

A MORPHOLOGICAL STUDY ON THE QUESTION OF THE HYBRID ORIGIN OF *ASTROPHYTUM COAHUILENSE* (CACTACEAE)

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ABSTRACT

Two alternative hypotheses may explain the origin of *Astrophytum coahuilense*. One hypothesis is that *A. coahuilense* is an ancient diploid hybrid derived from interbreeding between *A. capricorne* and *A. myriostigma*. An alternative is that *A. coahuilense* arose via allopatric speciation and its similarity to *A. myriostigma* in vegetative characters is due to convergent evolution. The two hypotheses are tested, applying univariate analysis and Non-metric Multidimensional Scaling analysis to morphological datasets. Univariate analysis, which is based on 13 vegetative and 10 reproductive characters, shows that the morphology of *A. coahuilense* is complex, consisting of a combination of vegetative and reproductive characters shared with both *A. capricorne* and *A. myriostigma* as well as several intermediate traits. Furthermore, the morphology of *A. coahuilense* lacks unique, fixed characteristics. Non-metric Multidimensional Scaling analysis of 15 characters (three vegetative and 12 reproductive traits) produced a two-dimensional scatterplot in which the cluster representing *A. coahuilense* occupies a position intermediate between the clusters of the two parental species along Dimension 1. Thus, both analyses support the hypothesis that *Astrophytum coahuilense* originated through hybridization between *A. capricorne* and *A. myriostigma*. Biological and ecological factors that may have facilitated homoploid hybrid speciation are discussed.

RESUMEN

Dos hipótesis alternativas pueden explicar el origen de *Astrophytum coahuilense*. Una hipótesis es que *A. coahuilense* es un antiguo híbrido diploide derivado del mestizaje entre *A. capricorne* y *A. myriostigma*. Una alternativa es que *A. coahuilense* surgió mediante especiación alopatrica y su similitud con *A. myriostigma* en caracteres vegetativos se debe a una evolución convergente. Las dos hipótesis se prueban aplicando análisis univariado y análisis de escala multidimensional no métrico a conjuntos de datos morfológicos. El análisis univariante, que se basa en 13 caracteres vegetativos y 10 reproductivos, muestra que la morfología de *A. coahuilense* es compleja y consiste en una combinación de caracteres vegetativos y reproductivos compartidos tanto de *A. capricorne* como de *A. myriostigma*, así como varios rasgos intermedios. Además, la morfología de *A. coahuilense* carezca de características únicas. El análisis de escalamiento multidimensional no métrico de 15 caracteres (tres vegetativos y 12 reproductivos) produjo un diagrama de dispersión bidimensional en el que el grupo que representa *A. coahuilense* ocupa una posición intermedia entre los grupos de las dos especies parentales a lo largo de la Dimensión 1. Por lo tanto, ambos los análisis apoyan la hipótesis de que *A. coahuilense* se originó a través de la hibridación entre *A. capricorne* y *A. myriostigma*. Se discuten los factores biológicos y ecológicos que pueden haber facilitado la especiación de híbridos homoploides.

INTRODUCTION

The “Coahuila Bishop’s Cap,” *Astrophytum coahuilense*, is an enigmatic cactus species; its stem morphology is like that of the Common Bishop’s Cap, *A. myriostigma* (subgenus *Astrophytum*), but several fruit and floral characters are shared with those of the Goat horn Cactus, *A. capricorne* (subgenus *Neoastrophytum*). H. Möller (1927) originally described *A. coahuilense* as a subspecies of *A. myriostigma*, despite finding differences in fruit and floral traits and cross-incompatibility between them. The striking similarity in vegetative characters between the two taxa may have been the reason that Möller assigned subspecific status to *A. coahuilense*. However, Kayser (1932) argued that despite their close resemblance, the two taxa were reproductively isolated and therefore should be recognized as two distinct species. Although Kayser’s argument is logical, many researchers, especially in Mexico, the United States, and Great Britain, have continued to treat *A. coahuilense* as a subspecies or synonym of *A. myriostigma* until quite recently.

Different interpretations of the phylogenetic position of *Astrophytum coahuilense* have been offered by various authors. Megata (1944) regarded it as a species “transitional” between his proposed subgeneric sections *Austrastrophytum* and *Septentriastrophytum* (invalid names; see Montanucci 2009). Backeberg (1961)

established two subgenera, *Neoastryphytum* and *Astryphytum* and placed *A. coahuilense* in the former subgenus without speculating about its evolutionary origin. Haage and Sadovský (1957) and Sadovský and Schütz (1979) did not recognize subgeneric categories, but informally described two species groups within *Astryphytum* based on “certation cross-pollination” experiments. The authors placed *A. coahuilense* in the group which included *A. asterias* and *A. capricorne* and its varieties, thus essentially agreeing with Backeberg’s (1961) interpretation.

The unusual admixture of traits in *Astryphytum coahuilense* led Hoock (1993) to propose that this species is an ancient hybrid originating through hybridization between *A. myriostigma* and *A. capricorne*, or their immediate progenitors. Hoock cited sterility barriers between the putative ancient hybrid and its parents as evidence of a close relationship to both species. He theorized that climatic oscillations during the Pleistocene enabled *A. myriostigma* to spread northward from the Central Plateau of Mexico, where it encountered and hybridized with *A. capricorne* in the region of southern Coahuila. This hybrid population became stabilized and established, acquiring the spineless, five-ribbed “star cactus” vegetative form of *A. myriostigma*, and some of the floral and fruit characteristics of *A. capricorne*. Hoock’s hypothesis seemed plausible, but it would require comprehensive and quantitative morphological analysis.

Vázquez-Lobo et al. (2015), apparently unaware of Hoock’s paper, offered their own hypothesis for the origin of *Astryphytum coahuilense*. The authors presented a biogeographic history and phylogeny for the genus *Astryphytum* based on an analysis of three gene sequences of chloroplast DNA (*rbcl* gene, *trnL-trnF* intergenic spacer, and *trnK-matK* region). The authors used Bayesian and Maximum likelihood methods to generate a phylogenetic tree for the genus. They determined that *Astryphytum* is monophyletic and contains six species grouped into two clades. The clades corresponded to the “northern” and “southern” radiations recognized as subgenera by Backeberg (1961) and defined by fruit and floral characters (Montanucci 2009). Vázquez-Lobo et al. (2015) determined that *A. caput-medusae* (formerly *Digitostigma caput-medusae*) originated within the genus, a conclusion previously supported only by morphological evidence (Montanucci 2009). The cladogram of Vázquez-Lobo et al. (2015) placed *A. coahuilense* within the “northern radiation” clade (i.e., subgenus *Neoastryphytum*) and placed *A. myriostigma* within the “southern radiation” clade (i.e., subgenus *Astryphytum*). Their cladogram depicted the lineage of *A. coahuilense* as having separated from the common ancestor of *A. asterias* and *A. capricorne* approximately 3.3 mya. The authors attributed the close similarity in stem morphology between *A. coahuilense* and *A. myriostigma* to convergent evolution.

In this study, the hypothesis that *Astryphytum coahuilense* is an ancient hybrid derived from hybridization between *A. capricorne* and *A. myriostigma* and an alternative hypothesis of allopatric speciation are tested by comparative morphological analyses. The morphology of *A. coahuilense* is also compared with synthetic F_1 hybrids derived from the putative parental species. In addition, results of cross-pollination experiments between *A. coahuilense* and its putative parents are interpreted in the context of homoploid hybrid speciation theory, and aspects of the biology of these taxa that may have facilitated homoploid hybrid speciation are discussed.

MATERIALS AND METHODS

Morphological analysis.—The univariate analysis of *Astryphytum coahuilense* (COA) and its putative parental species, *A. myriostigma* (MYR) (including subsp. *tulense*) and *A. capricorne* (CAP) as well as synthetic F_1 hybrids (MYRCAP) between the two species, is based on 13 vegetative characters and 10 reproductive characters. These include quantitative (meristic and mensural) characters, and qualitative (color, shape, presence/absence) characters. Morphological data from synthetic F_1 hybrid seedlings (MYRCAP) were obtained for comparisons with the seedlings of *A. coahuilense* and the putative parental species. Morphological comparisons with mature synthetic hybrids (MYRCAP) were based on literature descriptions because the hybrids produced in this study could not be grown to maturity due to slow growth rates.

The vegetative characters studied included stem height (in cm); stem diameter (in cm, at mid-height); number of ribs; rib angle (acute, intermediate, or obtuse); rib profile (angular or rounded); presence or

absence of spines; length of wool on new areoles (short or long); density of trichomes on stem epidermis (sparse, moderate, dense); color of the seedling hypocotyl; color, shape, and length (in mm) of seedling spines, and number of spines per areole.

The reproductive characters studied included diameter of the perianth (in mm); diameter of the red center of the flower (in mm, as a percentage of the diameter of the perianth); number of stigma lobes; type of nectar chamber protection, i.e., woolly “jamming hairs” or only anther filaments; floral scale length (in mm); density of lint on the floral tube; color and texture of the fruit (green, semi-dry vs. purplish red, semi-fleshy); mode of fruit dehiscence (basal or apical); type of floral remnant (persistent or deciduous).

In *Astrophytum*, the nectar chamber is a shallow groove that encircles the base of the style. Presumably, the nectary lies within or near the groove. Access to the nectar by small, non-pollinating insects may be impeded by tufts of woolly jamming hairs situated superior to the nectar chamber and interwoven among anther filaments and the style (Hooek 1988). To determine the presence or absence of jamming hairs, a median, longitudinal cut was made through a fresh flower. The flower’s interior was examined with a hand lens (14 ×), or in questionable cases, viewed under a stereoscopic microscope.

The statistics of dispersion, including sample mean (\bar{x}) \pm 95% confidence limits, and observed limits of variation were calculated for perianth diameter, number of stigma lobes, and diameter of colored flower center. These three quantitative floral characters were tested for significance of difference using the SAS system Mixed procedure t-tests. A correlation analysis was also performed to determine the potential relationship between perianth diameter and number of stigma lobes. The data for all characters (vegetative and reproductive) and sample sizes, were summarized and tabulated for direct comparisons among the taxa.

