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Species delimitation in *Neolloydia* Britton & Rose (Cacteae, Cactaceae)

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Abstract
Background: *Neolloydia* is a genus widely distributed in Mexico with notable morphological variation. In recent lists of Cactaceae, only *Neolloydia conoidea* has been recognized; however, previous reviews consider it to comprise a species complex. No studies have included statistical analyses of morphological variation, so species boundaries and recognition are uncertain.
Questions: How many species should be recognized in the genus *Neolloydia*?
Studied species: Genus *Neolloydia* Britton & Rose
Study site and dates: Twenty-nine sites located in the Mexican states of Coahuila, Guanajuato, Hidalgo, Nuevo León, Querétaro, San Luis Potosí, Tamaulipas, and Zacatecas were explored between 2022 and 2024.
Methods: Previous taxonomic proposals and a review of morphological traits allowed us to identify seven *a priori* groups. Ten quantitative and four qualitative traits were analyzed and tested using Random Forest classification analysis. Niche characterization was performed using GIS data (landform and soil type) from 189 records, followed by a chi-square goodness-of-fit test.
Results: Random Forest classification analysis identified the seven *a priori* designated groups as independent. According to the chi-square goodness-of-fit test, the results are significant for landform and soil type. For two taxa, it was not possible to determine whether their distribution was non-random.
Conclusions: A combination of morphological and niche characteristics supports the recognition of four species previously proposed by other authors; additionally, three new species are proposed.
Keywords: Cactaceae, species complex, Random Forest, MDS, new species, niche characterization.

Resumen
Antecedentes: *Neolloydia* es un género ampliamente distribuido en México con una variación morfológica notable. En listados recientes de Cactaceae solo ha sido reconocida *Neolloydia conoidea,* sin embargo, revisiones previas consideran que comprende un complejo de especies. No se han realizado estudios que incluyan análisis estadísticos que analicen la variación morfológica, por lo que los límites y reconocimiento de especies son inciertos.
Pregunta: ¿Cuántas especies deben ser reconocidas en el género *Neolloydia*?
Especie de estudio: Género *Neolloydia* Britton & Rose.
Sitio y fechas de estudio: Se exploraron 29 sitios localizados en los estados mexicanos de Coahuila, Guanajuato, Hidalgo, Nuevo León, Querétaro, San Luis Potosí, Tamaulipas y Zacatecas, visitados entre 2022 y 2024.
Métodos: Las propuestas taxonómicas previas y la revisión de caracteres morfológicos nos permitieron reconocer siete grupos determinados *a priori.* Se analizaron diez caracteres cuantitativos y cuatro cualitativos, los cuales se pusieron a prueba mediante el análisis clasificatorio Random Forest. A partir de 189 registros se realizó la caracterización de nicho usando datos SIG's (geoforma y tipo de suelo), enseguida, se aplicó una prueba de bondad de ajuste chi-cuadrada.
Resultados: El análisis clasificatorio Random Forest permitió reconocer como independientes a los siete grupos designados *a priori*. De acuerdo con la prueba de bondad de ajuste chi-cuadrada, los resultados son significativos para geoforma y tipo de suelo. Para dos taxones no fue posible determinar si su distribución no es aleatoria.
Conclusiones: Una combinación de caracteres morfológicos y de nicho soporta el reconocimiento de cuatro especies previamente propuestas por otros autores, adicionalmente, se proponen tres nuevas especies.
Palabras clave: Cactaceae, complejo de especies, Random Forest, MDS, especies nuevas, caracterización de nicho.

The genus *Neolloydia* was described by Britton & Rose (1922), with the type species being *Mammillaria conoidea* DC., collected by Thomas Coulter near Zimapán in Mexico (De Candolle 1828, Anderson 1986). The members have tuberculate stems, differentiated spines in radial and central, emerging flowers in the apical region, globose fruits that dry when ripe, and black tuberculate seeds (Britton & Rose 1922). Phylogenetic studies differ regarding its recognition as an independent genus (Breslin *et al*. 2021, Chincoya *et al*. 2023). On one hand, Breslin *et al*. (2021) propose merging *Neolloydia* Britton & Rose, *Ortegocactus* Alexander, and a group of *Mammillaria* Haw. species into *Cochemiea* (K. Brandegee) Walton., under the argument of a correlated geographical distribution. On the other hand, Chincoya *et al*. (2023) disagree with this proposal, since these lineages show disparity in their biogeography and ecological affinities, as well as a distinctive morphological variation that does not allow a practical delimitation at the generic level. These authors explain that *Neolloydia* diversified in the Chihuahuan Desert, while *Ortegocactus* diversified in the Chihuahuan Desert and subsequently migrated to the Serranías Meridionales, and *Cochemiea* diversified in the Baja California province. Hence, they should be recognized as three independent lineages; this argument is accepted for the present work.

The number of species recognized within *Neolloydia* has been controversial. Britton & Rose (1923) recognized seven species, noting that in this proposal four species were classified in other genera in subsequent taxonomic revisions (Table 1). Three taxa were retained within the genus: *N. conoidea*, *N. ceratites* (Quehl) Britton & Rose and *N. texensis* Britton & Rose, which were all recognized as varieties of the former taxon, in addition to including *N. matehualensis* Backeb. as a variety (Kladiwa & Fittkau 1971). A comparative analysis by Anderson (1986) concluded that variation in spine length and total number per areole did not form discrete groups, referring to one set of taxa as the *N. conoidea* complex (Table 1). This taxonomic complex is distributed in the Mexican states of Hidalgo, Querétaro, Guanajuato, San Luis Potosí, Durango, Zacatecas, Coahuila, Nuevo León and Tamaulipas, as well as in Texas in the United States (Anderson 1986, Hernández & *Gómez*-Hinostrosa 2011). Finally, Breslin *et al*. (2021) only recognizes *Cochemiea* (=*Neolloydia*) *conoidea* (DC.) P.B. Breslin & Majure because no samples of the previously recognized taxa were included, therefore, the names were listed in the synonymy (Table 1). However, Bravo-Hollis & Sánchez-Mejorada (1991) took up the proposal of Kladiwa & Fittkau (1971), again recognizing *N. matehualensis* at the specific level (Table 1). Later revisions, based only on morphological comparisons between taxa, stopped recognizing varieties and accepted to *N. conoidea* and *N. matehualensis* (Anderson 2001, Hunt *et al*. 2006). More recently, Donati (2012) described *Neolloydia inexpectata* D. Donati based on a comparison of seedlings of the new taxon with *N. conoidea*; however, due to the lack of updated data on the biology of this species, its *status* is considered uncertain (Korotkova *et al*. 2021).

Morphological analysis methods with multivariate statistics have been applied in different plant groups to understand interspecific boundaries. With these methods, it has been possible to resolve taxonomic complexes such as *Cestrum* L. [Solanaceae, Castillo-Batista *et al*. (2017); *Danielia* (DC.) Lem. (Fabaceae, De la Estrella *et al*. (2009), *Asteropyrum* J.R. Drumm. & Hutch. (Ranunculaceae, Cheng *et al*. 2021)], to mention some genera. Particularly, in Cactaceae, numerous studies have focused on the characterization and subsequent statistical analysis of vegetative structures, providing valuable information to distinguish between species in the absence of reproductive structures (Tapia *et al*. 2016, Arroyo-Cosultchi *et al*. 2017, Sánchez *et al*. 2020).

Like morphometry, niche characterization has provided evidence of environmental differences between localities to recognize separate evolutionary lineages, reinforcing morphological results, and even molecular analyses (Kozak & Wiens 2006, Ortiz-Martínez *et al*. 2024). Ecological niche characterization has been relevant in reinforcing species delimitation studies in lineages such as *Dioon* Lindl. (Zamiaceae, Gutiérrez-Ortega *et al*. 2020) and *Nolina* Michx. (Asparagaceae, Ruiz-Sanchez & Specht 2014). The application of environmental variables to support species delimitation in Cactaceae has been practical to support circumscription derived from morphological and molecular information in lineages of the tribes Cacteae and Cereeae (Aquino *et al*. 2021, Franco-Estrada *et al*. 2022, Cervantes *et al*. 2023).

In this study, we statistically analyzed the morphological variation within the *N. conoidea* complex throughout its distribution in Mexico, as well as the preference for soil type and landform among the recognized groups, to identify potential barriers that promote lineage isolation and propose an objective delimitation of the number of species in this genus.

Table 1. Taxonomic history of the *Neolloydia conoidea* complex according to proposed revisions. The symbol ** indicates that the taxon was transferred to a different genus in later studies. "=" synonym. "?" *status* uncertain.

Britton & Rose (1923)	**Kladiwa & Fittkau (1971)**	**Anderson (1986)**	**Bravo-Hollis & Sánchez-Mejorada (1991)**	**Anderson 2001, Hunt *et al*. 2016**	**Breslin *et al*. (2021), Korotkova *et al*. (2021)**
Neolloydia conoidea	*Neolloydia conoidea*	*Neolloydia conoidea*	*Neolloydia conoidea*	*Neolloydia conoidea*	*Cochemiea* (*Neolloydia*) *conoidea*
	N. conoidea var. *matehualensis*	= *N. matehualensis*	*N. matehualensis*	*N. matehualensis*	= *N. matehualensis*
N. ceratites	*N. conoidea* var. *ceratites*	= *N. ceratites*	*N. conoidea* var. *ceratites*	= *N. ceratites*	= *N. ceratites*
	N. conoidea var. *grandiflora*	= *N. grandiflora*	*N. conoidea* var. *grandiflora*	= *N. grandiflora*	= *N. grandiflora*
N. texensis	*N. conoidea* var. *texensis*	= *N. texensis*	*N. conoidea* var. *texensis*	= *N. texensis*	= *N. texensis*
					? *N. inexpectata*
N. clavata **					
N. horripila **					
N. pilispina **					
N. beguinii **	*N. beguinii* **		*N. beguinii* **		

Materials and methods

Taxon sampling. Taxonomic information available on *Neolloydia* was reviewed and compiled. Records were obtained from the herbaria ASU, IBUG, GBH, MEXU, and TEX (Thiers 2023) and from the digital platform iNaturalistMX (www.naturalist.mx 2023). All records were organized in a database and georeferenced manually using Google Earth v. 7.3.4, resulting in 1,218 records. Fieldwork was carried out in the states of Coahuila, Guanajuato, Hidalgo, Nuevo León, Querétaro, San Luis Potosí, Tamaulipas, and Zacatecas (Mexico), between 2022 and 2024, visiting 29 localities (see Supplementary material 1). For each site, ten adult individuals were selected, identified by the presence of flowers, fruits, or remains of these. For each individual, five to seven photographs were taken at three angles of the plant using a millimetric scale as a reference for posterior measurements. Additionally, three individuals were collected per locality and kept in cultivation to make additional measurements and observations. Altitude and coordinates were recorded for each locality using a GPS (Garmin 62S). In addition to the sampling of *N. ceratites*, *N. conoidea*, *N. matehualensis* and *N. inexpectata*, in two locations it was observed that the shape of the stem differed from that described in the consulted literature, so they were determined *a priori* as *Neolloydia* sp. 1 and *Neolloydia* sp. 2. Similarly, in a third location the arrangement of the spines differed from that observed in individuals collected during field work, so it was called *Neolloydia* sp. 3.

