002) and in the monophyly of the remaining species. These remaining species of *Grusonia* sensu Anderson (2001) should be treated under the genus *Corynopuntia* sensu Knuth (1935), including *G. marenae* (Parsons) Anderson (but excluding *G. pulchella*). Morphological support for this generic concept exists in the form of seed characters used to circumscribe *Grusonia* subg. Corynopuntia sensu Stuppy (2002), in addition to the characters discussed above. A summary of the generic circumscriptions proposed here is presented in Table 2. The other solution would be to include *Corynopuntia*, *Cylindropuntia*, *Marenopuntia*, and *Micropuntia* into a broadly circumscribed *Grusonia* (which has priority). I am not in favor of this option, although this broad circumscription would certainly recognize a monophyletic group. However, it would unnecessarily obscure the natural morphological diversity of the lineage as well as the morphological cohesiveness of many of its members.

I am aware of the lack of strong statistical support for many of the relationships suggested by the DNA sequence data. However, as there is even less support for the monophyly of *Grusonia* sensu Anderson (2001), I propose the recognition of these older generic concepts. Ongoing investigations involving additional DNA sequencing of nuclear and chloroplast genes and anatomical work may further elucidate these relationships, allowing for the most perceptive taxonomy possible. Further investigations may also elucidate whether specific rank is warranted for the various morphotypes (Daston, 1946; Benson, 1957) currently circumscribed within *Micropuntia pulchella*.

Nomenclatural Changes

The following new combinations are needed in support of this manuscript:

Corynopuntia aggeria (Ralston and Hilsenbeck) M. P. Griffith, comb. nov. Basionym: *Opuntia aggeria* Ralston and Hilsenbeck, Madroño 4: 226. 1989.

Corynopuntia emoryi (Engelm.) M.P. Griffith, comb. nov. Basionym: *Opuntia emoryi* Engelm., Proc. Am. Acad. 3: 303. 1857.

Corynopuntia kunzei (Rose) M.P. Griffith, comb. nov. Basionym: *Opuntia kunzei* Rose, Smithsonian Misc. Collect. 50: 505. 1908.

Corynopuntia marenae (Parsons) M.P. Griffith, comb. nov. Basionym: *Opuntia marenae* Parsons, Desert Pl. Life 8: 10. 1936

Micropuntia pulchella (Engelm.) M.P. Griffith, comb. nov. Basionym: *Opuntia pulchella* Engelm., Trans. Acad. Sci. St. Louis 2: 201. 1863.

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Table 2. Summary of generic circumscriptions presented here

Genus	Included species	Synapomorphies, Geography
Corynopuntia Knuth	*C. aggeria* (Ralston & Hilsenbeck) M.P. Griffith *C. agglomerata* (Berger) Knuth *C. bulbispina* (Engelm.) Knuth *C. clavata* (Engelm.) Knuth *C. dumetorum* (Berger) Knuth *C. emoryi* (Engelm.) M.P. Griffith *C. grahamii* (Engelm.) Knuth *C. invicta* (Brandegee) Knuth *C. kunzei* (Rose) M.P. Griffith *C. marenae* (Parsons) M.P. Griffith *C. moellerina* Knuth *C. parishii* (Orcutt) Knuth *C. reflexispina* (Wiggins & Rollins) Backeberg *C. schottii* (Engelm.) Knuth *C. vilis* (Rose) H. Robinson	Mat-forming habit with growth to 50 cm (60 cm in *C. marenae*) composed of clavate segments (terete in *C. marenae*). Spines of fruits retrorsely barbed, stiff. Deserts and grasslands of Northern Mexico and Southwestern United States to Nye County, Nevada.
Grusonia Reichenbach	*G. bradtiana* (Coulter) Britton & Rose	Mat-forming habit with erect, ribbed, jointed growth to 1 m. Flowering areoles without glochids. Chihuahuan Desert of Coahuila, Mexico.
Micropuntia Daston	*M. pulchella* (Engelm.) M.P. Griffith	Cushion habit to 20 cm high; storage tuber with areoles, glochids. Spines of fruit antrorsely barbed, flexible. Great Basin of Nevada, Utah, and California, United States.

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