×*Cylindronia*, a new nothogenus representing the first reported hybrid between *Grusonia* and *Cylindropuntia* (Cactaceae)

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Abstract: The rare Baja California Sur endemic cactus *Grusonia robertsii* was originally hypothesized to be an intergeneric hybrid between *G. invicta* and *Cylindropuntia alcahes* subsp. *alcahes* but was described as a *Grusonia* due to its overall closer resemblance to *G. invicta*, except in the fruit. A more comprehensive analysis of the morphology and phylogenetic placement of *G. robertsii* based on plastid and nrDNA sequences has revealed that Rebman's original hypothesis was correct, and this taxon represents the first documented intergeneric hybrid between *Cylindropuntia* and *Grusonia*, with *G. invicta* as the maternal parent and *C. alcahes* subsp. *alcahes* as the paternal contributor. We here describe a new nothogenus, ×*Cylindronia*, and provide a new combination for the nothospecies ×*Cylindronia robertsii* comb. nov.

INTRODUCTION

Individuals of Grusonia robertsii Rebman were first discovered in 1992 by Jon Rebman, who initially thought it to be a naturally occurring intergeneric hybrid between G. invicta (Brandegee) E. F. Anderson and Cylindropuntia alcahes (F. A. C. Weber) F. M. Knuth subsp. alcahes (Rebman 2006) (Figure 1). After further morphological evaluation of the original specimen and five additional individuals found near the type locality, Rebman determined that G. robertsii represented a new, rare species of Grusonia F. Rchb. ex Britton & Rose. He based this decision on the presence of deciduous spine sheaths occurring only at the spine apex, major spines that were flattened to angled at the base, pericarpel areoles with long tufts of wool, and a low growth habit, all of which are characteristics of the genus Grusonia. He noted, however, that the narrowly obconic to ellipsoid shape of the fertile fruits were more similar to those found in many species of Cylindropuntia (Engelm.) F. M. Knuth.

Baker and Cloud-Hughes revisited the type locality in 2017 and noted at least some main stems of *Grusonia robertsii* with younger stems branching in whorls of three or four. Such whorled stems are characteristic of *Cylindropuntia* but generally not of *Grusonia*, although the dense branching of *G. invicta* makes this character difficult to assess. Other character values, such as those for stem shape, tubercle shape, and spine number, length, and thickness also appeared intermediate between those of the local *G. invicta* and



Figure 1. Paratype specimen of *Grusonia robertsii*, with Rebman's notes indicating it may be an intergeneric hybrid.



Figure 2. Comparison of stem, tubercle, spine, and fruit morphology of *Grusonia robertsii* (center) to its putative parents *Cylindropuntia alcahes* subsp. *alcahes* (left) and *Grusonia invicta* (right).

C. alcahes subsp. *alcahes* (Figure 2). Generally, within Cactaceae, F_1 hybrid progeny exhibit character states intermediate between their putative parents (Baker and Cloud-Hughes 2014).

The biogeography and demographics of the species also suggest spontaneous hybridization, in that there are only a few individuals of Grusonia robertsii known among thousands of individuals of the putative parents. Although herbarium data indicate that Cylindropuntia alcahes subsp. alcahes generally flowers earlier (February-May) than G. invicta (April-May), their flowering times do overlap. These morphological, demographic, and phenological data led us to revisit Rebman's original hybrid hypothesis through DNA analyses, as a DNA study of the tribe Cylindropuntieae Doweld by Majure et al. (2019) was already underway. Live material of G. robertsii was obtained from a clone of Rebman's holotype plant that had been in cultivation at the San Diego Natural History Museum, as well as living material of one accession from the Huntington Botanical Garden. We also conducted a quantitative morphological comparison among Grusonia robertsii and its putative parents, as explained in the Methods below.