Character selection. From a series of morphological attributes recognized by Kladiwa & Fittkau (1971), Anderson (1986), and Bravo-Hollis & Sánchez-Mejorada (1991), 14 vegetative characters were selected (Table 2). Ten were discrete quantitative, which were recorded by manual counting, while the continuous quantitative variables were measured in ImageJ v. 1.53n (Schneider *et al*. 2012). In the particular case of tubercles, the spines were removed to facilitate their measurement with a digital vernier (Mitutoyo, Inc.). Four qualitative variables were considered and proposed in alternate states. In the specific case of epidermis color, the coding was based on the RHS Large Color Chant® palette (Six Revised Edition). Additionally, it was observed that individuals of two localities (Aramberri and Rayones, Nuevo León, Mexico) presented a constriction in the basal part of the stem, which was called "neck" (Table 2).

Table 2. Characters and acronyms used in morphological analysis. The symbol * is used for qualitative characters.

Character	Acronym	Character	Acronym
Stem ratio (height/diameter)	SR	Middle part of the tubercle	MdTub
Stem apex	StAp	Apex of the tubercle	ApTub
Areola length/width ratio	AR	Neck (presence/absence)*	Neck
Number of central spines	NCS	Space between tubercles* (space/no space)	ST
Length of central spines	LCS	Color* (opaque-green, bright-green and glaucous-green)	Color
Number of radial spines	NRS	Orientation of the central spine* (porrect/appressed)	OCS
Length of radial spines	LRS		
Tubercle shape (height/diameter)	TS		

Statistical analysis. For each variable, the mean and standard deviation were calculated (Table 3). Spearman's correlation coefficient (SCC) was calculated for quantitative variables. If, SCC ≥ ±0.6, the probability of correlation was considered high. (Aquino *et al*. 2019). Stem height and diameter, as well as the length and width of both the areole and the tubercle are correlated, therefore, they were transformed into stem height/diameter ratio (SR, Table 2), areole length/width ratio (AR, Table 2) and tubercle length/width ratio (TS, Table 2). The retained characters were added to a matrix analyzed with the Random Forest (RF) classification method (Breiman 2001). The database was partitioned to generate a training matrix (70 %) and a validation set (30 %). Different combinations of hyperparameters were evaluated to optimize the classification model. Several trees (ntree) in the range of 100 to 1,000 were tested, increasing by 100, and mtry values between 1 and 10 were explored. The best model was selected based on the accuracy obtained by repeated cross-validation. The multidimensional scale (MDS) was calculated using randomForest v. 4.6-14 (Liaw & Wiener 2002). Together with the classification model, calculations were performed with R (R Core Team 2021) in the RStudio environment (RStudio Team 2019).

Niche characterization. From 1,218 records, 189 were selected, of which 158 correspond to *N. conoidea*, eleven to *N. ceratites*, five to *N. inexpectata* and *N. matehualensis*, four to *Neolloydia* sp. 1, and three to *Neolloydia* sp. 2 and *Neolloydia* sp. 3. Only localities with precise coordinates were considered; therefore, ambiguous localities and duplicate records were eliminated, as well as those that were less than 5 km from each other to avoid spatial correlation (Aquino *et al*. 2021). Soil type according to FAO classification (Fischer *et al*. 2008) and landform (Aquino *et al*. 2021) were analyzed, where each layer has a spatial resolution of approximately 1 km^2. The variables were extracted using QGIS v. 2.1.8 (Quantum GIS Development Team 2024). Categorical variables were arranged in a contingency matrix from which the expected observation matrix and Pearson residuals were calculated, and a chi-square (χ^2) goodness-of-fit test was applied. Pearson residuals and χ^2 were calculated with dplyr (Wickham *et al*. 2020) and plotted using corrplot (Wei & Simko 2017). Since multiple comparisons were made per each species, *p*-values were adjusted using the Bonferroni correction. For each species, the occurrence proportions in each category were estimated, along with their 95 % confidence intervals, using Wilson's method (García-Pérez 2023). The analysis was conducted using the tidyverse (Wickham *et al*. 2019) and Binom (Dorai-Raj 2022) packages. All calculations were performed using the R programming language (R Core Team 2021) within the RStudio environment (RStudio Team 2019).

Conservation. Area of occupancy (AOO) and extent of occurrence (EOO) were estimated for taxa that could be considered new species using the Geospatial Conservation Assessment Tool (GeoCAT, Bachman *et al*. 2011). This analysis was applied only to taxa that were resolved as new species.

Table 3. Mean and standard deviation values of quantitative variables. SR: stem ratio; StAp: stem apex; AR: areola ratio; NRS: total number of radial spines per areole; LRS: length of radial spines; NCS: total number of central spines per areole; LCS: length of central spine; TS: tubercle height-to-width ratio; ApTub: tubercle apex; MdTub: middle of tubercle. "**N**" corresponds to the number of individuals analyzed.

ESPECIE	N	SR		StAp (mm)		AR		NER		LRS (mm)	
		x̄	SD	x̄	SD	x̄	SD	x̄	SD	x̄	SD
N. ceratites	30	10.23	±3.784	12.56	±1.767	10.45	±0.897	11.6	±1.610	8.94	±1.198
N. conoidea	190	13.70	±3.942	13.03	±1.723	10.64	±1.956	13.52	±3.002	7.69	±1.583
N. inexpectata	10	12.23	±1.698	13.09	±1.370	10.81	±0.904	13.9	±0.994	5.96	±0.111
N.matehualensis	20	11.99	±4.153	15.67	±3.297	10.05	±1.295	9.75	±1.371	8.23	±0.306
N.sp. 1	10	21.61	±0.565	14.03	±2.023	10.84	±0.932	18.9	±2.469	5.78	±1.522
N.sp. 2	20	13.00	±1.677	15.16	±1.815	12.07	±1.136	20.95	±1.700	6.81	±0.748
N.sp. 3	10	14.27	±1.852	9.51	±1.369	10.10	±0.583	19.7	±1.494	5.08	±1.936

ESPECIE	N	NCS		LCS (mm)		TS		MdTub (mm)		ApTub (mm)	
		x̄	SD	x̄	SD	x̄	SD	x̄	SD	x̄	SD
N.ceratites	30	6.73	±0.691	14.84	±3.224	1.05	±0.280	7.93	±1.262	5.34	±0.645
N.conoidea	190	2.20	±0.800	12.06	±3.088	1.15	±0.277	8.09	±1.465	5.50	±0.795
N.inexpectata	10	2.6	±0.516	7.60	±0.999	1.30	±0.141	7.55	±0.395	7.87	±0.107
N.matehualensis	20	2.5	±0.512	14.80	±2.821	0.53	±0.052	11.46	±0.226	5.84	±0.577
N.sp. 1	10	3.1	±0.567	9.00	±0.638	1.29	±0.124	7.63	±0.457	5.92	±0.430
N.sp. 2	20	3.45	±0.686	13.08	±1.296	1.15	±0.171	7.43	±0.659	4.69	±0.472
N.sp. 3	10	5.7	±0.823	11.46	±1.280	1.27	±0.391	6.07	±2.503	4.36	±1.487

Results

Morphometric analysis. The best model for the data had an accuracy value of 99 % (optimal value of ntree = 100 and mtry = 2, Figure 1A). The confusion matrix showed the number of correctly classified individuals and the classification error of 0.47 %; only one individual determined *a priori* as *Neolloydia* sp. 1 was classified in *N. conoidea* (Table 4). The most critical variables for the model are the space between tubercles, total number of central spines, arrangement of central spines, and the color of the epidermis. (Figure 1B). The model's overall performance is represented in figure (1C). The Y-axis represents the true positive rate (TPR), while the X-axis shows the false positive rate (FPR). Each colored line represents a distinct class, and the model performance is quantified by the area under the curve (AUC), whose value is close to 1.0, indicating a better discrimination capacity of the model.

Multidimensional scaling (MDS) analysis showed that there is no morphological proximity between the *a priori* recognized groups (Figure 1D). *Neolloydia ceratites*, *N. conoidea,* and *N. matehualensis* are clearly distinguishable groups in the MDS graph with 100% correct classification (Table 4). On the other hand, individuals determined *a priori* as *N. inexpectata* were segregated in the negative values of the MDS-1, close to *Neolloydia* sp. 2 (Figure 1D). However, both groups had 100 % of correctly classified individuals (Table 4). Similarly, a cloud of points corresponding to *Neolloydia* sp. 1 and *Neolloydia* sp. 3 are apparently interspersed (Figure 1D). However, the classification analysis indicated that only one individual determined *a priori* as *Neolloydia* sp. 1 was classified in *N. conoidea* (Table 4). On the other hand, *Neolloydia* sp. 3 obtained a classification percentage of 100 % (Table 4).