Multivariate analysis was applied to 15 informative characters. The characters included: fruit color, fruit texture, mode of dehiscence, presence/absence of woolly jamming hairs at the nectary, presence/absence of spines on mature stem, number of ribs on mature stem, length of woolly hair on areoles, amount of lint on exterior of floral tube, number of floral tube scales, length of floral tube scales, perianth diameter, number of stigma lobes, color of flower center, diameter of colored flower center, and floral remnant deciduous or persistent. Since most of these characters were qualitative in nature and encoded as nominal variables, the analysis performed was Non-metric Multidimensional Scaling (NMDS). This method provided a visualization of the level of similarity among individuals. NMDS found both a non-parametric monotonic relationship between the dissimilarities in the individual–individual matrix and the Euclidean distances between individuals, as well as the location of each individual in the low dimensional space. All individuals were placed into N-dimensional space ($N = 2$) such that the between-individual distances were preserved as well as possible and the resulting data points were displayed as a two-dimensional scatter plot. For this analysis, the following samples were used: 22 *Astrophytum capricorne*, 26 *A. coahuilense*, four *A. myriostigma*, and 29 *A. myriostigma* subsp. *tulense*.

Cross-pollination experiments.—Cross-compatibilities between *Astrophytum coahuilense* (COA) and its putative parental species *A. capricorne* (CAP) and *A. myriostigma* (MYR) were previously tested by Montanucci (2008, 2015). In this study, four crosses (COA \times CAP) and three reciprocal crosses (CAP \times COA) were performed to supplement data from Montanucci (2015). Eight crosses (COA \times MYR) and the reciprocal crosses (MYR \times COA) were made to supplement data from Montanucci (2008). Percent survivorship of seedlings was calculated and compared with homospecific crosses used as controls. The seeds were germinated, and seedlings raised following experimental procedures (substrate, light cycle, temperature) described in Montanucci (2015).

Also, experimental cross-pollinations between *Astrophytum myriostigma* (MYR) and *A. capricorne* (CAP) were performed to obtain data on fertility relationships between the two putative parental species. Seven cross-pollinations (MYR \times CAP) resulted in seed production; four reciprocal crosses (CAP \times MYR) were attempted but fruit set failed.

RESULTS

Comparative morphology of the stem.—*Astrophytum capricorne* has a globose stem, but with age it becomes increasingly columnar, reaching a height of at least 52 cm (Kleszewski 2010). This species has eight acute ribs, but rarely, plants with seven or as many as 11 ribs have been found (Hook 2008). The edges of the ribs have areoles which have (0)5–8(10) rather flattened spines which are flexible, curly, and twisted. However, in some plants the spines are less curly, and instead are directed upwards from the areoles. New spines from areoles near the apex are dark brown to black, but older spines (lower on the stem) become whitish gray. New areoles have yellowish-brown wool composed of relatively long hair; older areoles lose some of the wool.

The epidermis of the stem is covered with white trichomes, but their density and pattern vary among individuals and local populations. In some plants the flecking is moderately sparse giving the epidermis a greenish appearance; in others, the flecking is dense giving the plant a strikingly white appearance. Generally, the tufts of white trichomes, which appear as white dots, are rather evenly spaced; but in some plants narrow, transverse bands of green epidermis separate the white-dotted areas.

Astrophytum myriostigma (the nominotypical subspecies) has a broad, somewhat depressed stem, becoming barrel-shaped in age and reaching a height of at least 52 cm. The stem has a modal number of five ribs but plants with additional ribs (six or seven, rarely greater) have been noted. The rib angle varies from obtuse to acute, and the rib profile varies from angular to rounded (Montanucci & Kleszewski 2021). The dorsal edges of the ribs have a series of areoles which typically lack spines. New areoles which appear at the stem apex have light brown to reddish brown wool composed of relatively short hairs; as the areoles age, the wool is lost. The subsp. *tulense* has a globose or an attenuated, club shaped stem in youth, which becomes tall, columnar in age, reaching a height of at least 90 cm. The stem usually has five ribs, but additional ribs may appear with increasing age; six to 11 ribs have been noted in old plants. The rib angle varies from obtuse to acute, and the rib profile varies from angular to rounded (Montanucci & Kleszewski 2021). Plants with obtuse, rounded ribs are relatively uncommon. The dorsal edges of the ribs have a series of spineless areoles that typically are closely spaced. New areoles have whitish to light brown or pinkish wool composed of short hairs.

The epidermis of the stem in *Astrophytum myriostigma* and its subsp. *tulense* is usually uniformly covered with white trichomes. In some plants the flecking is moderately sparse giving the stem a greenish appearance; in others, the flecking is dense giving the plant a white appearance. Dense flecking is especially characteristic of subsp. *tulense*.

Astrophytum coahuilense has a broad, somewhat depressed stem, becoming barrel-shaped to short columnar in age. Large plants may reach a height of 65 cm and a diameter of 20 to 25 cm, but exceptionally tall plants, ca. 80 cm or more have been recorded (P. Momberger, pers. comm., April 27, 2012). This species typically has five ribs, and this number is extraordinarily constant; in a sample of 226 plants, only four plants had six ribs. Rarely, plants with nine, seven or four ribs, or even three, have been found growing in their habitat. In mature plants the rib angle varies from acute to obtuse or obtusely rounded. Hook (2008) reported that seedlings have acute ribs which become broad with age, but in this study, small seedlings with obtuse ribs and others with acute ribs were noted, suggesting that rib angle can be determined early in development. The rib edges have a series of areoles that lack spines. New areoles have whitish wool composed of relatively short hairs. The epidermis is typically densely covered with trichomes that produce a white, felted texture and obscure the underlying green color of the stem. However, moderately flecked plants with a greenish appearance have also been observed, although they are less typical of the species.

Comparative morphology of the seedlings.—Newly germinated seedlings of *Astrophytum capricorne* are usually light green. As they grow, ribs develop, and spines appear on the areoles. At six to 12 months of age, the seedlings have white to pale yellow spines (1 or 2 per areole). The spines are straight, slender, and vary from 1–4(6) mm in length.

Newly germinated seedlings of *Astrophytum myriostigma* are reddish brown, or initially greenish, later turning reddish brown. The hypocotyl is relatively short. As the seedling grows, 1 or 2 black spines emerge

from each areole, but in many cases, spines may be absent; the spines are short (0.5–1[2] mm), and straight or curving slightly upward. In subsp. *tulense* the hypocotyl of newly germinated seedlings is relatively elongated and reddish brown, or greenish, later turning reddish brown. As the seedling grows, 1 or 2 black spines emerge from each areole, but in many cases, spines may be absent; the spines are short (0.5–1[3] mm), and usually straight, or less typically, curving slightly upward.

Newly germinated seedlings of *Astrophytum coahuilense* are typically pale green. As they grow and develop ribs, spines appear on the areoles; the spines are white to pale yellow, and are straight, slender and vary from 1–3 mm in length. Usually 1–2 spines appear on each areole, but as many as 3–4 per areole have been noted in a few seedlings. See Table 1 for comparisons of the vegetative characters among the studied taxa.

Comparative morphology of the flower.—The flower of *Astrophytum capricorne* is pale, satiny yellow to golden yellow, and its center (throat) varies only slightly from medium red to dark crimson; the red pigment typically has a magenta cast. The maximum recorded perianth diameter is 140 mm. The number of stigma lobes varies from 5–13. In a total of 61 flowers from 18 plants, jamming hairs were found protecting the nectar chambers of 56 flowers; in five flowers the jamming hairs were very sparse and considered “functionally absent.”

The exterior surface of the floral tube has elongated, lanceolate, brown to black scales with grayish lint in the intervening areas. The scales are flat, moderately narrow, and taper to a fine, needle-like point. The scales vary from 9–14 mm long (\bar{x} = 11.4 mm) in 29 withered flowers from 12 plants. The amount of lint on the withered flower varies from sparse to moderately sparse and the scales are numerous and not hidden by the lint.

The flower of *Astrophytum myriostigma* is entirely satiny yellow, lacking a red center; it is relatively large, with a maximum recorded perianth diameter of 80 mm. The flowers have multiple tepal rows, usually three to five. The number of stigma lobes varies from 4–9. The nectar chamber is protected by the anther filaments which are tightly pressed against the base of the style. A total of 20 flowers from 8 plants were examined, and all lacked tufts of woolly jamming hair. Hooek (1988) examined 64 flowers from 46 *A. myriostigma* and noted the absence of jamming hairs in 63 flowers; one flower had only a few jamming hairs.

The exterior surface of the floral tube has elongated, lanceolate, brown to black scales and a covering of grayish-white lint. The scales are flat, rather narrow, and taper to a fine, needle-like point. The scales vary from 6–10 mm long (\bar{x} = 7.9 mm) in 38 withered flowers from four plants. The lint covering the withered flower is quite dense and the embedded scales are usually partly obscured, in some cases with only the distal points of the scales visible.

The flower of subsp. *tulense* is pale satiny yellow and relatively small; the maximum recorded diameter is 50 mm. The flower has one to two, less commonly three, tepal rows. The number of stigma lobes varies from 1–8. The nectar chamber is protected by the anther filaments. In 35 flowers from 14 plants, all lacked tufts of woolly jamming hairs near the nectar chamber.

The floral tube scales are smaller than those of the nominotypical subspecies of *Astrophytum myriostigma*. The length of the scales varies from 3.5–6 mm long (\bar{x} = 4.6 mm) in 62 withered flowers from eight plants. The covering of lint on the exterior of the withered flower is quite dense, like that of the nominotypical subspecies.

The flower of *Astrophytum coahuilense* is yellow, and its center (throat) varies from pale orange to dark crimson; but occasional plants have entirely yellow flowers or have only slight evidence of reddish color. In a sample of 26 plants, some shade of orange color was recorded in 23 plants (88.5% of sample). The maximum recorded perianth diameter is 90 mm. The number of stigma lobes varies from 4–10. In 51 of 61 flowers (from 24 plants), abundant woolly jamming hairs were found protecting the nectar chamber, and in 10 flowers the woolly hairs were very sparse and considered “functionally absent.” Hooek (1988) reported jamming hairs in 31 of 34 flowers (from 24 plants), sparse jamming hairs in one flower, and none in two flowers.