Several naturally occurring intergeneric hybrids have been reported for Cactaceae (reviewed in

Machado 2008). Two of these are from the Baja California Peninsula: ×Pachgerocereus orcuttii (K. Brandegee) Moran, which is the putative hybrid between Bergerocactus emoryi Britton & Rose and Pachycereus pringlei Britton & Rose; and ×Myrtgerocereus lindsayi Moran, which is the putative hybrid between B. emoryi and Myrtillocactus cochal Britton & Rose (McGill 1977, Moran 1962a, 1962b). Only four individuals of ×P. orcuttii were originally reported by Moran, however, McGill was unable to relocate any of these. Similarly, only one of the three individuals of ×M. lindsayi originally reported by Moran was relocated by McGill. Other examples of naturally occurring intergeneric hybrids in North American cacti are ×Myrtillenocereus G. D. Rowley from Hidalgo, Mexico, which is known only from two individuals and is morphologically intermediate between Myrtillocactus geometrizans Console and Stenocereus dumortieri (Scheidw.) Buxb. (Glass 1964, Glass and Foster 1964); and xPachybergia apicicostata S. Arias & Terrazas, which is known only from a single individual and is morphologically intermediate between *Pachycereus pecten-aboriginum* (Engelm.) Britton & Rose and *Backebergia militaris* (Audot) Bravo ex Sánchez-Mej (Rowley 1982).

Table 1. Description of characters measured. Plant height and width were measured only once per individual; all other chara	۱C-
ters used in the morphological analyses were measured three times per individual.	

Character	Description
Plant height	Height of plant from soil level to the tallest stem, excluding spines
Plant width	Width of plant at its widest point, excluding spines
Branch angle	Mean angle between one or more of the main trunks and the primary branches, a divaricate branch equaling 90°
Trunk internode length	Mean length along a main trunk between two trunk nodes, identified by one or more living branches or branch scars
Stems per node	Mean number of stems branching from one trunk node
Stem length	Mean length of mature terminal stem segments, excluding spines
Stem diameter	Mean diameter at mid-stem of mature terminal stem segments, excluding spines
Stem tubercle length	Mean length of penultimate tubercles from mature terminal stem segments.
Stem tubercle width	Mean width of penultimate tubercles from mature terminal stem segments
Stem tubercle height	Mean height of penultimate tubercles from mature terminal stem segments
Central spine number	Mean number of central spines from areoles of tubercles as described above
Radial spine number	Mean number of radial spines from areoles of tubercles as described above
Central spine length	Mean length of longest central spines from areoles of tubercles as described above
Radial spine length	Mean length of longest radial spine from areoles of tubercles as described above
Central spine thickness	Mean thickness of longest central spine from areoles of tubercles as described above
Fruit length	Mean fruit length
Fruit width	Mean fruit width measured at widest portion
Fruit uppermost tubercle length	Mean length of the uppermost fruit tubercles
Fruit uppermost tubercle width	Mean width of the uppermost fruit tubercles
Fruit uppermost tubercle height	Mean height of the uppermost fruit tubercles
Fruit lowermost tubercle length	Mean length of the lowermost fruit tubercles
Fruit lowermost tubercle width	Mean width of the lowermost tubercle of fruit
Fruit lowermost tubercle height	Mean height of the lowermost fruit tubercles
Fruit areole number	Mean number of fruit areoles
Fruit spine number	Mean number of spines of uppermost fruit areoles
Fruit spine length	Mean spine length of uppermost fruit areoles
Fruit wall thickness	Mean width of fruit walls measured at widest portion

METHODS

MORPHOLOGY

Mean values for 15 stem characters and 12 fruit characters were calculated for *Cylindropuntia alcahes* subsp. *alcahes*, *Grusonia invicta*, and *G. robertsii*. This set of characters has been used successfully in other morphometric studies of *Cylindropuntia* (Baker and Cloud-Hughes 2014, 2018; Baker 2016). Measuring a large number of characters provides greater statistical resolution for distinguishing between taxa and allows for the production of more accurate keys and more complete descriptions of taxa for identification in the field. The characters measured are described in Table 1. Locality and voucher information is presented in Table 2. Stem character data for *Cylindropuntia alcahes* subsp. *alcahes* were collected from 30 individuals in 2017, and fruit character data were collected from 16 individuals at the same location in 2022. Five individuals of *G. robertsii* (Figure 3) and ten of *G. invicta* were measured at and near the type locality of *G. robertsii*. The five *G. robertsii* measured are all of the known individuals of the species for which we were able to obtain accurate locations.