Niche characterization. The association between landform type and *Neolloydia* spp. members was statistically significant ($\chi^2 = 34.181$, df = 36, $p < 0.001$, p-adjusted < 0.005553). Multiple comparisons per species, using Bonferroni correction, were used to adjust p-values. In the case of *Neolloydia* sp. 3, it was not possible to determine whether the distribution was random, since the three records were concentrated in a single variable (Table S1). The remaining six taxa followed a significantly non-random distribution (Table S1).

For three taxa, Pearson's r was estimated; the distribution is not random, since the confidence index values are > 0.2. The positive association of *N. conoidea* with volcanic reliefs is not evident since the Pearson' r is close to zero (0.63, Table S2); however, the proportion of occurrence for this landform (6.96 %) is not significant (95 % CI: 0.393 - 0.1203 %, Table S3). On the contrary, it is significant for karstic system with a percentage of 38.6 % (95 % CI: 0.3137-0.46.38, Table S3). *Neolloydia matehualensis* exhibited a high association with plains (Pearson's $r = 1.731$), followed by karstic system (Pearson's $r = 0.862$). With an occurrence proportion of 60 %, it is confirmed that karstics system is the landform with which it presents the greatest affinity (95 % CI: 0.2307-0.8823, Table S3). Because the greatest number of observations occur in this landform (Table S3), the association of *Neolloydia* sp. 2 is strong for fold mountains (Pearson $r = 1.28$). The occurrence proportion for this landform (66.67 %) is significant (95 % CI: 0.2076-0.9385, Table S3). Pearson's r was estimated for three taxa; however, the distribution is random, since the

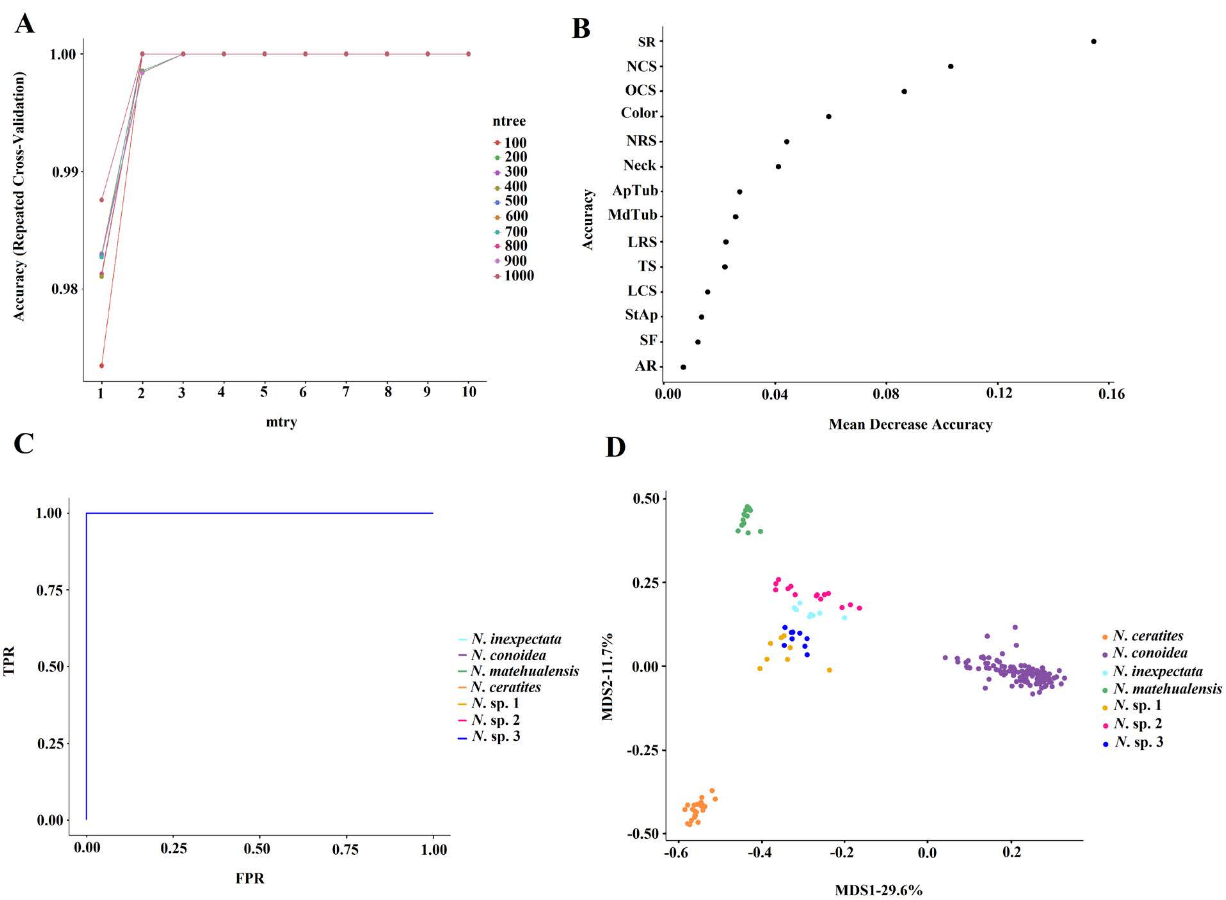


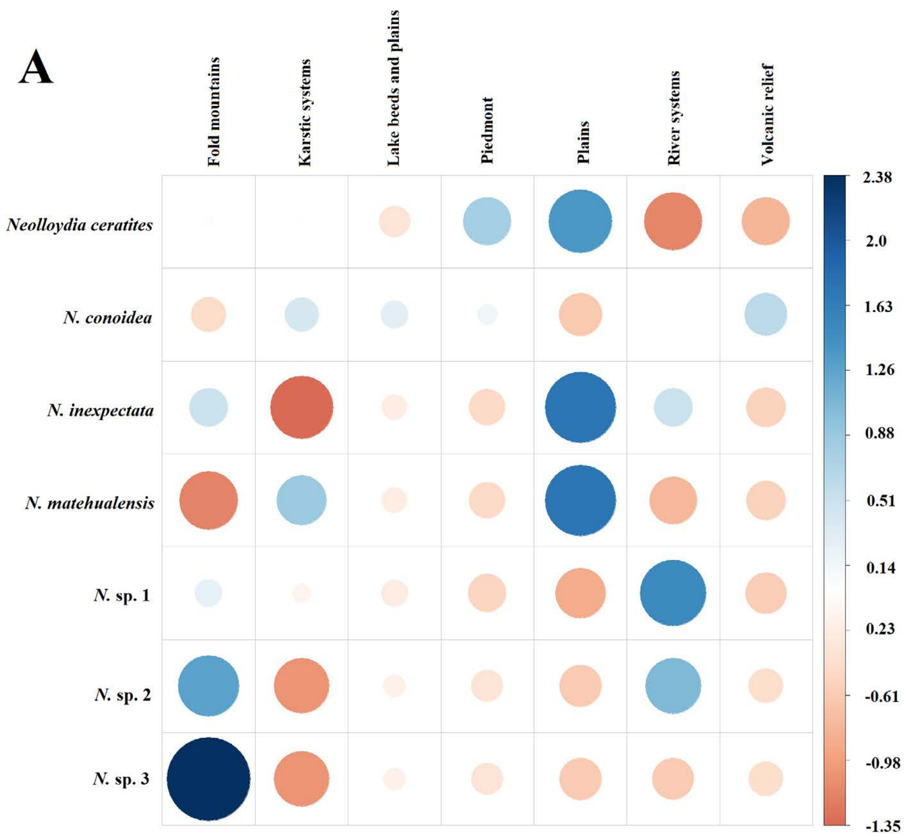
Figure 1. Results of the Random Forest (RF) classification analysis using 14 morphological variables. A) Accuracy with different parameters (ntree and mtry) to determine the best model; B) Variable importance plot; C) Receiver operating characteristic (ROC) curves of the cross-validated on test data; D) Multidimensional scaling (MDS) plot to illustrate the morphological clustering of the species. The meaning of the acronyms in section B is included. SR: stem ratio; StAp: stem apex; AR: areola ratio; NRS: number of radial spines per areole; LRS: length of radial spines; NCS: number of central spines per areole; LCS: length of central spine; TS: tubercle height-to-width ratio; ApTub: tubercle apex; MdTub: middle of tubercle.

confidence index values are < 0.2. *Neolloydia ceratites* is strongly associated with plains (Pearson's r = 1.731) and piedmont systems (Pearson's r = 0.764); however, the occurrence proportion is not significant (Table S3). This pattern is repeated with *N. inexpectata*, since the Pearson's r indicated that although it is strongly associated with plains (Pearson's r = 1.731, Figure 2, Table S2), the proportion of occurrence is not significant (Table S3). *Neolloydia* sp. 1 showed an association with river systems (Pearson's r = 1.503), while there was a negative association with plains (Pearson's r = -0.868) and the proportion of occurrence is not significant (Table S3). In the case of *Neolloydia* sp. 3, only Pearson residuals could be estimated; in this case, the positive association was with fold mountains (Pearson's r = 2.376). However, it is not possible to estimate the proportion of occurrence because observations are restricted to a single variable (Table S3).

Table 4. Confusion matrix obtained from the Random Forest (RF) analysis; the best model is shown.