The exterior surface of the floral tube has elongated, lanceolate, dark brown to black scales and a covering of grayish-white lint. The scales are flat, rather narrow, and taper to a fine, needle-like point. The scales vary from 5–12 mm long (\bar{x} = 8.4 mm) in 28 withered flowers from nine plants. The lint covering the withered

TABLE 1. Comparison of vegetative characters in *Astrophytum capricorne*, *A. coahuilense*, *A. myriostigma* subsp. *myriostigma* and *A. myriostigma* subsp. *tulense*.

| Character | <i>A. capricorne</i> | <i>A. coahuilense</i> | <i>A. myriostigma</i> | subsp. <i>tulense</i> |
|---------------------------|--------------------------------|---|--|---|
| Stem shape | globose; in age short columnar | globose; in age barrel-shaped or columnar | globose; in age short columnar | globose or club-shaped; in age tall, columnar |
| Stem height | ≥ 52 cm n = 92 | 65–80 cm n = 74 | ≥ 52 cm n = 55 | 90 cm n = 52 |
| Rib number | (7)–8–(11) n = 121 | (3)–5–(9) n = 226 | (3)–5–(12) n = 146 | (4)–5–(11) n = 141 |
| Wool on new areoles | long hair | short hair | short hair | short hair |
| Spine number | (0)5–8(10) | 0 | 0 | 0 |
| Seedling color | green | green | reddish-brown, or green then reddish-brown | reddish-brown, or green then reddish-brown |
| Seedling spine color | yellowish | yellowish | black | black |
| Seedling spine shape | straight | straight | straight, or curving slightly upward | straight, or curving slightly upward |
| Spine number in seedlings | 1–2 n = 281 | 1–2(3–4) n = 186 | 1–2 n = 148 | 1–2 n = 342 |
| Spine length | 1–4(6) mm | 1–3 mm | 0.5–1(2) mm | 0.5–1(3) mm |

flower is quite dense and the embedded scales are usually partly obscured, in some cases with only the distal points of the scales visible.

Comparative morphology of the fruit.—The fruit of *Astrophytum capricorne* is bright red to dark purplish red, seldom greenish with a pink or reddish base. The fruit wall has a semi-fleshy texture and when ripe, the seeds are released by basal dehiscence; a circular split develops around the base and the fruit lifts upward, exposing the seeds. The dried floral remnant is persistent.

The fruit of *Astrophytum myriostigma* is green when mature, and the walls are not semi-fleshy but rather firm and semi-dry. Dehiscence is apical, with several vertical splits opening into a star-shaped structure with four or five flaps. The fruit of subsp. *tulense* is green, and the base of the fruit capsule may become pink when mature. However, the fruit walls are not semi-fleshy but rather firm and semi-dry. Dehiscence is apical, with several vertical splits opening into a star-shaped structure with three (rarely two) to five flaps. In both taxa, the dried floral remnant is deciduous, usually easily falling from its point of attachment at the top of one of the flaps. The point of attachment is marked by a small, smooth, pale brown scar.

The fruit of *Astrophytum coahuilense* is bright red to dark purplish red, but some plants produce a green to gray-green fruit with pink to medium red color around the base. The fruit wall has a semi-fleshy texture, and when ripe the seeds are released by basal dehiscence; a circular split develops around the base and the fruit lifts upward, exposing the seeds. The fruit has a persistent floral remnant at its top, although very rarely (4.9% of sample) the floral remnant is deciduous. See Table 2 for comparisons of the floral and fruit characters among the taxa.

Morphology of synthetic hybrids.—The morphology of MYRCAP F₁ hybrid seedlings has not been described previously. In the present study, the number of hybrid seedlings produced were as follows: nominotypical *Astrophytum myriostigma* × CAP (72 seedlings); subsp. *tulense* × CAP (two groups with 2 and 31 seedlings), and from the Jaumave Valley, subsp. *quadricostatum* × CAP (two groups with 18 and 66 seedlings).

All hybrid seedlings were pale green in color, with whitish to yellowish, straight spines about 1–3 mm in length. The hypocotyls of the hybrid seedlings derived from subsp. *tulense* and from subsp. *quadricostatum* were elongated, but hypocotyls of the hybrid seedlings from crosses involving subsp. *myriostigma* were shorter. After two years of growth, most hybrid seedlings had developed five ribs. At three years of age, the rib number had increased in some seedlings and their spines had started to disappear. The subsp. *tulense* × CAP hybrid seedlings grew very slowly, and except for two seedlings, never developed more than four ribs (the

TABLE 2. Comparison of floral and fruit characters in *Astrophytum capricorne*, *A. coahuilense*, *A. myriostigma* subsp. *myriostigma* and *A. myriostigma* subsp. *tulense*. Sample mean (\bar{x}) \pm 95% confidence limits; observed limits in parentheses.

| Character | <i>A. capricorne</i> | <i>A. coahuilense</i> | <i>A. myriostigma</i> | subsp. <i>tulense</i> |
|--------------------------------|---|--|---|---|
| Perianth diameter | \bar{x} = 92 (max. 125) mm 52 flowers ¹ \bar{x} = 73 \pm 3.12 mm (max. 95 mm) 15 plants; 39 flowers ² | \bar{x} = 68 (max. 90) mm 18 flowers ¹ \bar{x} = 57.5 \pm 2.31 mm (max. 75 mm) 22 plants; 50 flowers ² | \bar{x} = 63 (max. 76) mm 26 flowers ¹ \bar{x} = 50.5 \pm 1.57 mm (max. 64 mm) 4 plants; 55 flowers ² | \bar{x} = 38 (max. 50) mm 21 flowers ¹ \bar{x} = 30.3 \pm 0.76 mm (max. 43 mm) 16 plants; 171 flowers ² |
| Stigma lobes | \bar{x} = 8.4(6–12) 45 flowers ¹ \bar{x} = 8.8 \pm 0.25 (5–13) 20 plants; 102 flowers ² | \bar{x} = 7.6 (6–10) 25 flowers ¹ \bar{x} = 6.9 \pm 0.19 (4–10) 25 plants; 150 flowers ² | \bar{x} = 6.8 (5–9) 21 flowers ¹ \bar{x} = 5.75 \pm 0.25 (4–9) 5 plants; 82 flowers ² | \bar{x} = 5.9 (5–7) 30 flowers ¹ \bar{x} = 4.7 \pm 0.13 (1–8) 17 plants; 250 flowers ² |
| Flower center color | bright medium red to dark crimson; with magenta cast | yellow to pale orange red to bright red; no magenta cast | yellow | yellow |
| Flower colored center diameter | \bar{x} = 32.9 % \pm 1.10 (26.2–41.1%) 33 plants; 46 flowers ² | \bar{x} = 19.2% \pm 2.10 (0–36.5%) 38 plants; 41 flowers ² | \bar{x} = 0% 5 plants; 82 flowers ² + 23 photos | \bar{x} = 0% 17 plants; 250 flowers ² + 19 photos |
| Floral scales | long (9–14 mm) | short (5–12 mm) | short (6–10 mm) | very short (3.5–6 mm) |
| Floral lint | sparse to moderate | dense | dense | dense |
| Nectar chamber protection | woolly hairs | woolly hairs | anther filaments | anther filaments |
| Fruit color | bright red to purplish red; some green with reddish base n = 47, 20 plants | bright red to purplish red; some green with reddish base n = 41, 18 plants | green, n = 28, 14 plants | green; some green with pink base; n = 83 22 plants |
| Fruit texture | somewhat fleshy | somewhat fleshy | somewhat dry, firm | somewhat dry, firm |
| Dehiscence | basal, circular | basal, circular | apical, linear | apical, linear |
| Floral remnant | persistent | persistent, rarely deciduous | deciduous | deciduous |

¹Hoock (2008); ²this study

usual number at the early seedling stage). The *Astrophytum myriostigma* \times CAP hybrid seedlings developed no more than five ribs. In the group of 66 subsp. *quadricostatum* \times CAP hybrid seedlings, three surviving seedlings had five ribs, and one had seven ribs. In the group of 18 subsp. *quadricostatum* \times CAP seedlings, 16 had five ribs, one seedling had six ribs, and another had eight ribs. Unlike typical *Astrophytum coahuilense* seedlings, the largest hybrid seedlings had only a sparse to moderate covering of epidermal trichomes. Morphological comparisons between *A. coahuilense* and the synthetic MYRCAP F₁ hybrids are summarized in Table 3.

The descriptions of adult synthetic hybrids (MYRCAP) and the reciprocal cross (CAPMYR) are presented in Haage and Sadovský (1957) and Sadovský and Schütz (1979). The former authors stated that the MYRCAP F₁ hybrids were intermediate between *Astrophytum capricorne* and *A. myriostigma*. In the F₂ generation all possible combinations were obtained, but most hybrids were densely covered with trichomes, and occasional hybrids had rather long, slender black spines, which never reached the length of the spines of *A. capricorne*. Additionally, there were many hybrids with five to eight ribs and short spines. According to Haage and Sadovský (1957) the reciprocal cross (CAPMYR) produced mainly eight-ribbed intermediate hybrids which otherwise did not differ from the MYRCAP cross. In older plants, there was a tendency for the spines to drop. In the F₂ generation, all possible combinations appeared as in the MYRCAP cross.

Sadovský and Schütz (1979) provide a similar description to the foregoing. The authors stated that the MYRCAP F₁ hybrids were intermediate between the two parental species. Nearly all the F₁ plants had five ribs. They also mentioned that the trichomes were not as tightly attached if the maternal parent (MYR) originated from the Jaumave Valley, as compared to nominotypical *Astrophytum myriostigma* or subsp. *tulense* from

TABLE 3. Comparison between *Astrophytum coahuilense* and synthetic MYRCAP F₁ hybrids.

| Character | <i>Astrophytum coahuilense</i> | F ₁ MYRCAP hybrids |
|--------------------------------|---|---|
| Number of ribs | 5(3–9) | 5(7–9) |
| Trichome density | sparse to dense | sparse to moderately dense |
| Flower center color | pale orange red to dark red, rarely yellow | pale orange red |
| Colored flower center diameter | relatively narrow, or absent | relatively narrow |
| Fruit texture | semi-fleshy | somewhat dry |
| Fruit color | bright red to purplish red, or greenish with reddish base | pale red |
| Dehiscence | basal, with single, horizontal, circular split | apical, with several vertical splits producing four flaps |
| Seedling color | pale green | pale green |
| Seedling spine color | white to pale yellow | white to pale yellow |
| Seedling spine shape | ± straight | ± straight |
| Seedling spine length | 1–3 mm | 1–3 mm |
| Spines per areole | 1–2(3–4) | 1–2 |

the Central Plateau region of Mexico. The authors stated that in the F₂ generation quite limited combinations were obtained because nearly two-thirds of the plants were eight-ribbed. Most were densely covered with trichomes. The F₂ plants had slender, black spines that were shorter than those of *A. capricorne*.