Taxon	Locality	Latitute/ Longitude	N	Voucher
Cylindropuntia alcahes subsp. alcahes	Mexico, Baja California Sur, Mesa Los Crestones, Sierra San Francisco, 15 km SW of San Francisco, ca. 16 km due east of Hwy 1.	27.51°,113.13°	30 (stem characters, 2017) 16 (fruit characters, 2022)	Baker 15187, ASU0054194
Grusonia invicta	Mexico, Baja California Sur, Along MEX 1; ca. 1 km NW of Palo Verde Wash on the Mesa la Cantina.	27.29°,113.06°	10	<i>Baker</i> 8708, ASU0058928
Grusonia robertsii	Mexico, Baja California Sur, Vizcaíno Desert: between San Ignacio and Vizcaíno Junction; ca. 1 mile west of Highway 1 and just north of the road to Punto Abreojos.	27.33°,113.13°	5	Rebman 7795, SD00000097

Table 2. Localities for morphological measurements of Grusonia robertsii and its putative parents.

PHYLOGENETIC ANALYSIS

DNA sequences were sampled broadly across diploids of the tribe Cylindropuntieae based on available plastome data (Majure et al. 2019), and two accessions of *Grusonia robertsii* were sequenced using the genome skimming method (Straub et al. 2012). DNA extraction was carried out using a modified CTAB extraction buffer (Doyle and Doyle 1987) on either fresh or silicadried tissue. Library building and paired-end sequencing (150 bp) on the Illumina HiSeq XTen platform was carried out by Rapid Genomics (Gainesville, FL, USA). DNA accessions sampled are presented in Table 3.

Our raw data from genome skimming for available diploid taxa from Majure et al. (2019) were reference mapped to the large single copy unit of the Cylindropuntia bigelovii plastome (Majure et al. 2019), and an ETS-ITS sequence generated for C. abyssi (Majure unpubl. data) based on reference mapping against available ITS data in GenBank was used for reference-mapping all taxa for ETS-ITS. ETS-ITS is relatively conserved across Cylindropuntieae (Majure, unpubl. data), thus, any species within the clade could be used successfully to reference map ETS-ITS. A majority consensus sequence was generated for the two accessions of Grusonia robertsii to determine whether a clear majority genotype existed among the ETS-ITS sequences. Majority genotypes were determined based on mutations at specific sites within ETS-ITS sequences and were considered "majority" when they contained more raw reads than the contrasting genotype, which was considered the minority genotype (Majure 2022). Next, a 95% consensus sequence was generated for both accessions to illuminate those sites in the ETS-ITS sequences where conflicting bases occurred. At those sites, the Find Variations/SNPs function in Geneious Prime version 11.0.14.1+1 (http://www.geneious.com) was used to determine the percentage of base occurrence at that site, with the minority genotype expected



Figure 3. *Grusonia roberstii* individuals measured for morphometric analysis. Type locality is the westernmost location.

to have a smaller percentage of occurrences at a given site. Once identified, the minority bases at each site were used to generate an overall minority genotype for each of the two accessions, and the majority sequence was used as the majority genotype. Both the majority and minority genotypes were included as separate sequences in our final ETS-ITS alignment.

Our plastome and ETS-ITS alignments were carried out using MAFFT[®] (Katoh and Standley 2016), and we analyzed our datasets using the RAxML (Stamatakis 2014) plugin in Geneious using the default GTR+G model of molecular evolution and undertaking 1000 rapid bootstraps. *Micropuntia pulchella* was used as an outgroup for both datasets based on its sister placement to the *Grusonia* + *Cylindropuntia* clade (Majure et al. 2019). Because we were mainly interested in fine scale relationships between *Grusonia* and *Cylindropuntia*, a more distantly related outgroup taxon was considered unnecessary.