	N. ceratites	*N. conoidea*	*N. inexpectata*	*N. matehualensis*	SP1	SP2	SP3	Class.error
N. ceratites	22	0	0	0	0	0	0	0.000
N. conoidea	0	136	0	0	0	0	0	0.000
N. inexpectata	0	0	8	0	0	0	0	0.000
N. matehualensis	0	0	0	15	0	0	0	0.000
N. sp. 1	0	1	0	0	7	0	0	0.125
N. sp. 2	0	0	0	0	0	15	0	0.000
N. sp. 3	0	0	0	0	0	0	8	0.000

The association between *Neolloydia* species and soil type was significant (χ^2 = 95.054, df = 48, p < 0.001, p-adjusted < 0.006). Multiple comparisons were made by species, and p-values were adjusted using the Bonferroni correction. *Neolloydia ceratites*, *N. conoidea*, *N. inexpectata*, *N. matehualensis*, *N.* sp. 1, showed a significantly non-random distribution among soil types (Table S4). In contrast, it was not possible to determine whether the distribution was random or not for *Neolloydia* sp. 2 and *N.* sp. 3 because the variables were concentrated in one category (Table S4). Five taxa showed a strong association with a soil type, *N. ceratites* showed positive although moderate association values for Calcic xerosol (Pearson's r = 1.965, Table S5), while the occurrence proportion is 45.4 % (95 % CI: 0.2127-0.7199, Table S6) confirming this preference. Additionally, the occurrence proportion (45.5 %) is significant for lithosol (95 % CI: 0.2127-0.7199, Table S6), even when the Pearson's residual is close to zero (Table S5). The Pearson's r values for *N. conoidea* are close to zero (Table S5). The highest value is for Haplic kastanozems (Pearson's r = 0.773), a value close to zero and which when graphed is not significant (Figure 2). However, the distribution of *N. conoidea* in Lithosols is not random, the occurrence proportion is 45.4 % (95 % CI: 0.2127-0.7199) confirming this preference, since the highest number of observations are found in this soil type (Table S6). Another taxon that follows a similar pattern is *N. matehualensis* since Pearson's r values are close to zero and it is not possible to determine the occurrence by soil type with this method (Table S5, Figure 2). On the contrary, the occurrence proportion (80 %) is significant for Lithosols (95 % CI: 0.3755-0.9637, Table S6). *Neolloydia inexpectata* was associated with Eutric regosols (Pearson's r = 5.044) and the occurrence proportion is 60.0 % (95 % CI: 0.2307–0.8823, Table S6). The association by Rendzines is next in importance (Pearson's r = 2.565), however the occurrence proportion (40 %) is not significant for that soil type (95 % CI: 0.1176-0.7692, Table S6). *Neolloydia* sp. 1 showed a strong association with Rendzines (Pearson's r = 5.141), with an occurrence proportion of 66.67 % (95 % CI: 0.2999-0.9032, Table S6), confirming a strong association. Finally, Pearson's residuals indicate that the frequency is positive for Lithosols in the case of *Neolloydia* sp. 2 and *N.* sp. 3 (Pearson's r = 1.841, in both cases, Figure 2B, Table S5). On the other hand, it was not possible to estimate the proportion of occurrence, since the observations are restricted to a single variable (Table S6).



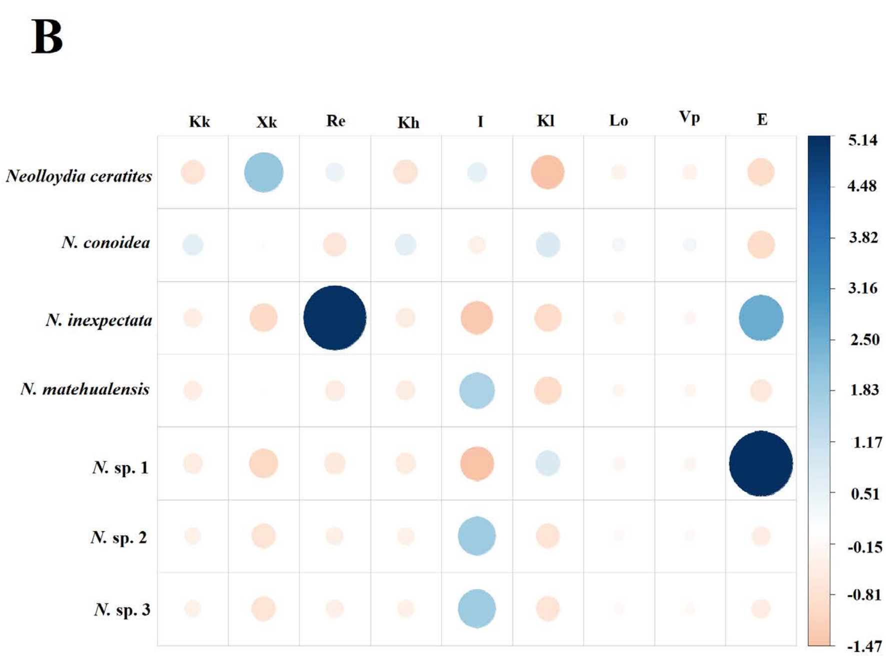


Figure 2. Correlograms of *Neolloydia* morphotypes/species for landform (A) and soil type (B). Positive correlations are shown in blue, and negative correlations in red. Key to acronyms B: Kk: Calcic kastanozems, Xk: Calcic xerosols, Re: Eutric regosols, Kh: Haplic kastanozems, I: Lithosols, Kl: Luvic kastanozems, Lo: Orthic livisols, Vp: Pelic vertisols, E: Rendzines.

Discussion

Species delimitation with morphological characters. Three of the six taxa recognized by Bravo-Hollis & Sánchez-Mejorada (1991) plus (Donati 2012) were included, resulting in four taxa. *Neolloydia conoidea* is a taxon with light to dark green stems with (0-) 2-3 (-4) straight central spines (Figures 3A and 4A). Based on our results, we consider *N. texensis* to be part of the variation of *N. conoidea*. This taxon is characterized by globose to short-oblong stems with 10-15 radial spines and 1-3 central spines (Britton & Rose 1923). The number of radial and central spines is consistent with the variation reported here and therefore does not merit recognition at the species level. Within the variation observed here, specimens with (0-) 1-2 central spines were included [Y. Moredia *et al*. 11 (CHAPA); supplementary material 1; Figures 3B and 4B]. These specimens match the description of *Neolloydia grandiflora* (Otto ex Pfeiff.) F.M. Knuth: cylindrical stems, number of central spines 0-1 (-2) and 16-20 radial spines (Pfeiffer 1837, Schumann 1896, Backeberg 1961). Statistical analysis did not show significant differences with respect to the rest of the individuals identified as *N. conoidea*, since both the number of radial and central spines agree with the pattern observed here.

Three *Neolloydia* taxa were distinguishable with the analyses performed. The first is *N. ceratites* (= *Mammillaria ceratites* Quehl), described with the diagnostic characters 5-6 central spines and up to 15 radial spines (Quehl 1909). The original description only mentioned the type locality as "Mexico", however, the description is accompanied by a good quality photograph, so authors such as Krainz (1972) and Bravo-Hollis & Sánchez-Mejorada (1991) mention localities in Coahuila and Zacatecas with very similar characteristics. In particular, the number of central spines ranging from (0-) 2-3 (-4) in *N. conoidea* (Figure 3A) allows it to be distinguished from *N. ceratites*, which has 6-7 (-8) central spines (Figure 3C), so the set of points representing *N. ceratites* and *N. conoidea* are located at opposite extremes of the MDS1-axis (Figure 1D). On the other hand, *N. matehualensis* was described from specimens collected in Matehuala, SLP (Mexico), the diagnostic characters are the color of the epidermis, radial spines 8-12 (-14) cm long, and 1-2 central spines of ca. 22 mm long (Backeberg 1948). Kladiwa & Fittkau (1971) as well as Anderson (1986) considered that it should be considered as part of the variation of *N. conoidea*. However, Hunt *et al*. (2006) recognize this taxon independently. Our results confirm the independence of *N. matehualensis* (Table 4). One of the morphological attributes with weight in RF is the color of the epidermis; in *N. matehualensis* the stems are glaucous-green, a unique characteristic not shared by the other members (Figure 3D). For this reason, determined individuals *a priori* segregate at the upper end of the MDS2 (Figure 1D). Finally, *N. inexpectata* was described and compared with *N. conoidea* based on the seedling morphology (Donati 2012). The *status* of this taxon had been uncertain, as it is not included in the synonymy of *N. conoidea* but also not recognized as an independent species (Korotkova *et al*. 2021). The appressed central spines allow distinguishing *N. inexpectata* from *N. conoidea*, *N. matehualensis* and *N. ceratites* whose central spines are porrect (Figure 3E), so the set of individuals are located in the middle part of the MDS2-axis (Figure 1D). Additionally, the length of the central spines in *N. inexpectata* is 0.50-1.11 cm, while in *N. conoidea* it is 0.43-2.0 cm. The scope of this work does not allow to include data on seedling morphology, however, Paria & Bose (2017) consider that the information obtained from seedling morphology offers a specific combination that serves both for the delimitation and identification of species.

Recognition of new species in Neolloydia. During the herbarium review and subsequent fieldwork, two morphotypes were detected: *Neolloydia* sp. 1 and *Neolloydia* sp. 3. The stems are characterized by the presence of a neck (Figures 4C and 4D), which makes it possible to distinguish them from *N. conoidea* (Figures 4A and 4B). However, the MDS graph indicates that the presence of a neck forms a single cloud; in contrast, the confusion matrix indicates that there are no individuals wrongly classified between both morphotypes (Table 4). We accept two independent morphotypes because a set of characters allows them to be differentiated. *Neolloydia* sp. 1 has (2-) 3-4 central spines and orbicular to suborbicular areoles (Figure 3F), while in *Neolloydia* sp. 3 the number of central spines is 5-6 (-7) and elliptical areoles (Figure 3H). On the other hand, *Neolloydia* sp. 2 differs from *N. conoidea* by the arrangement of the appressed central spine. This last character is shared with *N. inexpectata*; however, *Neolloydia* sp. 2 presents (18-) 22-19 (-23) radial spines and a length of central spines of (1.0-) 1.25-1.40 (-1.50) cm, while *N. inexpectata* has 13-15 (-16) radial spines and the length of the central spines is (0.57-) 0.62-0.80 (-0.86) cm (Figure 3G).