A photo of the MYRCAP F₁ hybrids is presented in Haage and Sadvoský (1957); the plants lack spines. Curiously, the same photo appears in Sadvoský and Schütz (1979), but it is reversed, the plant label is altered, and the photo is retouched to depict plants with short, stout spines. Haage and Sadvoský (1957) also allude to spines in CAPMYR F₁ hybrids, but a spineless hybrid is shown in a photo. The same photo is presented in Sadvoský and Schütz (1979), but the plant is identified as a MYRCAP F₂ hybrid. Both publications mention spines in their descriptions of the F₂ hybrids. Neither publication provides any detailed description of the flowers in MYRCAP or CAPMYR hybrids. Also, the authors of both works do not compare synthetic F₁ hybrids with *Astrophytum coahuilense*. Examination of the photo of MYRCAP F₁ hybrids in Haage and Sadvoský (1957) reveals that the hybrids closely resemble *A. coahuilense* in terms of stem morphology and flower color.

Heinz Hooek (pers. comm., October 21, 2004) produced synthetic MYRCAP F₁ hybrids using *Astrophytum myriostigma* from the Jaumave Valley as the maternal parent, and *A. capricorne* var. *minus* from La Rosa, Coahuila, as the pollen donor. He obtained a total of seven hybrids, five of which had eight ribs, one with seven ribs, and one with five ribs. At maturity, none of the plants had spines, nor could minute spines be detected by touching the areoles. Based on photos received from Hooek, the hybrids have moderately dense epidermal trichomes, somewhat less than that typical of *A. coahuilense*. The flower is yellow with a relatively narrow, orange-red center and thus closely resembles the flowers of *A. coahuilense*. The fruit produced by the MYRCAP F₁ hybrid is pale red but appears to be somewhat dry and firm. Fruit dehiscence is apical, with several splits producing a capsule with four flaps. Hence, the fruit of MYRCAP F₁ hybrid differs from that of *A. coahuilense* in having a pale red color, a slightly dry, firm texture, and apical dehiscence.

Summary of morphological comparisons.—The stem of *Astrophytum coahuilense* closely resembles that of *A. myriostigma* (Fig. 1). Both species have a modal number of five ribs which are angular or rounded in profile. In plants with more than five ribs, the rib angle is usually acute. Both species have spineless areoles, and the new areoles have wool composed of relatively short hairs in contrast to the longer hairs noted in *A. capricorne*. Also, both species show similar developmental trends; small plants tend to have a globose, somewhat depressed stem, which later grows into a barrel-shaped stem. But some examples of *A. coahuilense* may grow into a columnar form which resembles subsp. *tulense*.

The young seedlings of *Astrophytum coahuilense* are very similar to seedlings of *A. capricorne*. In both species the seedlings are pale green and have relatively long, straight, whitish, or yellowish spines. By contrast, the seedlings of *A. myriostigma* and subsp. *tulense* are reddish brown or initially greenish, later turning



FIG. 1. **A.** *Astrophytum capricorne* (Saltillo, Coahuila), **B.** *Astrophytum myriostigma* (Las Tablas, San Luis Potosi), **C.** synthetic F₁ MYRCAP hybrid, **D.** *Astrophytum coahuilense* (Cerro Bola, Coahuila).

reddish brown. The seedling spines are short, either straight or curving slightly upward; some seedlings lack spines. The morphology of MYRCAP F₁ hybrid seedlings closely resembles that of *A. capricorne* and *A. coahuilense*.

Astrophytum coahuilense is intermediate between *A. capricorne* and *A. myriostigma* (and subsp. *tulense*) for the perianth diameter and the number of stigma lobes. All pair-wise comparisons among the four taxa are statistically significant for the diameter of the perianth ($p < 0.05$) and for the number of stigma lobes ($p < 0.0001$). The possible relationship between perianth diameter and number of stigma lobes was tested by correlation analysis. The following correlation coefficients (r) were obtained: 0.176 for *A. capricorne*; 0.043 for *A. coahuilense*; 0.345 for *A. myriostigma*; -0.023 for subsp. *tulense*. A significant correlation ($p < 0.001$) was found between the two variables only in *A. myriostigma*. In *A. coahuilense* the flower's center varies from dark crimson (as in *A. capricorne*) to pale orange or orange red, but occasional plants have entirely yellow flowers (as in *A.*

myriostigma) or have only slight evidence of reddish color (Fig. 2). The colored center of the flower of *A. coahuilense* has a smaller mean diameter than that of *A. capricorne* ($df = 85$, $t = 11.69$, $p < 0.0001$), and the diameter of the colored center has greater variability (coefficient of variation 35.8% vs. 11.6%). The nectar chambers of *A. capricorne* and *A. coahuilense* are protected by tufts of woolly jamming hairs, and the fruit of the two species have the same color, texture, and mode of dehiscence. A comparison of the withered flowers reveals that *A. capricorne* has longer floral scales than those of *A. coahuilense* and *A. myriostigma*. In the latter two species, the floral tube scales are similar in length, and shorter and slightly narrower than those of *A. capricorne*. Additionally, in *A. capricorne* the floral scales are numerous with sparse lint, but both *A. coahuilense* and *A. myriostigma* have fewer scales and a dense covering of lint on the surface of the withered flower (Fig. 3).

Both *Astrophytum coahuilense* and *A. capricorne* have the same fruit morphology. The fruit is bright red to dark purplish red, but some plants produce a green to gray-green fruit with pink to medium red color around the base. The fruit wall has a semi-fleshy texture, and when ripe the seeds are released by basal dehiscence; a circular split develops around the base and the fruit lifts upward, exposing the seeds. In both species, the fruit typically has a persistent floral remnant at its top.

Non-Metric multidimensional scaling analysis.—Non-metric Multidimensional Scaling analysis of the distance matrix based on 15 variables recorded from a total of 81 *Astrophytum* plants produced a two-dimensional scatterplot (Fig. 4). The plot reveals that *A. capricorne* and *A. myriostigma* form two widely separated clusters along Dimension 1. All 15 variables contributed to this separation. The cluster representing *A. myriostigma* is slightly more variable than that of *A. capricorne* along Dimension 1 due to the inclusion of two infraspecific taxa (the nominotypical subspecies and subsp. *tulense*). The cluster representing the putative hybrid, *A. coahuilense*, occupies a position between the two clusters of the putative parental species along Dimension 1. The plot reveals that *A. coahuilense* displays an intermediate morphology with respect to *A. capricorne* and *A. myriostigma*, thus supporting the hypothesis of a hybrid origin. Perusal of the raw data for this analysis reveals that six characters (spines on mature stem, number of ribs, length of hair on new areoles, lint on floral tube, number of floral scales, length of floral scales) separate *A. coahuilense* from *A. capricorne*. Four characters (color of fruit, texture of fruit, mode of dehiscence, type of nectary protection) separate *A. coahuilense* from *A. myriostigma*. Five characters (diameter of perianth, number of stigma lobes, color of flower center, diameter of colored center, floral remnant deciduous or persistent) display variability and contribute to the separation of *A. coahuilense* from one, or the other, or both putative parental taxa. Note also that the cluster representing *A. coahuilense* is more variable than the clusters of the two putative parental species along Dimension 2. Higher morphological variability is often seen in populations of plants that are presently hybridizing or are of hybrid origin.

Crosses between *Astrophytum coahuilense* and *A. capricorne*.—In the present study three *Astrophytum coahuilense* \times *A. capricorne* crosses (COA \times CAP) produced morphologically normal seeds with relatively high germination rates. But a fourth cross-pollination failed completely (no seeds were produced). In the first cross, only seven seeds were produced, indicating that many ovules of the *A. coahuilense* failed to develop in the ovary. In the second cross, 100 of 102 seedlings had abnormal hypocotyls with lumps and/or longitudinal clefts, and the epicotyl was small, narrow, or pointed. In the third cross, out of 75 seedlings only one was abnormal (hypocotyl with a longitudinal cleft). Mortality from chlorophyll deficiency began to appear at four months of age. All long-term survivors (ca. 24 months) suffered from very slow growth rates.

Three reciprocal crosses (CAP \times COA) produced morphologically abnormal seeds. In two crosses producing 18 seeds and 50 seeds, the seeds were about 2 mm in length, smaller than normal. A third cross that produced 42 seeds included 12 seeds ca. 1 mm or slightly less, 14 seeds ca. 2 mm in length, and 16 seeds ca. 3 mm in length. Average normal seed length is ca. 3 mm. The testa color of all CAPCOA seeds was pale to medium brown, rather than the dark brown of fully developed seeds of *Astrophytum capricorne*. Also, the normally convex surface of the testa was indented (concave) in all the seeds, indicating the embryo had not fully developed. Germination of the seeds of all three crosses failed except for one seed; the seedling had normal chlorophyll but did not grow and died 20 days after sowing.

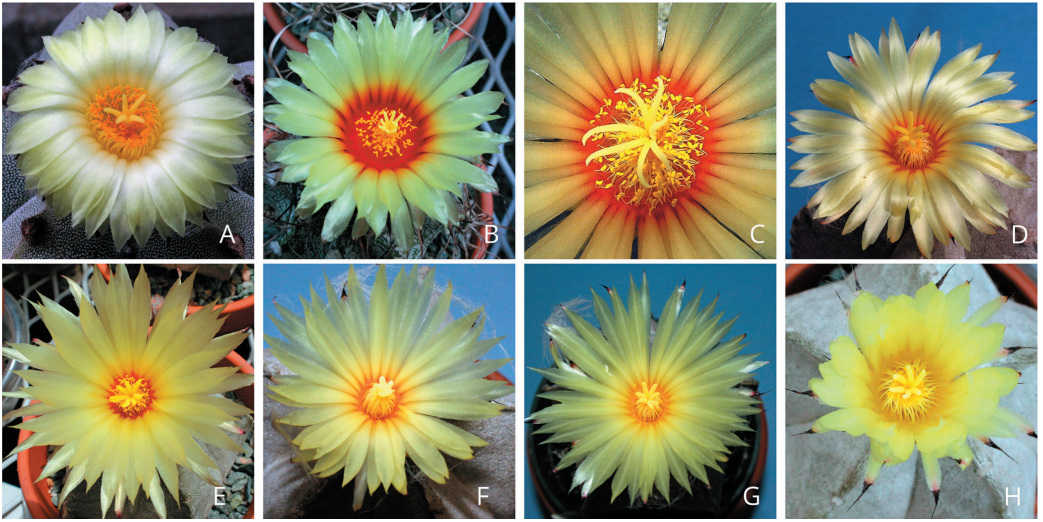


FIG. 2. Flowers of A. *Astrophytum myriostigma*, B. *A. capricorne*, C. synthetic F₁ MYRCAP hybrid, and D.–H. *A. coahuilense*, showing variation in the extent and hue of the flower center.



