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Morphometric and Cytogenetic Studies in *Mimosa diversipila* (Mimosoideae, Leguminosae) and Their Taxonomic and Evolutionary Inferences

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Abstract—Here we describe the morphological variability, chromosome number, and chromosome size in *Mimosa diversipila*. This species comprises two varieties, which are distinguished by the indumentum. However, this character is insufficient for circumscription of these infraspecific taxa. Using multivariate techniques, we found that quantitative characters were useful for identification of the varieties, which also have a different geographic distribution. Cytogenetic studies revealed that these taxa form a polyploid complex and that the polyploidy may contribute to the morphological variability observed.

Keywords—*Brevipedes,* cytogenetics, Fabaceae, infraspecific complex, legumes, morphometric study, polyploidy.

Micheli (1883) described *Mimosa diversipila* Micheli based on the indument type: stellate trichomes and plumose setae. Later, Burkart (1948) studied the Argentinean *Mimosa* species and recognized *M. diversipila*, with an indument of plumose setae and stellate trichomes all over the plant (from northeastern Argentina, Paraguay, and Brazil), and *M. setistipula* Benth., with smooth, unbranched trichomes on the leaflets (from northeastern Argentina). He included *M. diversipila* in *Mimosa* Series *Meticulosae* Benth. and found high variation in foliar characters of this species, but the presence of intermediate forms prevented him from proposing infraspecific division.

More recently, Barneby (1991) studied the Neotropical species of *Mimosa* and provided a new taxonomic treatment. In this new classification, this author included *M. diversipila* in *Mimosa* L. Section *Mimosa* (because of its haplostemonous fertile flowers and lack of a petiolar nectary) Series *Mimosa* (because of its pauci-jugate leaves and non-contracted pinnae) Subseries *Brevipedes* Barneby (because of the erect habit, heads clustered in long racemes, and craspedia). This author also concluded that Burkart (1948) wrongly identified the Argentinean specimens as *M. setistipula* Benth., and considered that they constituted a new variety: *M. diversipila* var. *subglabriseta* Barneby & Fortunato (Barneby 1991).

M. diversipila var. *subglabriseta* and *M. diversipila* var. *diversipila* were distinguished exclusively by the indument on the stems and leaflets (Barneby 1991). However, in previous studies, we observed some specimens intermediate between them (Luna-Castro et al. 2012), and high variation in quantitative characters at the infraspecific level (Morales 2011).

The aim of this work is to characterize morphological and cytogenetic variation and the geographical distribution of the *M. diversipila* complex. We applied morphometric multivariate techniques to analyze character variability and the grouping of individuals, and studied the chromosome number and chromosome size of representatives of the two infraspecific taxa.

Materials and Methods

Plant Material and Morphological Studies—Specimens of *Mimosa diversipila* were collected from throughout its distribution: northeastern Argentina, Paraguay, and Brazil. In total, 100 herbarium specimens of *M. diversipila* were included in this study. The specimens analyzed are deposited in BAB, BAF, CTES, G, LP, MBM, MO, and SI. This study included the nomenclatural types of *M. diversipila* and *M. diversipila* var. *subglabriseta.* All specimens were identified according to Barneby (1991).

Measurements were taken from 79 individuals; qualitative and quantitative characters were observed and analyzed (Table 1). Habit and height of plants were recorded in the field and/or taken from the herbarium specimen labels, where possible. The remaining quantitative characters were analyzed using a stereoscopic microscope WILD M5−26530 (Wild, Heerbrugg, Sankt Gallen, Ostchweiz, Switzerland) and measured with a ruler. Floral organs were boiled in water to study morphology. Five to ten flowers, leaves and fruits (craspedium) were measured or counted from each individual.

To study the geographic distribution of the infraspecific taxa, data from herbarium specimens were used. When information from coordinates was lacking, the localities were georeferenced using gazeteers or following the georeferencing procedures of Chapman and Wieczorek (2006).

Morphometric Analysis—The median, maximum, and minimum values were calculated for all ordinal or discrete variables, and the mean and standard deviation were calculated for all continuous variables (Table 2). A Shapiro-Wilk test with modifications (Mahibbur and Govindarajulu 1997) was used to determine if the data deviated from a normal distribution. All of the above were conducted using Infostat (Di Rienzo et al. 2009).

The quantitative, continuous variables were standardized to reduce the effect of different scales and a dataset based on the variables was assessed. Several multivariate techniques were performed on 74 individuals: principal coordinates analysis (PCoA), cluster analysis (CA), canonical variance analysis (CVA), and non-parametric multivariate analysis of variance (np-MANOVA). These techniques were conducted using PAST (Hammer et al. 2001) (PCoA and np-MANOVA) and R (CA) (La Grange et al. 2010; Borcard et al. 2011; Maechler et al. 2012).

Principal coordinates analysis (PCoA) and np-MANOVA are techniques appropriate for the analysis of multiple types of variables. These approaches were used because not all variables exhibited a normal distribution, even after applying different transformations. This constraint prevented us from using other exploratory techniques such as principal component analysis.