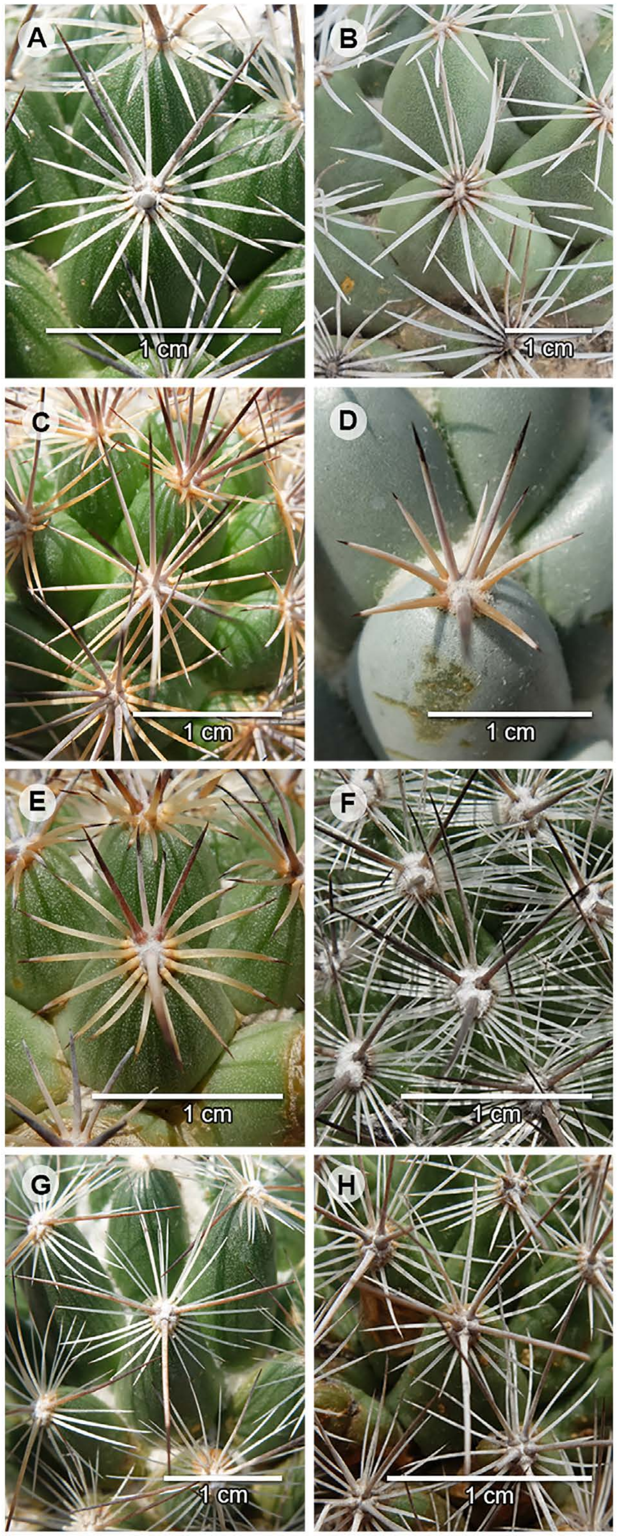


Figure 3. Detail of epidermis and areoles in two localities of *Neolloydia conoidea*: A) Y. Moredia & M. Vázquez-Sánchez 22 (CHAPA) and B) Y. Moredia *et al*. 11 (CHAPA). C) *N. ceratites* Y. Moredia *et al*. 03 (CHAPA). D) *N. matehualensis* (Y. Moredia *et al*. 26 CHAPA); E) *N*. *inexpectata* (Y. Moredia *et al*. 13 CHAPA); F) *Neolloydia* sp. 1 (Y. Moredia *et al*. 12 MEXU, CHAPA); G) *Neolloydia* sp. 2 (Y. Moredia *et al*. 24 MEXU, CHAPA) and H) *Neolloydia* sp. 3. D. Aquino *et al*. 574 (MEXU, CHAPA). Photographs D. Aquino.

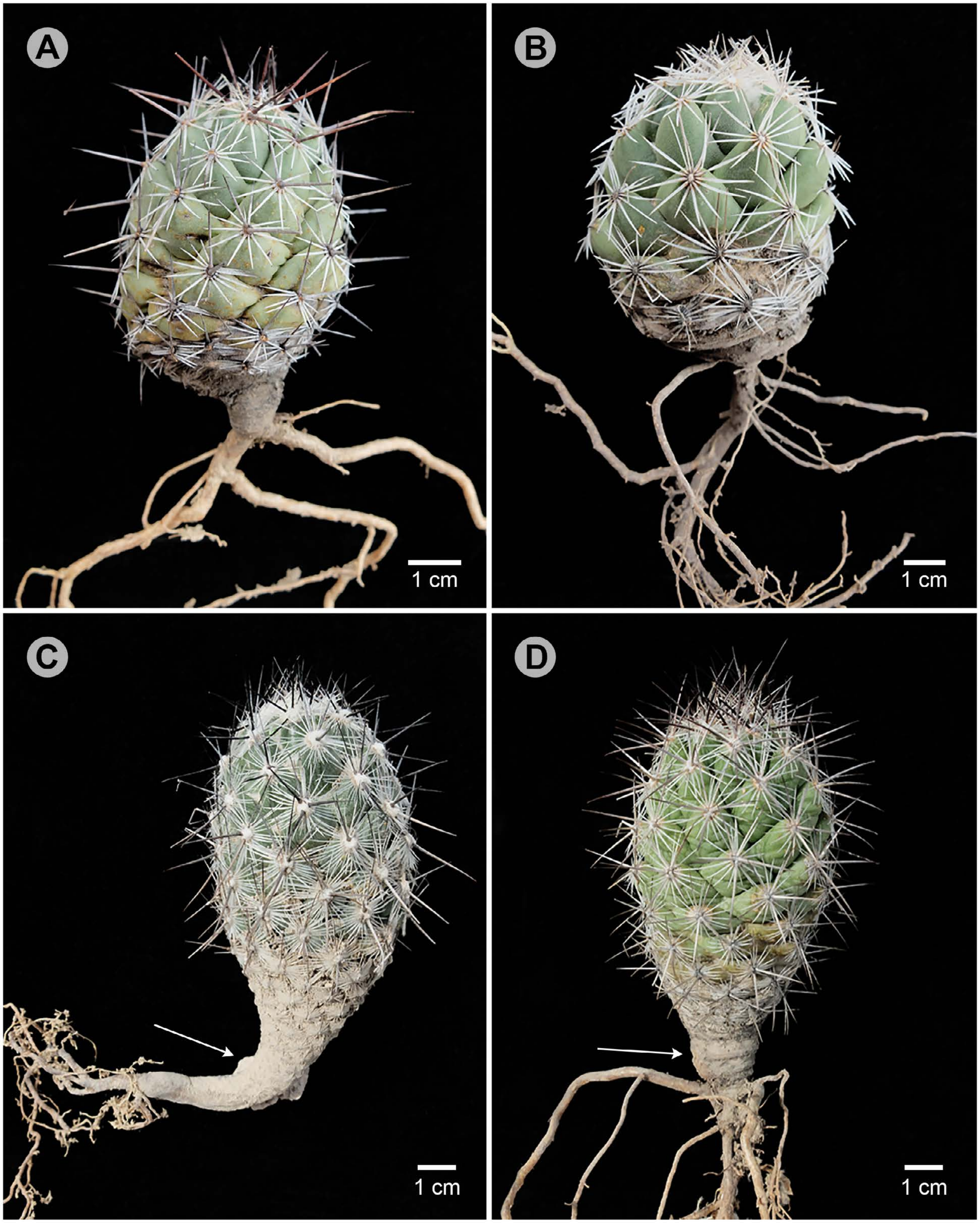


Figure 4. Detail of the lower stem portion of *N. conoidea*: A) D. Aquino *et al*. 580 (MEXU) and B) Y. Moredia *et al*. 11 (CHAPA). Presence of a neck in C) *Neolloydia* sp. 1 (Y. Moredia *et al*. 12 MEXU, CHAPA) and D) *Neolloydia* sp. 3 (D. Aquino *et al*. 574 MEXU, CHAPA). Photographs by D. Aquino.

Niche characterization supports species delimitation. *Neolloydia matehualensis* is a soil- and landform-specific taxon; Pearson's r indicate that it establishes in karstic systems on Lithosols. The occurrence proportion confirms that its distribution is not random, unlike what occurs with plains (Tables S2 and S3). A similar result occurs with *N. conoidea*, where the occurrence proportion is significant for Lithosols in karstic systems (Tables S3 and S6), and contrary to what occurs with Pearson's r, whose values are close to zero (Table S2). The establishment of a taxon in a type of landform does not occur randomly, since processes such as microclimatic changes and tectonism promote the isolation of lineages (Huggett 2016, Aquino *et al*. 2021, Cervantes *et al*. 2023). However, *N. conoidea* is able to establish in volcanic systems and tolerate iron-rich soils with a pH lower than 7.0 and high clay content, as in Ortic luvisols (Acevedo-Sandoval *et al*. 2004). This suggests that *N. conoidea* possesses a greater number of strategies that allow it to successfully compete (Denelle *et al*. 2018). In contrast, *N. matehualensis* likely possesses very specific strategies to tolerate stress in a karstic system (Denelle *et al*. 2018). This type of habitat, where factors such as water content, nutrient availability, and the presence of carbonates limit the distribution of vegetation, acting as an ecological barrier favoring the isolation of lineages (Estrada-Medina *et al*. 2019, Franco-Estrada *et al*. 2022), is a soil-specific species. Three taxa are soil-specific: *Neolloydia* sp. 1 (Rendzines), *N. ceratites* (Calcic xerosols and Lithosols), and *N. inexpectata* (Eutric regosols), the soil types to which the three taxa are restricted are considered shallow, up to 15 cm deep, and are characteristic of arid zones (FAO 2014). According to Baldwin (2005), the upwelling of a soil type generates edaphic habitats; understanding these characteristics is key to proposing hypotheses that explain the diversification of lineages (Bárcenas-Argüello *et al*. 2013, Anacker 2014). *Neolloydia* sp. 2 presents specificity by landform (fold mountains); however, the Bonferroni correction and consequently the occurrence proportion cannot be applied to *Neolloydia* sp. 3 because the number of registered localities is restricted to a single soil type and landform (Tables S3 and S6), in contrast, Pearson's residuals indicate that the association with fold mountains is strong (Table S2).