FIG. 3. Comparison of the withered flowers of *Astrophytum capricorne* (upper), *A. coahuilense* (center), and *A. myriostigma* (lower). Note the smaller floral scales and presence of dense lint in the latter two species. Scale in mm.

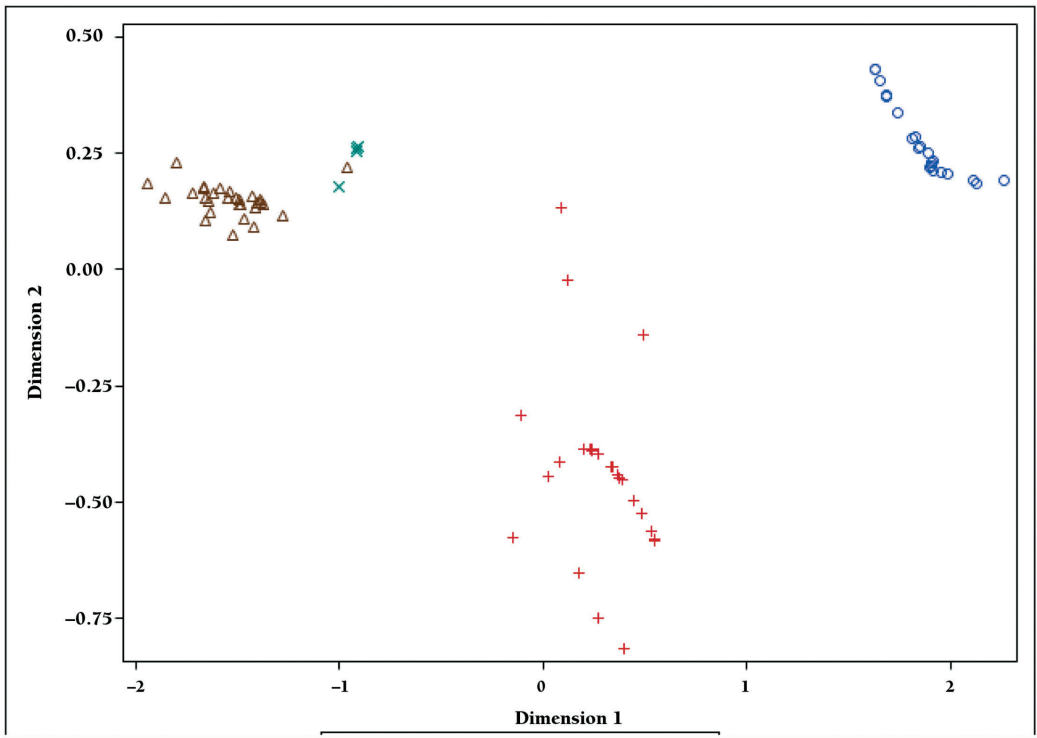


FIG. 4. Two-dimensional scatterplot based on Non-metric Multidimensional Scaling analysis. The clusters are: *Astrophytum myriostigma* (x), subspecies *tulense* (Δ), *A. coahuilense* (+), and *A. capricorne* (\circ). See text for discussion.

The cross-pollinations between *Astrophytum coahuilense* and *A. capricorne* resulted in different outcomes depending on the individual plants used and the direction of the cross. Generally, the CAP \times COA crosses produced small, abnormally shaped seeds which failed to germinate or had low rates of germination (2% to 20%). The F₁ hybrids that successfully sprouted displayed morphological abnormalities, chlorophyll deficiency, and impaired growth; all seedlings eventually died. The reciprocal cross (COA \times CAP) produced seeds with normal morphology and higher germination rates (85.7% to 100%), although pollination failed in one case. The F₁ hybrids had morphological abnormalities in some cases, and all seedlings eventually developed chlorophyll deficiency and died. Overall, there is strong postzygotic incompatibility between *A. coahuilense* and its putative parent *A. capricorne* (Table 4).

Montanucci (2015) noted different levels of incompatibility between the reciprocal crosses. The COA \times CAP cross resulted in 86.6% germination. No morphological abnormalities were noted among the 39 seedlings produced. Thirty seedlings survived for 12 months, and six seedlings survived more than 12 months, but all eventually died due to chlorosis. In the reciprocal cross (CAP \times COA) about 20.1% germination occurred. Among the 45 germinating seedlings, nine had abnormalities, e.g., hypocotyls with longitudinal grooves and small epicotyls. Twenty-six seedlings survived more than 12 months, but all eventually died due to chlorophyll deficiency.

Megata (1944: 25) found abnormal seed setting, germination, and growth in hybrids between the two species, but provided no further details. Sadovský in Sadovský and Schütz (1979) produced some 800 F₁ generation hybrids and produced F₂ hybrid plants but did not discuss chlorosis, vigor, or survivorship.

TABLE 4. Results of cross-pollination experiments between *Astrophytum coahuilense* (COA) and the putative parental species *A. capricorne* (CAP). The pollen donor is the second plant listed for each cross. Locality data for collection numbers are listed below.

| Cross | Number of seeds | Number (%) germinated | Remarks |
|--|-----------------|-----------------------|--|
| COA (RRM 15-2) × CAP (SB 323D) | 77 | 75(97.4%) | last seedling died at 21 months, growth impaired |
| COA (RRM 15-6) × CAP (SB 323F) | 119 | 102(85.7%) | growth impaired; last seedling died at 34 months |
| COA (SB 1474A) × CAP (SB 323H) | 7 | 7(100%) | all developed chlorosis and died within 8 months |
| COA (SB 1474A) × CAP (SB 323H) | 0 | – | fruit set failure |
| CAP (SB 323F) × COA (RRM 15-6) | 18 | 0 | seeds abnormally small, concave |
| CAP (SB 323H) × COA (SB 1474A) | 50 | 1(2%) | seeds abnormally small, concave, seedling died after 20 days |
| CAP (SB 323D) × COA (RRM 15-2) | 42 | 0 | seeds abnormally small, concave |
| CAP (RRM 80-1) × COA (RRM 81-2) ¹ | 223 | 45(20.1%) | 100% mortality at 36 months |
| COA (RRM 81-2) × CAP (RRM 80-1) ¹ | 45 | 39(86.6%) | 100% mortality at 36 months |

¹Data from Montanucci (2015)

Astrophytum capricorne: S. Coahuila: RRM 80-1; N. S altillo, Coah.: SB 323D, SB 323E, SB 323F, SB 323H. *Astrophytum coahuilense*: Cerro Bola, Coah.: RRM 15-2, RRM 15-5, RRM 15-6, RRM 81-2, RRM 84-5A, RRM 84-5B, RRM 84-5C, RRM 90-3A, RRM 90-3B, RRM 91-10A; Lerdo, Dgo.: RRM 85-2, SB 1474A, SB 1474B. Collection Acronyms: RRM (Richard R. Montanucci); SB (Steven Brack).

Crosses between *Astrophytum coahuilense* and *A. myriostigma*.—The present study supplements data from Montanucci (2008) with 16 additional crosses for a total of 23 crosses (Table 5). The results from both studies reveal that the two species are isolated by prezygotic (fruit set failure) and postzygotic isolating mechanisms (nonviable seed and seedling mortality). Ten crosses from the combined studies resulted in fruit set failure, six of which involved *Astrophytum coahuilense* as the maternal parent. Thirteen crosses produced seed, six resulting in 0% germination and seven resulting in 5.3% to 68.9% germination; all F₁ seedlings were chlorotic and eventually died. The data suggest a stronger reproductive barrier than that observed between *A. coahuilense* and its putative parent, *A. capricorne*.

Haage and Sadovský (1957) and Sadovský and Schütz (1979) briefly discussed the reproductive isolation between *Astrophytum coahuilense* and *A. myriostigma*, referring to “countless” experimental cross-pollinations that failed. These authors regarded the two species as having the strongest reproductive isolation among members of the genus.

Crosses between the putative parental species.—Seven cross pollinations were performed between *Astrophytum myriostigma* (MYR) as the maternal plant, and *A. capricorne* (CAP) as the pollen donor. The crosses involved *A. myriostigma* of three different subspecies: two crosses with the nominotypical subspecies, three crosses with subsp. *tulense*, and two crosses with subsp. *quadricostatum*. Among these seven crosses, seed germination ranged from 86 to 100%, but seedling survivorship was low: 0–1.4% for subsp. *myriostigma* × CAP; 0% for subsp. *tulense* × CAP; 5.5–6.1% for subsp. *quadricostatum* × CAP. The majority of the F₁ hybrid seedlings died from chlorosis. Chlorophyll loss began near the base of the stem and gradually moved toward the stem apex, with death occurring when the seedling became entirely yellow. At the time of this writing, one seedling of subsp. *myriostigma* × CAP and five seedlings of subsp. *quadricostatum* × CAP have survived nine years but have not yet reached sexual maturity.