Table 3.	DNA	accessions	samp	led.
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Taxon	Accession
Cylindropuntia alcahes subsp. alcahes	<i>Baker</i> 8690 (DBG 1993057901), Mexico, Baja California along highway Mex 5; km 15; 2.1 miles NW of Las Arrastras Wash, 29.5766°, -114.3703°
Grusonia invicta	<i>Haughey</i> 249 (DBG 1993004001), Mexico, Baja California Sur, 7 miles S of San Ignacio, 27.1934° -112.8547°
Grusonia robertsii	<i>Majure</i> 7173 (FLAS), obtained from Huntington Botanical Garden 30 Aug 2018 (HBG 93485), which was obtained as a seedling September 1980 from the Arizona-Sonora Desert Museum (AZDM 78-050-01), originally collected by R. Perrill et al., 3 Nov 1978, Mexico, Baja California Sur, 3 mi SW of San Ignacio, on road to El Alamo, 190 m elev., 27.2437°, -112.9252°. <i>Rebman</i> 7795 (ASU, HCIB, SD), 28 Oct 2001, Mexico, Baja California Sur, Vizcaíno Desert: between San Ignacio and Vizcaíno Junction; ca. 1 mile west of Highway 1 and just north of the road to Punto Abreojos, 27.3253°, -113.134°.

RESULTS

MORPHOLOGY

Table 4 shows character value means and standard deviations for the measured taxa as well as character means for a hypothetical F_1 hybrid between *Cylindropuntia alcahes* subsp. *alcahes* and *Grusonia invicta* calculated by averaging the character means of the parent species. For *G. robertsii*, all but four of the 27 characters sampled had mean character values intermediate to those of *C. alcahes* subsp. *alcahes* and *G. invicta*. The intermediate morphology of the habit, stem and fruit of *Grusonia robertsii* compared to its putative parents are illustrated in Figure 4.

The four exceptions were branch angle, stem length, and tubercle heights for both the upper and lower fruit tubercles. Branch angle from the main stem(s) was highest for *Grusonia robertsii*, although it was very similar to that of *Cylindropuntia alcahes*. Branch angles were estimated, and these estimates are likely somewhat inaccurate for *G. invicta*, due to the crowded nature of the stems. Stem length was highest for *G. robertsii* and was much closer to that of *G. invicta*. Fruit upper tubercle height was highest for *G. robertsii* and considerably exceeded that of both putative parents. Although fruit lower tubercle height was higher than the expected intermediate value, it matched that for *C. alcahes*, and the overall range among all three entities was small.

Standard deviations for the character means of *Gru*sonia robertsii were equal to or higher than one or more of its putative parents for 18 of the 27 characters. High variation is to be expected in rare cases of spontaneous hybridization across a large range, due to morphological variation in the parent species across large distances and variable habitats. This variation in the individual morphology of *G. robertsii* is particularly apparent in the fruits, as shown in Figure 5.

PHYLOGENETIC ANALYSES

Our plastome data, as in Majure et al. (2019) resolved a well-supported *Grusonia* sister to *Cylindropuntia*, and subclades were well-supported. ETS-ITS data did not resolve relationships among *Grusonia* and *Cylindropuntia*, and many relationships among subclades also were not well-resolved. The lack of resolution at these deeper levels is expected for ETS-ITS, and in general for Cactaceae (Ritz et al. 2012), where this locus may be homoplasious, and it is better suited for finer scale, species level relationships (Majure 2022).

Plastome and nrDNA data placed *Grusonia rob*ertsii in two separate clades. Our plastome dataset firmly placed *G. robertsii* as sister to *G. invicta* (bootstrap = 100, Figure 6), while our reference-mapped, majority consensus genotypes of ETS-ITS placed *G. robertsii* in a clade with members of the Californica clade (bs = 99) in an unsupported subclade consisting of *C. alcahes* s.l. (Figure 6). Our minority genotype placed *G. robertsii* as sister to *G. invicta* (bs = 99). Plastome and ETS-ITS trees showing the placement of *G. robertsii* with its putative parents are shown in Figure 6.

Based on the majority consensus sequences derived from ETS-ITS, four distinct nucleotides are shared among *Grusonia robertsii* and mostly members of the Californica clade: *Cylindropuntia alcahes* subsp. *alcahes* (E.A.C. Weber) F.M. Knuth, *C. alcahes* subsp. *burrageana* (Britton & Rose) U. Guzmán, *C. alcahes* subsp. *gigantensis* (Rebman) Rebman, *C. californica* (Torr. & A. Gray) F.M. Knuth subsp. *californica, C. californica* subsp. *rosarica* (G.E. Linds.) U. Guzmán, *C. delgadilloana* Rebman & Pinkava, *C. ganderi* (C.B. Wolf) Rebman & Pinkava, and *C. munzii* (C.B. Wolf) Backeb. These are at positions 67 (T), 247 (C), and 480 (T) in ETS and position 2492 (A) in ITS 1.