Of the seven taxa recognized here, *N. conoidea* has the largest distribution, spanning five ecoregions, with the largest concentration of localities in the Chihuahuan Desert (Figure 5). *Neolloydia matehualensis* is distributed between the boundaries of the Chihuahuan Desert and the Pine-Oak Forests of the Sierra Madre Oriental (Figure 5). The remaining taxa are restricted to one ecoregion: *N. ceratites* is endemic to the Chihuahuan Desert, while *N. inexpectata*, *N.* sp. 1, *N.* sp. 2, and *N* sp. 3 are endemic to the Pine-Oak Forests of the Sierra Madre Oriental (Figure 5). Geographic features such as the Sierra Madre Oriental are relevant for the diversification of different lineages within Cacteae (Vázquez-Sánchez *et al*. 2013). Santa-Ana-del-Conde *et al*. (2009) report that the rugged topography acted as a climatic refuge during the Pleistocene, in addition to exhibiting a great wealth of soil types, which has caused the isolation of numerous lineages of Cactaceae, resulting in a high number of endemisms (Hernández & Bárcenas 1995).

Three taxa (*N. ceratites*, *N. matehualensis*, and *N. inexpectata*) were considered as part of the *N. conoidea* variation (Breslin *et al*. 2021, Korotkova *et al*. 2021). Our results supported by RF classificatory analysis and niche characterization allow to distinguish four species. Furthermore, we recognize that the morphotypes *Neolloydia* sp. 1, *Neolloydia* sp. 2 and *Neolloydia* sp. 3 are new species and are described below. Finally, an identification key is provided.

Neolloydia guzmanii Vázquez-Sánchez & D. Aquino sp. nov. (Figure 6)

Type. México. Nuevo León, municipio de Rayones, Los Nogales, 1,665 m asl, 15 June 2024, *D. Aquino, M. Vázquez-Sánchez* & *M. Martínez-Flores 574* (Holotype: CHAPA; Isotype: MEXU).

Diagnosis. The number of central spines of *N. guzmanii* is 5-6 (-7) which differentiates it from *N. leccinumii* sp. nov. where the central spines are (2-) 3-4. This taxon has a neck on the lower part of the stem, which allows it to be distinguished from *N. conoidea*, *N. ceratites* and *N. terrazasiae* sp. nov., that lack this structure. *Neolloydia guzmanii* has porrect central spines and (17-) 19-21 (-22) radial spines, while in *N. inexpectata* the central spines are appressed and it has 13-15(-16) radial spines. Finally, glaucous-green stems and the number of radial spines of (8-) 9-11 (-12) in *N. matehualensis* allow to distinguish it from *N. guzmanii*.

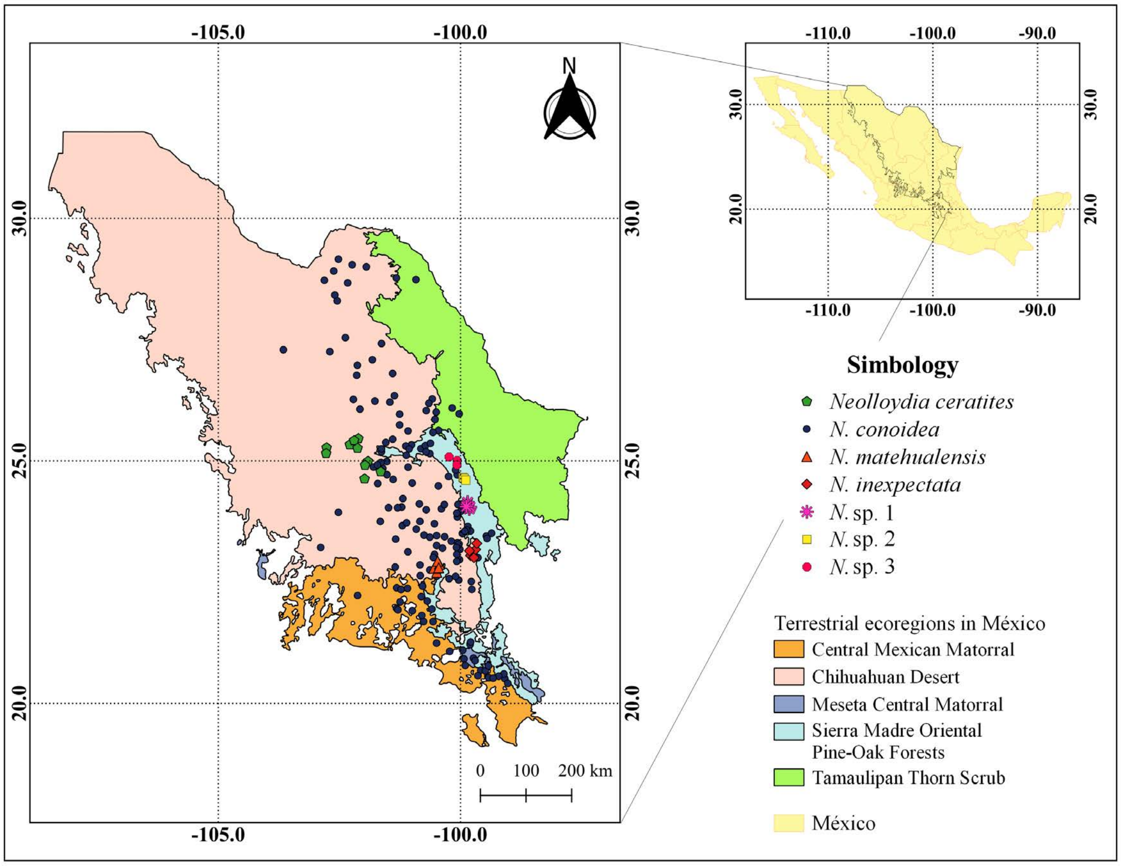


Figure 5. Distribution map of the *Neolloydia* species identified in this study.

Description. Plant simple to branched; stems 14 cm tall, 5-8 cm diameter, growth form globose to shortly cylindrical, opaque-green, with a basal neck. Tubercles 1.1-1.4 (-1.5) × 0.7-0.85 (-0.9) cm, with space between them; areoles 0.2-0.3 (-0.33) × 0.15-0.2 (-0.23) cm, elliptic; radial spines (17-) 19-21 (-22), (0.36-) 0.44-0.58 (-0.75) cm long, acicular, white; central spines 5-6 (-7), (1.04-) 1.08-1.11 (-1.23) cm long, acicular, porrect, light brown, greyish with age. Flowers infundibuliform, 3.0-4.0 (-4.3) cm long; outer tepals 1.0-1.8 (-2.0) cm long, linear-lanceolate, rounded, green with magenta edges, inner tepals 2.2-2.4 (-2.6) cm long, linear-lanceolate, acuminate, magenta; filaments 1.3-1.4 (-1.5) cm long, yellow, anthers yellow, style 1.7-1.8 cm long, whitish, stigma ca. 0.5 cm long, 5-6 lobed, white to yellowish. Fruits 0.5-0.6 cm in diameter, pinkish at the top, turning green towards the middle and white at the base, globose; seeds ca. 2 mm in diameter, suborbicular, black.

Distribution and ecology. *Neolloydia guzmanii* is endemic to Nuevo León, Mexico; it grows in xerophilous scrub on sloping sites, on Lithosols-type, and occupies an altitudinal range of 900 to 1,500 m asl, among the mountain folds of the pine-oak forests of the Sierra Madre Oriental. It coexists with *Agave lechuguilla* Torr., *Calliandra eriophylla* Benth., *Opuntia stenopetala* Engelm., and *Yucca* sp.

Conservation status. At least three localities are known, and *N. guzmanii* is estimated to occupy an extent of occurrence (EOO) of 88.262 km^2 (criterion B2) and an area of occupancy (AOO) of 16 km^2 (criterion B2). The approximate number of individuals is 350 adult plants and 100 juvenile plants (criterion C2). *Neolloydia guzmanii* establishes on rocky slopes unsuitable for agriculture; however, grazing may have a negative impact. It is proposed that this species be classified as Endangered (EN) according to criteria B2 + C2 (IUCN 2022). Although there is no evidence of illegal collection of specimens, it should be noted that this factor may affect the number of individuals recorded for this work in the future.

Figure 6. *Neolloydia guzmanii* sp. nov. A) General view of the plant, showing the basal neck. B) Branched individual showing the basal neck. C) Flower in cross section. D) Mature fruit. E) Seeds. Photographs by D. Aquino.

Phenology. *Neolloydia guzmanii* flowers in mid-June and fruits in late August and early September.

Etymology. This taxon is dedicated to Biol. Ulises Guzmán, a specialist in the Cactaceae family who participated in the development of the "Catálogo de Cactáceas Mexicanas" and who has rediscovered and described new species for science.

Additional specimens examined. México, Nuevo León, municipio de Rayones, 1 km al SE de Rayones, 911 m asl, 08 July 2018, *D. Aquino & L. García 433* (MEXU). Municipio de Rayones, 24 km al NW de Rayones rumbo a Ciénega del Toro, 1,523 m asl, 19 September 2005, *C. Gómez-Hinostrosa et al. 2432* (MEXU). Municipio de Rayones, cerca de 18 km al N de Galeana, camino a Rayones, 1,254 m asl, 07 September 2017, *C. Gómez-Hinostrosa et al. 2684* (MEXU). Municipio de Rayones, Rayones, 965 m asl, 30 May 2014, *G. B. Hinton 29485* (GBH).