DISCUSSION AND CONCLUSIONS

Vázquez-Lobo et al. (2015) analyzed three gene sequences of chloroplast DNA (*rbcl* gene, *trnL–trnF* intergenic spacer, and *trnK–matK* region), and used Bayesian and Maximum likelihood methods to generate a phylogenetic tree for the genus *Astrophytum*. The authors determined that *Astrophytum* is monophyletic and contains six species grouped into two clades. The clades correspond to the “northern” and “southern” radiations recognized by Backeberg (1961) as infrageneric taxa, i.e., *Neoastrophytum* and *Astrophytum*, respectively. The cladogram of Vázquez-Lobo et al. (2015) positioned *A. coahuilense* within the “northern radiation” clade (i.e.,

TABLE 5. Results of cross-pollination experiments between *Astrophytum coahuilense* (COA) and the putative parental species *A. myriostigma* (MYR). The pollen donor is the second plant listed for each cross. Locality data for collection numbers are listed below.

| Cross | Number of seeds | Number germinated | Percent germination | Remarks |
|---|-----------------|-------------------|---------------------|-------------------|
| MYR (HK 1925A) × COA (RRM 91-10A) | 19 | 1 | 5.3% | 100% mortality |
| COA (RRM 91-10A) × MYR (HK 1925A) | 0 | – | – | fruit set failure |
| MYR (HK 1925A) × COA (RRM 90-3A) | 19 | 5 | 26.3% | 100% mortality |
| COA (RRM 90-3A) × MYR (HK 1925A) | 0 | – | – | fruit set failure |
| MYR (RRM 06-2) × COA (RRM 85-2) | 29 | 20 | 68.9% | 100% mortality |
| COA (RRM 85-2) × MYR (RRM 06-2) | 0 | – | – | fruit set failure |
| MYR (GL 652A) × COA (SB 1474B) | 0 | – | – | fruit set failure |
| COA (SB 1474B) × MYR (GL 652A) | 1 | 0 | 0% | – |
| MYR (RRM 06-3) × COA (SB 1474B) | 39 | 11 | 28.2% | 100% mortality |
| COA (SB 1474B) × MYR (RRM 06-3) | 30 | 0 | 0% | – |
| MYR (RRM 62B) × COA (RRM 90-3B) | 0 | – | – | fruit set failure |
| COA (RRM 90-3B) × MYR (RRM 62B) | 22 | 2 | 9% | 100% mortality |
| MYR (HK 1925E) × COA (RRM 15-5) | 0 | – | – | fruit set failure |
| COA (RRM 15-5) × MYR (HK 1925E) | 0 | – | – | fruit set failure |
| MYR (NM 3194F) × COA (SB 1474B) | 39 | 10 | 25.6% | 100% mortality |
| COA (SB 1474B) × MYR (NM 3194F) | 0 | – | – | fruit set failure |
| MYR (RRM 95-1) × COA (RRM 84-5A) ¹ | 88 | 0 | 0% | – |
| COA (RRM 84-5A) × MYR (RRM 95-1) ¹ | 53 | 0 | 0% | – |
| MYR (RRM 95-1) × COA (RRM 81-2) ¹ | 31 | 0 | 0% | – |
| COA (RRM 81-2) × MYR (RRM 95-1) ¹ | 75 | 0 | 0% | – |
| MYR (RRM 06-2) × COA (RRM 84-5B) ¹ | 0 | – | – | fruit set failure |
| COA (RRM 84-5C) × MYR (RRM 06-2) ¹ | 0 | – | – | fruit set failure |
| MYR (SB 264) × COA (RRM 84-5C) ¹ | 10 | 1 | 10% | 100% mortality |

¹Data from Montanucci (2008)

Astrophytum coahuilense: Cerro Bola, Coah.: RRM 15-2, RRM 15-5, RRM 15-6, RRM 81-2, RRM 84-5A, RRM 84-5B, RRM 84-5C, RRM 90-3A, RRM 90-3B, RRM 91-10A; Lerdo, Dgo: RRM 85-2, SB 1474A, SB 1474B. *Astrophytum myriostigma*: S. of Tula, Tamps.: RRM 06-2, RRM 06-3; N. San Luis Potosi: RRM 62B, RRM 95-1; Huizache Jct. SLP: HK 1925A, HK 1925E, NM 3194F; Las Tablas, SLP: GL 652A, RRM 79-2; Jaumave, Tamps.: RRM 07-3, RRM 2708, SB 264. Collection Acronyms: GL (Gary Loos); HK (Horst Kuenzler); NM (New Mexico Cactus Research); RRM (Richard R. Montanucci); SB (Steven Brack).

subgenus *Neoastrophytum*) and placed *A. myriostigma* within the “southern radiation” (i.e., subgenus *Astrophytum*). Their cladogram depicts the lineage of *A. coahuilense* as basal to, and diverging from, the common ancestor of *A. asterias* and *A. capricorne* approximately 3.3 mya. The authors attributed the close resemblance in stem morphology between *A. coahuilense* and *A. myriostigma* to convergent evolution.

The hypothesis of allopatric speciation followed by convergent evolution is not supported by the results of this study. The results of the univariate analysis, which is based on 13 vegetative and 10 reproductive characters, indicate that the morphology of *Astrophytum coahuilense* is complex, consisting of a combination of vegetative and reproductive characters shared with both *A. capricorne* and *A. myriostigma* as well as several intermediate traits. In addition, Non-metric Multidimensional Scaling analysis (NMDS) of 15 characters (three vegetative and 12 reproductive traits) produced a two-dimensional scatterplot in which the cluster representing *A. coahuilense* occupies a position intermediate between the clusters of the two parental species along Dimension 1. Thus, both analyses support the hypothesis that *A. coahuilense* originated through hybridization between *A. capricorne* and *A. myriostigma*.

The morphological characters shared between *Astrophytum coahuilense* and parental *A. capricorne* include: 1) the fruit color is typically red to purplish red; 2) the fruit texture is semi-fleshy; 3) the fruit dehiscence is basal, involving circular splitting; 4) the floral remnant is persistent, although rarely deciduous in *A. coahuilense*; 5) the nectary is protected by woolly “jamming hairs”; 6) the seedling is usually pale green; 7) the seedling spines are whitish to pale yellow; 8) the seedling spines are straight and comparatively long.

The morphological characters shared between *Astrophytum coahuilense* and parental *A. myriostigma* are: 1) the adult stem lacks spines; 2) the stem has a modal number of five ribs; 3) the new areoles have wool

composed of short hair (compared to longer hair in *A. capricorne*); 4) the floral tube exterior has a dense covering of whitish lint (compared to the sparse lint in *A. capricorne*); 5) the floral tube has a reduced number of scales (compared to a higher number in *A. capricorne*); 6) the floral tube scales are relatively short as compared to the longer scales in *A. capricorne*.

Based on the morphological characters studied, the phenotype of *Astrophytum coahuilense* lacks fixed, unique characters; it is comprised only of character states shared with its parental species and intermediate character states. The intermediate character states include: 1) the diameter of the perianth, and 2) the number of stigma lobes. The variation in color of the flower throat in *A. coahuilense* also suggests hybridization as it varies from the bright red of *A. capricorne* through shades of orange to the pure yellow of *A. myriostigma*. The orange color represents an intermediate character state between bright red and pure yellow. Various shades of orange color are predominant in *A. coahuilense* (88.5% of sample) and closely resemble the color noted in synthetic F₁ hybrids. Also, the colored flower center of *A. coahuilense* typically has a narrow diameter which agrees with the narrow-colored center of synthetic F₁ hybrids.

In phylogenetic studies, hybridization between plant taxa is often revealed by discordance between nuclear and cytoplasmic gene markers, or between different nuclear markers (Rieseberg & Soltis 1991; Rieseberg et al. 1996b). Also, in many cases discordance is found between molecular markers (e.g., cpDNA) and morphological data sets (Wendel & Doyle 1998). Other causal factors, such as incomplete lineage sorting or gene duplication and loss, can also explain phylogenetic discordance (Galtier & Daubin 2008). However, hybridization is often considered the most probable cause of phylogenetic discordance because it is so widespread among angiosperms (Rieseberg & Soltis 1991). Vázquez-Lobo et al. (2015) did not consider the possibility that *Astrophytum coahuilense* originated through hybridization and the authors did not analyze nuclear gene markers, nor study morphology.

The sharing of cpDNA markers between *Astrophytum coahuilense* and *A. capricorne* suggests that the latter species could be the maternal parent in the hybrid origin of the former, as cpDNA is maternally inherited in many plant taxa (Zhang et al. 2003). Moreover, chloroplast capture is a common phenomenon in plants and has been reported in numerous taxa (Rieseberg & Soltis 1991). Presently, little is known about the nuclear genome of the *Astrophytum* species, but research is planned to sequence the nuclear genes of *A. coahuilense* and its parental species to better understand their genetic relationships.

ASTROPHYTUM AND HOMOPLOID HYBRID SPECIATION

Astrophytum coahuilense presumably arose through the mechanism of homoploid hybrid speciation since this species and other members of the genus are diploid (see below). Homoploid hybrid speciation is less well-known than allopolyploid speciation because its detection in natural populations is difficult, and its prevalence among plant groups remains unclear (Rieseberg 1997). Although diploid hybridization is known in several genera in the subfamily Cactoideae, and several species and genera are suspected of having a hybrid origin (Machado 2008), no examples of homoploid hybrid speciation have yet been thoroughly investigated and substantiated. See Granados-Aguilar et al. (2022) for a comprehensive review of hybridization in the Cactaceae.

Theoretically, homoploid hybrid speciation occurs under rather stringent conditions. The incipient hybrid species, which has the same ploidy level as its parents, is initially sympatric with both parents and must escape the homogenizing effects of gene flow and become fertile and stabilized. The development of reproductive barriers and ecological divergence and/or spatial isolation of the hybrid population are necessary to facilitate the speciation process (Anderson 1948; McCarthy et al. 1995; Rieseberg & Carney 1998; Buerkle et al. 2000). In the present study, the results of experimental cross-pollinations between *Astrophytum coahuilense* and its parental species reveal the existence of reproductive barriers between the hybrid and its parents. These results suggest a history of contact and genetic interaction consistent with the model of homoploid hybrid speciation. In the case of *A. coahuilense* and its parent *A. myriostigma*, the two species display particularly strong reproductive barriers, predominantly classed as prezygotic mechanisms. It is considered likely that the two

species, although broadly allopatric, came into contact in the past, perhaps repeatedly, leading to the development of strong reproductive barriers. In the case of *A. coahuilense* and *A. capricorne*, the barriers seem less stringent, being largely, if not entirely, postzygotic mechanisms. However, the possibility of having had a history of genetic contact is considered very probable because the two species have closely adjacent allopatric or probably parapatric distributions. There are records [photo vouchers] for *A. capricorne* from Tanque Menchaca (25°16'29.4"N, -102°18'03.7"W), and for *A. coahuilense* some kilometers west (Pavel Tuma, PT 508). Also, further south, *A. coahuilense* has been recorded at General Cruz Maltos (25°07'59.1"N, -102°18'50.1"W) (Anton Hofer, HO 1490).

Two theoretical models of homoploid hybrid speciation are recognized, the recombinational speciation model (Stebbins 1957; Grant 1958, 1981) and the external isolation model (Gross & Rieseberg 2005 and references therein). The external isolation model and the recombinational model are not mutually exclusive and elements of both have been found in many cases of homoploid hybrid speciation (Gross & Rieseberg 2005; Buerkle et al. 2000). As discussed below, in the case of *Astrophytum coahuilense*, various aspects of its biology and ecology suggest that both models are relevant to its origin.

Recombinational speciation.—The recombinational speciation model of homoploid hybrid speciation involves hybridization between two species that differ by two or more chromosomal rearrangements (Stebbins 1957; Grant 1958, 1981). The resulting F_1 hybrids are heterozygous for structural differences and thus have reduced fertility. But interbreeding between F_1 individuals may produce a small number of F_2 individuals with novel, chromosomally balanced genotypes, and restored fertility. The novel structural homozygotes (homokaryotypes), which are at least partially reproductively isolated from the parental species, may persist and give rise to a new homoploid species.