Based on the minority genotypes of ETS-ITS of *Grusonia robertsii*, nine sites were shared with *Grusonia*, and in some cases were specific to *G. invicta*. Three sites in ETS were shared with *Grusonia*, at sites 21 (G), 121 (A), and 242 (T), and six sites were shared in ITS, at sites 2321 (G), 2413 (A), 2452 (T), and 2475 (A) in ITS 1 and sites 2726 (T) and 2839 (T) in ITS 2.

Based on the ranges of percentages of variable sites between the majority and minority genotypes, the majority genotype was present in 54.81-62%of reads, while the minority genotype was present in 38-45.19% of the reads. Thus, the majority and putatively paternal genotype is clearly derived from *Cylindropuntia*, while the minority and puta**Table 4.** Character value means for taxa measured and hypothetical F1 hybrid. Bold indicates values not intermediate between the putative parent species.

Character	<i>Cylindropuntia alcahes</i> subsp. <i>alcahes</i> (stems, n=30; fruit, n=16)		Grusonia invicta (n = 10)		Grusonia robertsii (n = 5)		Hypothetical <i>C. alcahes</i> subsp. <i>alcahes</i> × <i>G. invicta</i> F1
	Mean	SD	Mean	SD	Mean	SD	
Plant height	79.3	18.7	34.3	6.1	51.4	8.5	56.8
Plant width	123.8	40.3	63.2	24.7	116.0	64.3	93.5
Branch angle	62.3	14.2	49.8	13.6	65.0	7.3	56.1
Trunk internode length	10.9	3.2	6.0	2.4	10.8	4.4	8.4
Stems per node	2.8	0.7	1.3	0.4	2.3	0.3	2.1
Stem length	76.4	12.2	86.1	9.8	89.7	11.7	81.3
Stem diameter	29.7	3.4	57.4	5.6	37.3	1.6	43.5
Stem tubercle length	20.4	2.2	52.3	4.0	33.1	3.2	36.3
Stem tubercle width	10.2	1.4	17.5	3.5	11.5	1.1	13.8
Stem tubercle height	5.1	1.3	18.3	2.1	10.7	1.9	11.7
Central spine number	3.8	1.1	6.2	0.6	5.7	1.3	5.0
Radial spine number	4.4	1.0	8.9	1.4	7.7	1.2	6.7
Central spine length	12.7	2.2	50.2	4.4	41.7	5.0	31.4
Radial spine length	10.1	1.6	35.3	3.8	24.5	5.5	22.7
Central spine thickness	34.0	4.3	247.6	51.9	125.2	13.7	140.8
Fruit length	24.6	3.2	33.0	3.9	32.3	6.7	28.8
Fruit width	22.1	3.0	28.1	4.7	22.6	0.7	25.1
Fruit uppermost tubercle length	5.7	1.1	8.0	1.5	7.9	1.4	6.8
Fruit uppermost tubercle width	4.7	0.6	6.6	1.2	5.4	0.3	5.6
Fruit uppermost tubercle height	2.7	0.6	2.5	1.1	3.1	0.6	2.6
Fruit lowermost tubercle length	12.2	1.4	17.9	3.5	13.4	3.9	15.0
Fruit lowermost tubercle width	5.4	0.9	7.9	3.5	6.3	1.5	6.7
Fruit lowermost tubercle height	2.5	0.8	2.3	1.1	2.5	0.5	2.4
Fruit areole number	28.2	4.7	40.0	10.1	27.2	3.0	34.1
Fruit spine number	2.0	2.1	17.4	2.3	4.8	0.8	9.7
Fruit spine length	10.4	4.6	21.4	3.6	17.1	3.6	15.9
Fruit wall thickness	7.2	1.7	4.3	1.6	6.3	3.2	5.7



Figure 4. Intermediate morphology of the habit (top), stem (middle), and fruit (bottom) of *Grusonia robertsii* (center) compared to *Cylindropuntia alcahes* subsp. *alcahes* (left) and *Grusonia invicta* (right).

tively maternal genotype is derived from *Grusonia* and is most closely related to *G. invicta*. Given the plastome data, it is clear that the maternal parent in this case was *G. invicta*, and based on the nrDNA data, the paternal parent was a member of the Californica clade of *Cylindropuntia*, most likely *C. alcahes* based on our ML topology.