Discussion. *Neolloydia guzmanii* is one of the taxa with the highest number of central spines, 5-6 (-7) similar to *N. ceratites* with 6 to 7 (-8), but they differ in the presence of a neck in *N. guzmanii* (Table 5). The original descriptions of *N. ceratites*, *N. conoidea*, *N. inexpectata* and *N. matehualensis* were consulted to corroborate the absence of a neck (Quehl 1909, De Candolle 1828, Backeberg 1948, Donati 2012). The only taxon that presents a neck is *Neolloydia leccinumii*, but it differs from *N. guzmanii* by the number of central spines (2-) 3-4. Additionally, *N. leccinumii* fruits measure 0.7-0.8 (-1.0) cm in diameter, while *N. guzmanii* fruits measure 0.5-0.6 cm in diameter (Table 5).

Table 5. Morphological comparison between *Neolloydia leccinumii*, *N. terrazasiae* and *N. guzmanii* with respect to members of *Neolloydia* recognized here. Identification key for the ecoregions: Chihuahuan Desert (ChD), Tamaulipan Thornscrub (TT), Sierra Madre Oriental Pine-oak Forests (SMO), Central Plateau Scrubland (CPS) and Central Mexican Scrubland (CMS). Interpretation of acronyms. ST: Space between tubercles, NRS: Number of radial spines, OCS: Orientation of the central spine, and NCS: Number of central spines.

	Neolloydia ceratites	***N. conoidea***	***N. inexpectata***	***N. matehualensis***	***N. leccinumii***	***N. terrazasiae***	***N. guzmanii***
Stem color	Opaque-green	Opaque-green - to -bright	Green-bright	Green-glaucous	Opaque-green	Opaque-green	Opaque-green
Neck	Absent	Absent	Absent	Absent	Present	Absent	Present
ST	No space	With space	With space	No space	No space	With space	With space
NRS	(9-) 10-14 (-16)	(8-) 10-17 (-20)	13-15 (-16)	(8-) 9-11 (-12)	(15-) 17-21 (-22)	(18-) 22-19 (-23)	(17-) 19-21 (-22)
OCS	Porrect	Porrect	Adpresed	Porrect	Porrect	Adpresed	Porrect
NCS	6-7 (-8)	(0-) 2-3 (-4)	2-3 (-4)	2-3	(2-) 3-4	(2-) 3-4 (-5)	5-6 (-7)
Landform	Plains and piedmont	Karstic systems	Plains	Karstic systems	Rivers and, karstic systems and fold mountains	Fold mountains	Fold mountains
Soil	Calcic xerosols, and Lithosols	Lithosols	Eutric regosols	Lithosols	Rendzines	Lithosols	Lithosols
Ecoregion	ChD	ChD, TT, SMO, CPS y CMS	SMO	SMO and ChD	SMO	SMO	SMO

Neolloydia leccinumii Vázquez-Sánchez & D. Aquino sp. nov. (Figure 7)

Type. México, Nuevo León, municipio de Aramberri, W de Aramberri, 1,289 m asl, 27 February 2022, *Y. Moredia, M. Vázquez-Sánchez & D. Sánchez 12* (Holotype: CHAPA; Isotype MEXU).

Diagnosis. *Neolloydia leccinumii* has (2-) 3-4 central spines, differing from *N. ceratites*, which has 6-7 (-8) central spines and from *N. guzmanii* which has 5-6 (-7) central spines. *Neolloydia leccinumii* has porrect central spines, which allows it to be distinguished from *N. inexpectata* and *N. terrezasiae* sp. nov., whose central spines are appressed. *Neolloydia leccinumii* has a basal neck, which allows it to be distinguished from *N. conoidea* and *N. matehualensis*, which lacks this stem modification.

Figure 7. *Neolloydia leccinumii* sp. nov. A) General view of the plant in its habitat. B) Branched stems. C) Flowers in cross-section. D) Fruits. E) Seeds. Photographs by D. Aquino.

Description. Plant simple to branched; stems 12 (-15) cm tall, 5-7 cm diameter, growth form shortly cylindrical, opaque-green with a basal neck. Tubercles 1.7-1.8 (-2.0) × 0.5-0.6 (-0.7) cm, without space between them; areoles 0.2-0.25 (-0.28) cm, orbicular to suborbicular; radial spines (15-) 17-21 (-22), (0.4-) 0.49-0.61 (-0.70) cm long, acicular, straight, white; central spines 2-3 (-4), (0.79-) 0.81-0.90 (-0.93) cm long, porrect, acicular, straight, dark brown to black. Flowers infundibuliform, (3.0-) 3.2-3.5 cm long; outer tepals 1.2-1.5 (-1.6) cm long, lanceolate, acute, green with light magenta to white edges; inner tepals 2.0-2.3 (-2.5) cm long, lanceolate, acuminate, magenta; filaments 1.5-1.6 cm long, yellow, anthers yellow, style 1.9-2.0 cm long, greenish with slight whitish tones, stigma 0.4-0.5 cm long, with 5-6 lobed, white with slight greenish tones. Fruits 0.7-0.8 (-1.0) cm in diameter, reddish, with a white base, globose, the dry remains of the perianth are missing; seeds ca. 3 mm diameter, suborbicular, black.

Distribution and ecology. *Neolloydia leccinumii* is endemic to Nuevo León, Mexico. It grows in xerophilous scrub and in an altitude range of 1,100 to 1,650 m asl. It cohabits with *Agave lechuguilla* Torr., *Cordia boissieri* A.DC., *Cylindropuntia kleiniae* (DC.) F. M. Knuth, *Echinoagave striata* (Zucc.) A. Vázquez, Rosales & García-Mor., *Echinocactus platyacanthus* Link & Otto, and *Yucca filifera* Chabaud. The distribution of *N. leccinumii* is limited to Rendzines-type soils. Although our statistical analyses do not indicate landform specificity, *N. leccinumii* is frequently observed on folded mountains, karstic, and fluvial systems within the Sierra Madre Oriental pine-oak forests ecoregion.

Conservation status. Up to nine localities are known, it is estimated that *N. leccinumii* occupies an extent of occurrence (EOO) of 41.51 km^2 (criterion B1) and an area of occupancy (AOO) of 14.75 km^2 (criterion B1). The approximate number of individuals is 590 adult plants and 310 juvenile plants (criterion C2). *Neolloydia leccinumii* is established on slopes where erosion and, consequently, soil drag can eventually be a factor that negatively affects the survival of individuals. Additionally, in one of the localities, small-scale extraction of materials is carried out. It is proposed that this species be classified as Endangered (EN) according to criteria B1 + C2 a(i) (IUCN 2022). There is no evidence of illegal collection of *N. leccinumii* specimens, however, the possibility of this phenomenon occurring in the future should not be ruled out.

Phenology. Flowering begins in late April and concludes between mid- and late May; a second period occurs in mid-June and ends in early July. Fruit ripens from mid-July to early August; the second fruiting period occurs in mid-September.

Etymology. This species is dedicated to PhD. Leccinum J. García Morales, a Mexican botanist with extensive knowledge of the flora of northeastern Mexico, who has proposed new species in several plant groups.

Additional specimens examined. México, Nuevo León, municipio de Aramberri, carretera de La Escondida a Aramberri, 1,281 m asl, 07 July 2018, *D. Aquino & L. García 423* (MEXU). Municipio de Aramberri, cerros al oeste de Aramberri, 1,193 m asl, 16 June 2024, *D. Aquino et al. 578* (MEXU). Municipio de Aramberri, 4 km al N de la carretera La Escondida a Aramberri, 1,321 m asl, 30 August 2024, *D. Aquino et al. 583* (MEXU). Municipio de Aramberri, 4.7 km al N de la carretera La Escondida a Aramberri, 1,230 m asl, 30 August 2024, *D. Aquino et al. 586* (MEXU). Municipio de Aramberri, en la desviación a Dolores la cual está a 6 km al W de Aramberri rumbo a la carretera Dr. Arroyo-carretera No. 60, 1,158 m asl, 17 April 1991, *H. Hernández et al. 1934* (MEXU). Municipio de Aramberri, El Monal, 8 km al SE de Aramberri por carretera a Zaragoza, 1,247 m asl, 18 April 1991, *H. Hernández et al. 1944* (ASU, MEXU). Municipio de Aramberri, a 7 km al W de Aramberri por la carr. [carretera] 19, rumbo a la carr. [carretera] Dr. Arroyo-La Ascensión, 1,252 m asl, 18 April 1991, *H. Hernández et al. 1951* (ASU, MEXU). Municipio de Aramberri, al norte de La Florida, 1,910 m asl, 12 October 2020, *J. Ortíz-Brunel et al. 982* (IBUG). Municipio de Aramberri, km 13, 0.5 km antes de Aramberri, 1,171 m asl, 31 October 2007, *T. Terrazas & S. Arias 843* (MEXU). Municipio General Zaragoza, 12 km al sur de Aramberri por la carretera a Zaragoza, 700 m por terracería, cerros al oeste de la carretera, 1,650 m asl, 28 January 1998, *B. Goettsch et al. 86* (MEXU).

Discussion. *Neolloydia leccinumii* differs from members of *N. conoidea* complex by the presence of a basal neck. Anderson (1986) did not report the presence of this structure within the variation of the *N. conoidea* complex. Comparing *N. leccinumii* with *N. terrazasiae* sp. nov. there are differences besides the absence of a neck: central spines are appressed in *N. leccinumii* and in *N. terrazasiae* the central spines are porrect (Table 5). This new taxon shares the presence of a basal neck with *N. guzmanii* sp. nov. The main distinction is found in the number of central spines: 5-6 (-7) in *N. guzmanii* while in *N. leccinumii* the number is 2-3 (-4). Additionally, the areoles of *N. leccinumii* are orbicular to suborbicular, while *N. guzmanii* has elliptical areoles (Table 5). Ecological characterization indicates that both taxa occupy different niches; *N. leccinumii* is specific to a single soil type (Rendzines), while *N. guzmanii* is specific to soil type (Lithosols). For this reason, we propose that they be recognized as independent species (Table 5).

Neolloydia terrazasiae Vázquez-Sánchez & D. Aquino sp. nov. (Figure 8)

Type. México. Nuevo León, municipio de Galeana, El Palmito I, 1,665 m asl, 23 March 2023, *Y. Moredia*, *M. Vázquez-Sánchez*, *Z. Rodríguez & D. Aquino 24* (Holotype: CHAPA; Isotype MEXU).