Astrophytum asterias, *A. capricorne*, *A. myriostigma*, and *A. ornatum* are diploid, with a chromosome number of $2n = 22$ (Katagiri 1953; Toman 1978; Ross 1981; Das et al. 2000; Das 2008). *A. coahuilense*, not previously studied, also has a diploid number of $2n = 22$ based on 23 meristematic root tip cells from 8 plants (Montanucci, unpublished data). Detailed karyotype analyses of the species listed above (except *A. coahuilense*) were made by Das et al. (2000) and Das (2008). The authors recognized four categories of chromosomes based on size, position of the primary constriction, and position of a secondary constriction, if present. The four standard types of chromosomes were: Type A—chromosomes with two constrictions in median and submedian positions; Type B—chromosomes with two constrictions in sub-median and sub-terminal positions; Type C—chromosomes with a nearly median to median primary constriction; Type D—chromosomes with a nearly sub-median primary constriction. Das et al. (2000) and Das (2008) suggested that the karyotypic differences among the species were the result of duplications and translocations. But it is also possible that unequal pericentric inversions could explain changes from metacentric to sub-metacentric chromosomes, and vice versa.

The karyotype formulas for *Astrophytum capricorne* and *A. myriostigma* (the putative parents of *A. coahuilense*) were found to differ from each other, but there were disparities between the two studies. For *A. capricorne*, Das et al. (2000) gave a karyotype formula of $2B + 16C + 4D$, but Das (2008) reported a formula of $4B + 16C + 2D$. For *A. myriostigma*, Das et al. (2000) reported a formula of $4A + 12C + 6D$, but Das (2008) gave a formula of $6A + 12C + 4D$. In both studies, the two species were found to differ by the number of C and D type chromosomes. Also, type B chromosomes were found only in *A. capricorne* and type A chromosomes were found only in *A. myriostigma*. Because the two putative parental species differ by at least two structural rearrangements, they meet the preconditions for the recombinational speciation model.

Self-fertilization and out-crossing.—Grant (1958) argued that new structural homozygotes are more likely to arise in the offspring of F_1 hybrids under a system of inbreeding rather than out-breeding. According to Rieseberg (1997: 365), Grant's argument led to the prediction that recombinational speciation should be more common among self-fertilizing species than among out-crossing species. However, all confirmed cases of homoploid hybrid speciation listed in Rieseberg (1997: 378, 381) involve taxa which are out-crossers. To explain this unexpected result, Rieseberg (1997: 383) suggested that self-fertilizing species are less likely to

hybridize than out-crossers. All species of *Astrophytum* are essentially obligate out-crossers that depend on insect pollinators, and normally cannot self-fertilize (Megata 1944; Ross 1981).

Pollen and seed dispersal.—In a study of the floral visitors of *Astrophytum myriostigma* (= *A. coahuilense*) in the Sierra El Sarnoso, Durango, three species of bees were recorded as floral visitors: *Ancylscelis apiformis*, *Augochloropsis metallica*, and *Diadasia olivacea*, the latter being more prevalent than the former two (Martínez-Adriano et al. 2015). Also, small bees (genus *Perdita*) are pollinators of *A. asterias* and *A. myriostigma* (Blair & Williamson 2008; Montanucci, unpublished data). Blair & Williamson (2008) observed that *Perdita* (*Macrotera*) *lobata* was the most frequent floral visitor of *A. asterias*, but the medium-sized cactus specialist, *Diadasia rinconis*, was a more effective pollinator (in terms of percent fruit set, mean number of seeds per visit, mean seed set per fruit). The authors surmised that because of the high frequency of visitations, *P. lobata* probably contributed a small proportion of the total seed set in any given population of *A. asterias*.

Pollen dispersal by insect pollinators of *Astrophytum* appears to be restricted. Cactus bees (genus *Diadasia*) are capable of foraging flights of up to 1 km, but most forays occur only within a few hundred meters (McDonald & McPherson 2005). The foraging flights of the small bee, *Perdita* (*Macrotera*) *texana*, occurred between 20 and 40 m from their nest (Neff & Danforth 1991). These observations of restricted forays are further supported by the study of Blair & Williamson (2010) in which fluorescent dyes were used as a pollen analogue to track pollen dispersal within a local population of *A. asterias*. The authors applied dye to the recently opened flowers of 13 source plants and recorded the presence of dye in the flowers of 69 recipient plants. About 80% of the recipient plants were located within 30 m of the source plants. The dispersal of dye particles followed a leptokurtic distribution, and the longest dispersal distance recorded was 142.2 m.

Seed dispersal also appears to be restricted. *Astrophytum* seeds are hat-shaped with a deeply sunken hilum surrounded by a prominent collar. This morphology has been interpreted as an adaptation for dispersal by water (Bregman 1988; Becerra 2009). Hook (1990a, b) reported finding *Astrophytum* seedlings in the Sierra de Parras growing along the courses of ephemeral rivulets which had been created by rains during summer thunderstorms. Seed dispersal by rainfall would seem to be effective on sloping terrain, although established seedlings have been observed only short distances down slope from the maternal plant. On flat terrain seedlings are usually found growing immediately around the maternal plant.

Ants are also an important dispersal agent, being attracted to the lipid-rich eliasomes (the funiculus) attached to the seed (Bernhard 1987). After dehiscence of the fruit, ants remove the seeds from the fruit and carry them to the colony entrance. There they detach the eliasomes and take them below ground; the seeds are left discarded on a refuse heap near the colony entrance and eventually are further scattered by the elements.

Other animal vectors may play a role in seed dispersal and could potentially carry seeds greater distances than ants could. Birds are attracted to the red fruit of various cactus species (Gibson & Nobel 1986), and they may be agents of seed dispersal for *Astrophytum capricorne* and *A. coahuilense*, both of which typically have bright red to purplish-red, semi-fleshy fruit. Klaus (1976) observed a ground squirrel (possibly *Ictidomys mexicanus* or *Otospermophilus variegatus*) feeding on the red fruit of *A. coahuilense*. Presumably, these rodents also feed on the red fruit of *A. capricorne*. They remove the fruit from the stem apex and eat it nearby or take it some distance away, thus scattering the seeds. Unlike the two *Astrophytum* species just mentioned, *A. myriostigma* has greenish, semi-dry fruit, which is unattractive to birds and rodents, and dries uneaten on the plant.

Astrophytum coahuilense arguably has a competitive advantage over its parental species in terms of seed dispersal. *A. myriostigma* depends on ants and rainfall for dispersal of its seeds. But the red, semi-fleshy fruits of *A. coahuilense* and *A. capricorne* are attractive to rodents and presumably to birds as well. These animal vectors can quickly carry the fruit considerable distances. The fruit of *A. capricorne*, however, is often enclosed in a tangle of curly spines at the stem apex. Under such circumstances rodents and birds may have difficulty accessing and removing the fruit but they would have no difficulty removing fruit from the stem of *A. coahuilense* which lacks spines. Thus *A. coahuilense* would seem to have greater seed dispersal capability than either of its parents.

Inbreeding facilitates recombinational speciation.—As mentioned previously, under the model of recombinational speciation, the two parental species must differ by two or more chromosomal rearrangements (Grant 1981). The F_1 hybrid will be heterozygous for these rearrangements and partially sterile because 75% of its gametes will be unbalanced and inviable due to duplications and deletions. The remaining gametes will be viable, consisting of parental and recombinant karyotypes in a 50:50 ratio.

Inbreeding (defined as crossing between individuals more closely related than average pairs in a population) is an important factor in recombinational speciation. Through inbreeding a small number of interfertile F_2 individuals will be produced with novel homokaryotypes and which will be at least partially reproductively isolated from the parental species. Also, over successive generations of the hybrid line, recombination between the parental chromosomes will probably occur, leading to a progressive reduction in the size of the parental linkage blocks (Ungerer et al. 1998). This recombination between different parental linkage blocks is thought to lead to further reproductive isolation between the incipient hybrid species and its parents (Rieseberg et al. 1996a).

It can be argued that the restricted pollen and seed dispersal in *Astrophytum* contribute to inbreeding, and therefore would have facilitated the mechanism of recombinational speciation. In habitat, mature *Astrophytum* plants are often surrounded by a cohort of seedlings presumably comprised of full-sibs and half-sibs. Under a postulated hybridization event, the F_1 hybrid seedlings would become established within a relatively small area surrounding their maternal plant. When the F_1 hybrids reached sexual maturity (and assuming some synchrony in flowering), they would be cross-pollinated by visiting insect pollinators. Continual cross-pollinations between closely related hybrid plants would eventually lead to the emergence of a new homozygous chromosomal rearrangement and its maintenance as a true-breeding line.

The external isolation model.—Gross & Rieseberg (2005) reviewed the ecological theory of homoploid hybrid speciation and based on theoretical and empirical evidence they concluded that ecological selection is a major factor facilitating homoploid hybrid speciation. The authors noted that in all suspected and confirmed cases of homoploid hybrid speciation, some type of ecological divergence between the hybrid species and its parents has been found, e.g., habitat, pollinator and/or temporal divergence. Furthermore, in all well-documented examples, the hybrid occurs in a habitat that is characterized as “different,” or in some cases, “extreme,” compared to the habitats of the parental species. The successful occupation of different or extreme habitats is often attributed to adaptive physiological traits resulting from transgressive segregation (Rieseberg et al. 1999).

In the case of *Astrophytum coahuilense* there is evidence of ecological divergence, suggesting that ecological selection played a role in the evolution of this species. *A. coahuilense* occurs in the mountains bordering the southwestern perimeter of the Comarca Lagunera, a region of southern Coahuila and adjacent Durango characterized by low rainfall. The region occupied by *A. coahuilense* has an average annual rainfall of about 224 mm and has an average annual temperature of 21°C (Bernhard 1987; Czaja et al. 2014). The annual precipitation totals for four localities of *A. coahuilense* are generally less and have narrower limits (177–245 mm), than the localities (10 records) for *A. capricorne* (201–604 mm). The localities (12 records) for *A. myriostigma* (370–616 mm) have higher precipitation and do not overlap with those for *A. coahuilense*. The precipitation data for the localities of all three species are significantly different (*A. capricorne* vs. *A. coahuilense*: $df = 25$, $t = 2.78$, $p < 0.0103$; *A. capricorne* vs. *A. myriostigma*: $df = 25$, $t = -3.03$, $p < 0.0056$; *A. coahuilense* vs. *A. myriostigma*: $df = 25$, $t = -5.11$, $p < 0.0001$). Tests for average annual temperature for localities of the three species were not significantly different.