DISCUSSION

Both morphological and DNA sequence data provide evidence for a hybrid origin for *Grusonia robertsii* between *G. invicta* and *Cylindropuntia alcahes* subsp. *alcahes*. Given that only a few individuals of *G. robertsii* are known and occur among thousands of individuals of its putative parents with no evidence of backcrossing, we feel that individuals of *G. robertsii* represent a rare spontaneous hybrid, indicating the close relationship between *Grusonia* and *Cylindropuntia*. This is in agreement with Majure et al. (2019), which resolved *Grusonia* as a sister taxon to *Cylindropuntia*. In contrast, no hybrids have been reported between either *Grusonia* or *Cylindropuntia* and the genus *Opuntia*, which is a more distant relative.

The occurrence of a single intergeneric hybrid does not suggest that *Grusonia* and *Cylindropuntia* should be circumscribed as a single genus, as was suggested by Rowley (2006), and which has been contraindicated by multiple more recent studies (Barcenas 2015, Majure et al. 2019, Mayer and Rebman 2021). That thousands of



Figure 5. Variation in *Grusonia robertsii* fruit morphology among widely-scattered individuals, indicative of rare spontaneous hybridization events.

individuals of two closely related genera manage only on rare occasions to produce a hybrid individual that survives to maturity demonstrates the strength of the reproductive barriers between the genera. That those barriers may occasionally be circumvented does not indicate that the genera should be lumped; simply that the genera have not yet diverged enough to make reproductive isolation absolute.

The existence of the previously discussed Baja intergeneric hybrids ×Pacherocactus orcuttii and ×Myrtgerocactus lindsayi led McGill (1977) to suggest lumping Bergerocactus emoryi, Myrtillocactus cochal, and Pachycereus pringlei within the genus Cereus. However, phylogenetic studies in more recent years support the separation of Cereus s. l. into many genera. Guerrero et al. (2019), for example, concluded that the North American columnars comprise two clades, corresponding to subtribes: Pachycereinae (Pachycereus, Cephalocereus, Carnegiea, etc.) and Stenocereinae (Stenocereus, Echinocereus, Myrtillocactus). Thus, Pachycereus pringlei and Myrtillocactus geometrizans belong not only to separate genera but also separate subtribes. This is similar to the hybridization events uncovered by Majure et al. (2012) between the genera Consolea and Opuntia, where at least four intergeneric hybrids are known (Majure et al. 2012; Majure et al. unpubl. data).

While naturally occurring intergeneric hybrids are uncommon within the Cactaceae, interspecific hybridization is recurrent within many genera. Majure et al. (2012, 2014), Granados-Aguilar et al. (2021), and Majure (2022) uncovered rampant hybridization in Opuntia and suggested that many of the polyploids in that genus are likely derived from interspecific hybridization, although homoploid hybridization, i.e. hybridization without genome duplication, is much more poorly documented (Majure and Puente 2014). Arrojadoa Britton & Rose, Cylindropuntia, Grusonia, Melocactus Link & Otto, Pilosocereus Byles & G.D. Rowley, Tacinga Britton & Rose, and numerous other cactus genera have reported interspecific hybrids as well (e.g., Baker and Pinkava 1987, 1999; Fenstermacher 2016, Mayer et al. 2000, Pinkava 2002, reviewed in Machado 2008).