Diagnosis. *Neolloydia terrazasiae* has appressed central spines, while *N. conoidea* and *N. ceratites* are porrect. *Neolloydia terrazasiae* has opaque-green stems with (17-) 19-21 (-22) radial spines. It is distinguished from *N. matehualensis*, which has green-glaucous stems and (8-) 9-11 (-12) radial spines, and from *N. inexpectata*, which has green-bright stems with 13-15 (-16) radial spines. The absence of a neck and the straight central spines distinguish *N. terrazasiae* from *N. leccinumii* and *N. guzmanii.*

Description. Plant simple to branched; stems 16 (-17) cm high, 5-8 cm diameter, growth form shortly cylindrical, opaque-green without a basal neck. Tubercles 1.5-1.8 (-2.0) × 1.0-1.2 (-1.5) cm, with space between them, areoles 0.3-0.4 (-0.45) cm long, 0.2-0.3 (-0.34) cm wide, oval; radial spines (18-) 22-19 (-23), (0.54-) 0.60-0.70 (-0.83) cm long, acicular, white; central spines (2-) 3-4 (-5), (1.07-) 1.24-1.41 (-1.56) cm long, acicular, porrect, reddish, turning light brown with age. Flowers infundibuliform, 4.0-4.5 (-4.8) cm long; outer tepals 1.8-2.0 cm long, linear-lanceolate, rounded, green with white edges, inner tepals 2.2-2.4 (-2.6) cm long, linear-lanceolate, acuminate, magenta; filaments yellow, anthers yellow, style white, stigma 5-6 lobed, white to yellowish. Fruits 0.5-0.7 cm diameter, green with white base, globose; seeds ca. 2.5 mm diameter, suborbicular, black.

Distribution and ecology. *Neolloydia terrazasiae* is an endemic species of the xerophilous scrub of Nuevo León. It is located in the fold mountains, on Lithosols in the foothills of the pine-oak forests of the Sierra Madre Oriental. It occupies an altitude range of 1,510 to 1,670 m asl. It coexists with *Brahea* aff. *decumbens* Rzed., *Mammillaria pilispina* J.A. Purpus, *Opuntia stenopetala* Engelm., *Pinus arizonica* Engelm., and *Yucca carnerosana* (Trel.) McKelvey.

Conservation status. At least three localities are known, consequently, *N. terrazasiae* is estimated to occupy an extent of occurrence (EOO) of 1.787 km^2 (criterion B1) and an area of occupancy (AOO) of 12 km^2 (criterion B1). The approximate number of individuals is 310 adult plants and 100 juvenile plants (criterion C2). No risk factors directly affecting *N. terrazasiae* localities have been observed; however, small-scale logging for construction purposes was observed, and the lack of vegetation cover could eventually promote soil erosion. This species is proposed to be classified as Endangered (EN) according to criteria B1 + C2 (IUCN 2022). As with *N. guzmanii* and *N. leccinumii*, there is no early evidence of illegal harvesting. It should be noted that due to their high habitat specificity and the lack of knowledge about the processes of establishment of new individuals, intensive collection can have a severe impact on populations.

Phenology. Flowering occurs during June, fruiting occurs in August.

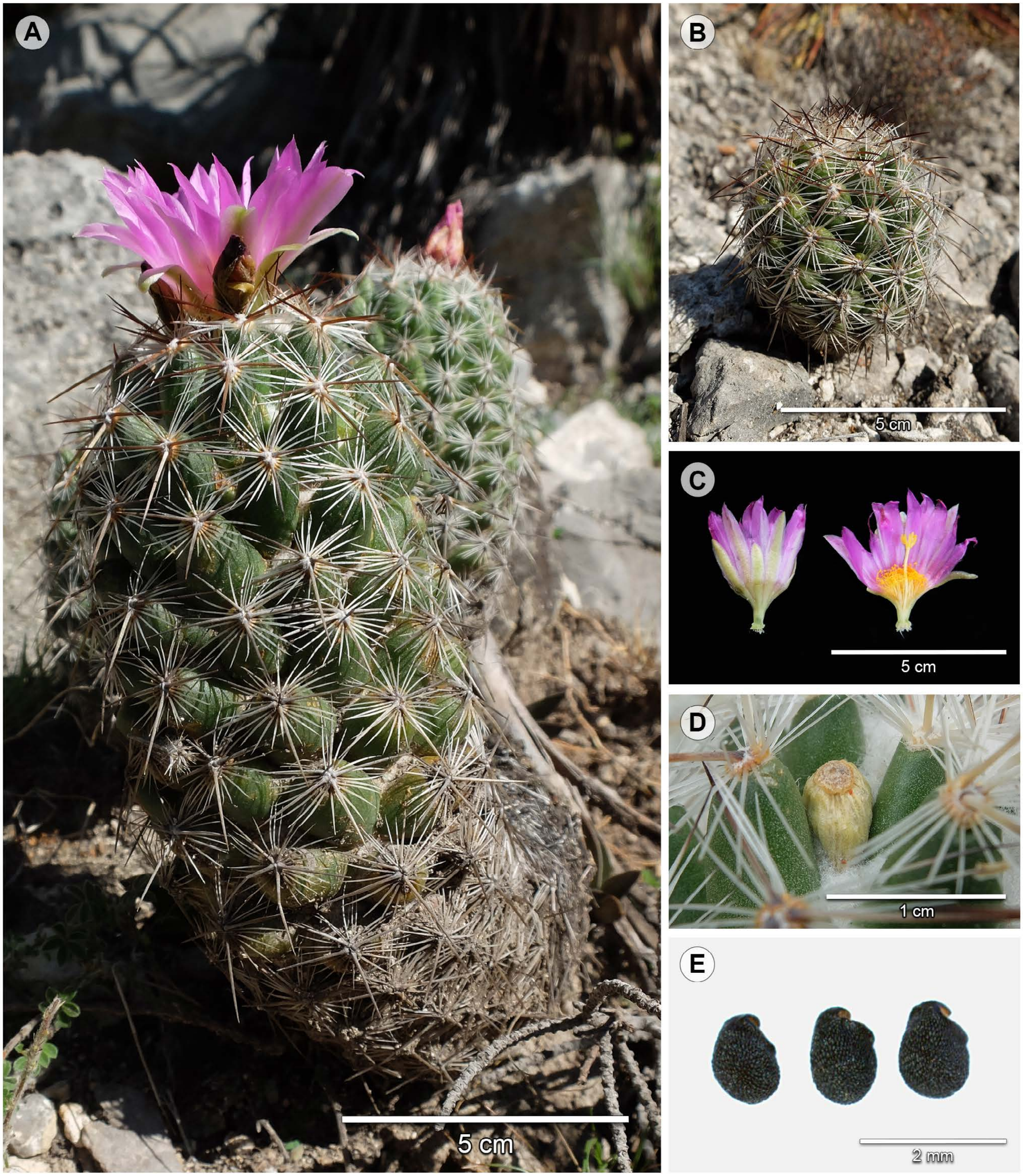


Figure 8. *Neolloydia terrazasiae* sp. nov. A) Adult flowering stems. B) Juvenile growing in its habitat. C) Flower in cross-section. D) Fruits. E) Seeds. Photographs by D. Aquino.

Etymology. This species is dedicated to PhD. Teresa Terrazas, whose studies on the anatomy of Cactaceae have been used in the systematics of the family, allowing for a better understanding of phylogenetic relationships, particularly among members of the tribes Cacteae, Echinocereeae, and Hylocereeae.

Additional specimens examined. México, Nuevo León, municipio de Galeana, al S de El Palmito, 1,674 m asl, 23 March 2023, *D. Aquino et al. 556* (MEXU). Municipio de Galeana, El Palmito II, 1,518 m asl, 23 March 2023, *Y. Moredia et al. 25* (CHAPA). Municipio de Galeana, San José del Río, 1,500 m asl, 09 April 2022, *M. Nevarez observation No. 111839179* (according to iNaturalist 2023+).

Discussion. The arrangement of the central spines was not included in Anderson (1986) as a character that would distinguish between species of the *N. conoidea* complex. According to our results, this character is significant because it allows distinguishing between a set of species. A combination of characters, among these, the appressed arrangement of the central spines in *N. terrazasiae*, allows to distinguish the new taxon from *N. guzmanii* and *N. leccinumii*, whose central spines are porrect, in addition to having a basal neck, unlike *N. terrazasiae*, which does not have this modification (Table 5). Finally, another set of characters that allows to distinguish the new taxon from *N. inexpectata* is the color of the external tepals: reddish with white margins with slight greenish tones, while in *N. terrazasiae* the tepals are green with white edges.

Identification key for the members of *Neolloydia*

1. Central spines appressed 2
2. Stems bright-green, radial spines 13-15 (-16), outer tepals reddish, with white margins with slight greenish tones .... *Neolloydia inexpectata*
2. Stems opaque-green, radial spines (18-) 22-19 (-23), outer tepals green with white edges….. *N. terrazasiae* sp. nov.
1. Central spines porrect 3
3. Central spines > 5 4
4. Stems with a neck at the bottom of the stem, areoles elliptical, central spines light brown, greyish with age .... *N. guzmanii* sp. nov
4. Stems without a neck at the bottom of the stem, areoles orbicular, central spines white, greyish to light brown..*N. ceratites*
3. Central spines < 4 5
5. Areoles elliptic to obovate .... *N. conoidea*
5. Areoles orbicular to suborbicular 6
6. Stems glaucous-green, radial spines (8-) 7-10 (-11), outer tepals dark pink, with the margins the same color in lighter shades. .... *N. matehualensis*
6. Stems opaque-green, radial spines (15-) 17-21 (-22), outer tepals green with magenta margins. .... *N. leccinumii* sp. nov.

Supplementary material

Supplemental data for this article can be accessed here: https://doi.org/10.17129/botsci.3701

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