The associated flora in the habitat of *Astrophytum coahuilense* has considerably lower species diversity than that of the habitats of the two parental species (Hooek 2008; Hooek & Baumann 1991, and unpublished records). The associated flora consists of only 53 species, including 37 cacti, six succulents, and ten trees and shrubs. The number of species recorded from the habitat of *A. capricorne* is 127, including 78 species of cacti, 11 species of succulents, and 38 species of trees and shrubs. The associated flora in the habitat of *A. myriostigma* has the highest diversity with 152 species, including 104 species of cacti, 11 species of succulents, and 37 species of trees and shrubs.

The region inhabited by *Astrophytum coahuilense* has a more open character with widely spaced perennial shrubs compared with the habitats of the parental species. Growing sites were recorded for 114 plants; 91 plants (79.8%) were growing in open sites exposed to full sun, and 23 plants (20.2%) were growing in partial shade. *A. capricorne* was also usually found growing in exposed locations. At four localities in eastern Coahuila and adjacent Nuevo León, 37 of 48 plants (77%) were growing in exposed sites; 11 plants (23%) were growing in partial shade. At six localities in southern Coahuila, 23 of 38 plants (60.5%) were growing in exposed sites, and 15 plants (39.5%) were situated in partial shade. The growing sites for *A. myriostigma* were recorded at three locations in the Central Plateau region and two locations in the Jaumave Valley. In the Central Plateau, 55 of 112 plants (49%) were growing in exposed sites, and 57 plants (51%) were in partially shaded sites. In the Jaumave Valley, a contrast was noted between growing sites in the southern and northern areas of the valley. In the south, 36 of 53 plants (67.9%) were growing in exposed sites, and 17 plants (32.1%) were growing in partial shade. In the northern area, out of a total of 70 plants, 28 plants (40%) were growing in exposed sites, and 42 plants (60%) were growing in partial shade.

The dense covering of epidermal trichomes on the stem of most specimens of *Astrophytum coahuilense* presumably protects the epidermis from intense solar radiation and moderates stem temperature. Experimental studies on various cacti have shown that thick apical pubescence protects the apical meristem from damage by high or low temperature extremes (Gibson & Nobel 1986). Epidermal trichomes have another adaptive function, namely gaseous phase water uptake by the stem. Lux & Kopunec (1992) demonstrated experimentally that gaseous phase water uptake by the stem was 10 times greater in *Astrophytum* species with epidermal trichomes as compared with those devoid of trichomes. The dense covering of epidermal trichomes of *A. coahuilense* may provide a survival advantage, especially for seedlings which can be susceptible to lethal desiccation during extended intervals of soil moisture deficit.

In summary, the habitat of *Astrophytum coahuilense* is significantly more arid than that of the parental species, has a relatively more open character, and the associated flora is less diverse. The habitat can be considered “different” if not “extreme” compared with the habitats of the parental species, and especially compared with the habitat of *A. myriostigma*.

A BRIEF HISTORICAL ACCOUNT OF HYBRIDIZATION

The Quaternary history of the Chihuahuan Desert is still poorly understood due to its complexities (Metcalf 2006). However, there is general agreement that Pleistocene glacial-interglacial oscillations and the more recent climatic fluctuations during the Holocene profoundly influenced the distribution and diversification of the flora of this region (Loera et al. 2012; Gándara & Sosa 2014; Scheinvar et al. 2017).

The Sierra Parras, Sierra Salsipuedes, and Sierra La Concordia comprise a transverse mountain axis that strikes westward from the northern segment of the Sierra Madre Oriental near 25°N latitude. This axis is an imposing physical barrier that has had a significant role in the evolutionary history of *Astrophytum*. Apparently, it was responsible for the primary phylogenetic division of *Astrophytum* in the late Miocene (5.7 mya) resulting in the separation of two clades, north and south of this geographic barrier (Vázquez-Lobo et al. 2015).

Astrophytum capricorne, a member of subgenus *Neoastrophytum*, occupies the region north of the transverse mountain axis, and *A. myriostigma* (a member of subgenus *Astrophytum*) occupies a region well to the south. Hybridization between the two species occurred when the latter species spread northward to reach this mountain barrier, and thence westward to contact populations of *A. capricorne* on the southern slopes of the transverse axis. Interbreeding between the two species produced a hybrid population which after becoming stabilized and true-breeding, spread westward along the mountains bordering Paleo-lake Irritila, a shallow drainage basin of immense size (15000 km²) (Czaja et al. 2014). This Pleistocene paleo-lake may have provided a partial barrier to gene exchange between *A. capricorne* and the incipient hybrid species (*A. coahuilense*), facilitating the latter's establishment (Fig. 5). Also, more efficient seed dispersal in the hybrid compared with its parental species and perhaps physiologically adaptive transgressive traits may have contributed to the hybrid's success in colonizing the region.

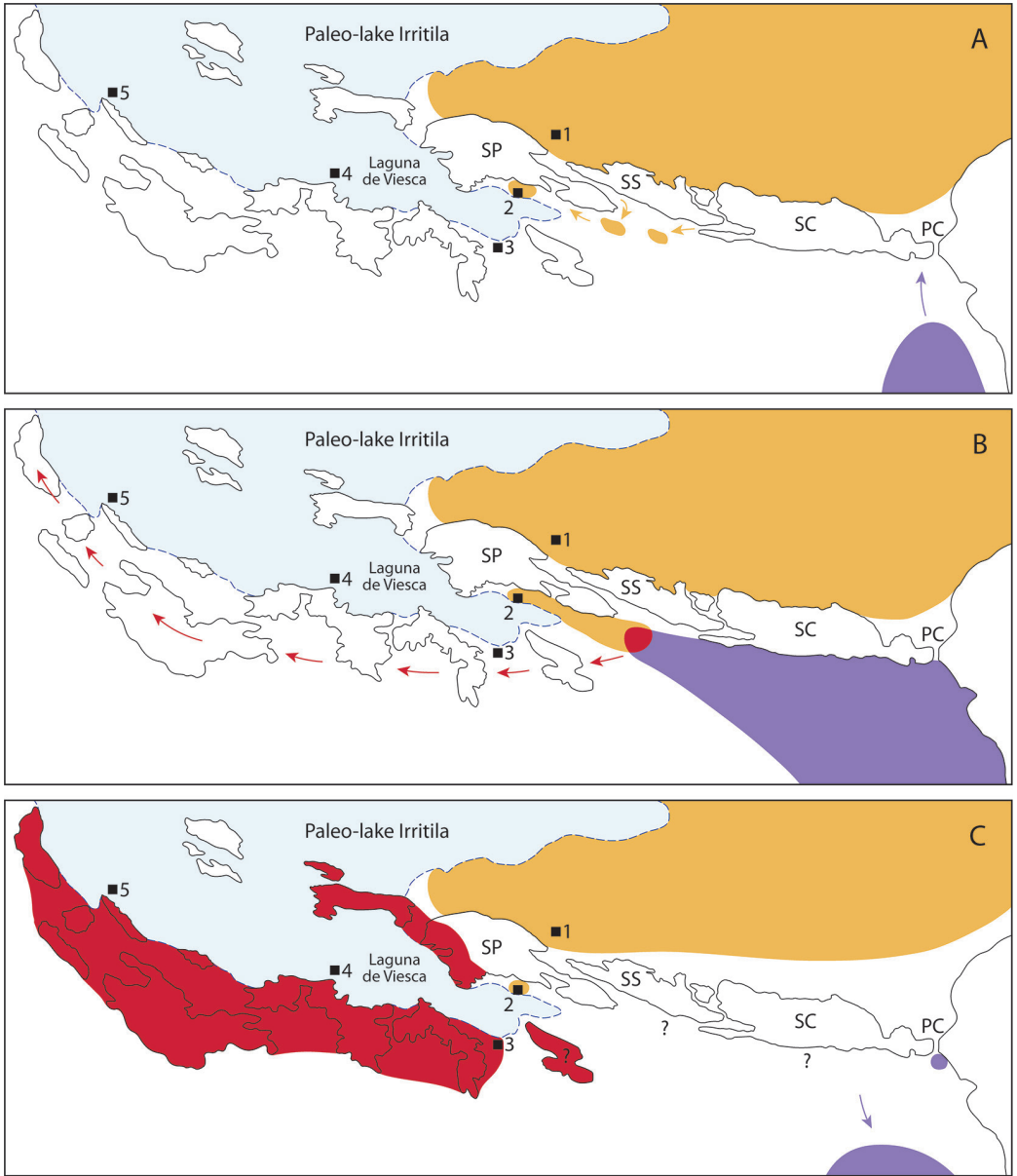


FIG. 5. Hypothetical changes in the distributions of *Astrophytum capricorne* (yellow) and *A. myriostigma* (blue) resulting in the hybrid origin of *A. coahuilense* (red). **A.** Northward spread of *A. myriostigma* and dispersal of *A. capricorne* through mountain passes. **B.** Contact and hybridization producing the incipient hybrid species *A. coahuilense* which disperses westward. **C.** Southward contraction of *A. myriostigma*. Geographic abbreviations: Paso de Careros (PC), Sierra la Concordia (SC), Sierra Salsipuedes (SS), Sierra de Parras (SP). Cities and agrarian settlements are: 1. Parras de la Fuente, 2. Ejido Tanque Menchaca, 3. General Cruz Maltos, 4. Viesca, 5. Torreón. Boundaries of Paleo-lake Irritila shown by dashed line (modified from Czaja et al. 2014).

Vázquez-Lobo et al. (2015) estimated (using BEAST) the time of divergence of *Astrophytum coahuilense* from its common ancestor with *A. capricorne* at about 3.3 mya (Pliocene). But distributional shifts resulting in interspecific contact and hybridization more likely would have occurred during the Pleistocene (1.6 mya) when profound climatic oscillations would have prompted such changes. Also, *A. coahuilense* is apparently absent (records are lacking) from the Sierra de San Lorenzo, Sierra Solis, and Sierra de Texas, which lie within the former boundaries of Paleo-lake Irritila. If the origin of *A. coahuilense* had predated the Pleistocene formation of this paleo-lake, this species would probably occupy these mountain ranges.

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