Very few studies in Cactaceae have outlined the percentage of parental genotypes in hybrid progeny, but this is of interest given the potential for gene conver-

sion or unequal crossing over in nrDNA loci, especially for later generation hybrids. In general, rates of gene conversion or unequal crossing over in nrDNA loci -- the components necessary for concerted evolution in these loci (Felliner and Roselló 2007) -- should be much higher in sexual species, where recombination is frequent, or those with longer evolutionary histories, which have had time for greater recombination. Thus, hybrids may show more equivalent percentages from the parents if the time since the initial hybridization event is more recent (i. e. early generation hybrids), or if they are not able to produce functional gametes with the possibility for genetic recombination. The minority genotype was present in 38-45.19% of the nrDNA reads for Grusonia robertsii. Majure (2022) found similar percentages in minority genotype occurrence for the two allopolyploid Opuntia species O. curvispina Griffiths and O. martiniana (L. D. Benson) B. D. Parfitt, with those taxa showing an average of 29% and 42% of raw reads, respectively. The average of 41.6% of reads pertaining to the minority genotype in G. robertsii contrasts with that of Opuntia studied, given that the minority genotype was apparently maternally inherited from G. invicta, while the majority genotype was paternally inherited from Cylindropuntia alcahes. The majority genotypes in O. curvispina and O. martiniana, by contrast, were maternally inherited. Majure (2022) considered that the higher percentage of minority genotypes in O. martiniana could reflect a younger age for that species, and thus reduced time for recombination and gene conversion (Feliner and Roselló 2007). This could also help explain the higher percentage of the minority genotype in G. robertsii. One of the putative parents, C. alcahes, belongs to one of the youngest clades within Cylindropuntia, the Californica clade, dating to the Pleistocene (Majure et al. 2019), thus, any hybrid derived from that species must necessarily be relatively young. Grusonia robertsii produces seed, although we have no data on viability. Apomixis could also play a role in the reduction of recombination in this hybrid species, further reducing the effects of concerted evolution in ETS-ITS.

That *Grusonia invicta* is the ovule donor in this hybridization scenario and *Cylindropuntia alcahes* the pollen donor is not surprising given the taller stature and



Figure 6. Plastome (left) and ETS-ITS (right) trees showing the placement of *Grusonia robertsii* with its putative parents. The plastome tree shows a well-supported and close relationship with the maternal parent *Grusonia invicta*, while the ETS-ITS tree shows support for the placement of the species with both *G. invicta* (minority genotype) and *Cylindropuntia alcahes* (majority genotype), suggesting *C. alcahes* as the paternal parent. Collector/institution initials and collection/accession number are included at the tips: DBG = Desert Botanical Garden; JPR = J. Rebman; LCM = L. Majure; MAB = M. Baker; MCH = M. Cloud-Hughes.

greater numbers of flowers and thus pollen load of *C. alcahes* compared to the lower-statured, fewer-flowered *G. invicta.* Similar patterns are seen in *Opuntia stricta* (Haw.) Haw. (including *O. dillenii* (Haw.)) and species with which it hybridizes. In all cases of hybridization studied to date of crosses between *O. stricta* and smaller-statured, fewer-flowered species of *Opuntia,* such as *O. abjecta* Small and *O. militaris* Britton & Rose, *O. stricta* is the paternal parent (Majure et al. 2014).

Our morphological results strongly support the origin of *Grusonia robertsii* through hybridization between *Cylindropuntia alcahes* subsp. *alcahes* and *Grusonia invicta*, with mean character values for 23 of the 27 characters measured falling between those of the parent species. The widely scattered distribution, morphological variation, and overall extreme rarity of *G. robertsii* suggest that this spontaneous hybridization event has occurred multiple times.

While small sample size likely explains some of the four *Grusonia robertsii* character means that equal or exceed those of its parents, there may be additional reasons in each case. That mean branch angle for *G. robertsii* exceeds that of its parents is likely due to a combination of the general similarity of the branch angle means in the parents and the difficulty of accurately measuring branch angle in low, compact plants such as *Grusonia*. The mean branch length of the ultimate stems of *G. robertsii* being longer than those of the parent species may be a function of stele morphology. Further investigation along these lines is warranted. The larger mean upper and lower fruit tubercle height for *Grusonia robertsii* is likely explained by the fact that both parent species have very similar mean values for these characters, as well as the difficulty in accurately measuring tubercle height, particularly in low-tubercled taxa. It is also possible that the higher fruit tubercles of *G. robertsii* may be a function of sterility, as sterile fruits in *Cylindropuntia* tend to have more prominent tubercles. However, half (3 of 6) of the *G. robertsii* fruits examined contained seeds that appeared normal and healthy.

Flowers of *Grusonia robertsii* have only been observed in cultivation, and there are no known data on pollen viability, seed viability, or seed genetic makeup for *G. robertsii*. Cross-pollination between *G. robertsii* individuals is unlikely given the extreme distances separating them. Seeds therefore probably result from either self-pollination, pollination by a related species, or apomixis. None of these hypotheses have been tested, and these data would provide useful insights into the evolutionary implications of hybridization in the Cactaceae.

CONCLUSION

Our morphological and molecular data indicate that *Grusonia robertsii* originated through spontaneous hybridization between *Cylindropuntia alcahes* subsp. *alcahes* and *G. invicta*. The extreme rarity of *G. robertsii* and the long distances between the few known individuals suggests that this hybridization event has occurred multiple

times. In the nomenclature section below, we describe a new nothogenus, *×Cylindronia*, and a new nothospecies, *×Cylindronia robertsii*. Future surveys for this hybrid and additional morphometric and genetic data may lead to further insights regarding higher-level hybridization events in the Cactaceae and other plant groups.

NOMENCLATURE

×Cylindronia M.A. Baker, Majure, Cloud-H., and Rebman nothogen. nov.

Etymology: The nothogenus name is derived from the parent genera, *Cylindropuntia* and *Grusonia*. The specific epithet is for Norman Roberts, a naturalist and explorer of the deserts of Lower California who contributed immensely to the knowledge and enjoyment of plants in Baja with his Baja California Plant Field Guide (1989) and through his promotion and facilitation of botanical studies and explorations of the region.

×Cylindronia robertsii (Rebman) M. A. Baker, Majure, Cloud-H. & Rebman (Cylindopuntia alcahes subsp. alcahes × Grusonia invicta) comb. nov.

Basionym: Grusonia robertsii Rebman, Type: Mexico, Baja California Sur: Vizcaíno Desert near 27.317N, -113.133W, between San Ignacio and Guerrero Negro, 28 October 2001, *Rebman* 7795 (holotype: SD 148287; isotypes: ASU268882, BCMEX, HCIB 22943)

Paratypes: MEXICO, Baja California Sur: Vizcaíno Desert, between San Ignacio and Guerrero Negro, 10 May 1992, *Rebman* 1361 (ASU 187542 [The words "intersubgeneric hybrid? J. Rebman" handwritten on the sheet.], (DES 37108) [The words "possible *Corynopuntia* × *Cylindropuntia* hybrid *O. invicta* × ? 1995 J. R." handwritten on the sheet], (SD 155542), (HCIB 3279); ibid, 10 March 1998, *Rebman* 4836 & N. *Roberts* (SD 143343).

Cytology: *n*=11 during meiosis of microgametogenesis, with meiosis appearing regular. Mexico, Baja California Sur, N27.3241° W113.1295°, 145m elevation, SW base of Cerro Las Mulas, 6 March 2017, *M. Baker* 18718 & *M. Cloud-Hughes* (BCMEX, SD).

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AUTHOR CONTRIBUTIONS

Marc Baker: Conceptualization; Methodology; Validation; Formal Analysis; Investigation; Resources; Data Curation; Writing – Original Draft; Writing – Review and Editing; Visualization; Supervision; Project Administration; Funding Acquisition. Lucas Majure: Conceptualization; Methodology; Validation; Formal Analysis; Investigation; Resources; Data Curation; Writing – Original Draft; Writing – Review and Editing; Visualization; Supervision; Project Administration; Funding Acquisition. **Michelle Cloud-Hughes**: Conceptualization; Methodology; Validation; Formal Analysis; Investigation; Resources; Data Curation; Writing – Original Draft; Writing – Review and Editing; Visualization; Supervision; Project Administration; Funding Acquisition. **Jon Rebman**: Conceptualization; Methodology; Validation; Formal Analysis; Investigation; Resources; Data Curation; Writing – Original Draft; Writing – Review and Editing; Visualization; Supervision; Project Administration; Funding Acquisition.

DATA AVAILABILITY

Morphological data: Contact corresponding author, Michelle Cloud-Hughes (mcloudhughes@gmail.com)

Genetic data: Figshare (DOI: 10.6084/ m9.figshare.21362700)

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