Principal coordinates analysis is an exploratory technique appropriate for taxonomic datasets with different types of variables (Henderson 2005). This technique finds the eigenvalues and eigenvectors of a matrix

Table 1. Quantitative morphological characters included in analyses of the *Mimosa diversipila* complex.

Continuous characters: Plant height, Petiole length, Pinna rachis length, Leaflet length, Leaflet width, Interfoliolar segment length, Stipule length, Peduncle length, Longest diameter of head, Shortest diameter of head, Craspedium length, Craspedium width
Discrete/ordinal: Leaflet pairs per pinna, Primary nerves per leaflet, Nodes per axis, Heads per axis, Articles per craspedium
Ratio: Length: width ratio of leaflet, Interfoliolar segment length: rachis length ratio, Length: width ratio of heads, Length: width ratio of craspedium

containing the distances or similarities between all data points; the Gower measure (Gower 1971), is normally used.

A canonical variates analysis (CVA) was used to determine which variables maximize the differences between the groups established a priori. CVA was performed with Biplot GUI (La Grange et al. 2010) in R (R Development Core Team 2009). Canonical variates analysis does not require multivariate normality when the objective is only descriptive. This analysis results in a graphical representation of the degree of separation between the groups in a biplot, based on the first two canonical variates (Rencher 2002).

A cluster analysis (CA) was performed to confirm the observations of PCoA. Hierarchical cluster was applied in order to classify the specimens. The methods used were: single linkage, complete linkage, unweighted pair group method with arithmetic mean (UPGMA), unweighted pair group method centroid (UPGMC), and Ward. The best cluster (UPGMA) was chosen based on the cophenetic correlation index and Gower's matrix distance. The cophenetic correlation index gives a comparison of the similarities according to the similarity matrix and the similarities according to the dendrogram.

The cophenetic distance between two objects in a dendrogram is the distance at which the two objects become members of the same group. A cophenetic matrix is a matrix representing the cophenetic distances among all pairs of objects. A Pearson's r correlation, called the cophenetic correlation in this context, can be computed between the original dissimilarity matrix and the cophenetic matrix. The method with the highest cophenetic correlation may be seen as the one that produced the best clustering model for the distance matrix. The Gower matrix distance is computed as the sum of squared differences between the original and cophenetic distances.

Two criteria, silhouette plot and binary and distance matrix comparison (Rousseeuw 1987; Kaufman and Rousseeuw 2005), were used to help identify an appropriate number of groups within the dataset.

The first technique, silhouette plot, is a graphical method, based on the comparison of the distance between one element and the closest cluster, and the distance between the same element and all elements of its cluster. Distance matrix comparison compares the original distance matrix to binary matrices computed from the dendrogram cut at several levels; it then chooses the level where the matrix correlation between the two is the highest.

A non−parametric analysis of variance (np−MANOVA) was used to test the hypothesis of the existence of two or three groups. This test analyzed differences between means or centroids of groups of multivariate observations, and does not require the assumption of multivariate normality (Anderson 2001).

***Cytogenetic Studies*—**Seeds from individuals growing in Argentina, Paraguay, and Brazil were collected. The seeds were germinated in Petri dishes at room temperature, and the roots were pretreated with 0.002 hydroxiquinoleine at 20−25°C for 4−7 h.

Following treatment with hydroxiquinoleine, the roots were 1) fixed immediately in 3:1 solution (100% ethanol: glacial acetic acid); 2) preserved in 70% ethanol until their use; 3) washed in a buffer solution of 0.01-M citric acid−sodium citrate at pH 4.6; 4) transferred to an enzymatic solution containing 2 mL 2% cellulase (Ozonuka R-10, Merck KGaA, Damstadt, Germany) and 20% liquid pectinase for 2−2.5 hr at 37°C; 5) washed again with buffer solution.

After this treatment, the root tips were macerated in a drop of dye (acetic haematoxylin), and the 'squash' technique was applied (Egozcue 1971). For each sample, 10−20 metaphases were counted.

To perform studies of chromosome size, ten cells per individual (seedlings germinated from the collected seeds) and five individuals per accession (plant collected in one locality) were studied.

Chromosome length per haploid genome (CLHG) was determined using Micromeasure ® (Reeves 2001). The interchromosomal asymmetry index (A_2) was calculated according to Romero Zarco (1986). The formula of the A_2 index is $A_2 = SX^{-1}$, where S represents the standard deviation and X is the mean length.

Means of CLHG and the A_2 index were evaluated using a Shapiro−Wilk test with modifications (Mahibbur and Govindarajulu 1997). Because the variables appeared normally distributed, they were examined with an analysis of variance (ANOVA) and the differences

Table 2. Intraspecific morphology in *M. diversipila*, including both continuous (mean ± standard deviation), discrete or ordinal (median and range of minimum and maximum values) and qualitative characters.

Character	*M. diversipila* var. *subglabriseta*	Intermediate specimens	*M. diversipila* var. *diversipila*
Plant height (cm)	80.31 ± 36.26	110.23 ± 45.82	143.40 ± 66.29
Petiole length (mm)	1.98 ± 1.37	1.69 ± 0.98	4.36 ± 3.08
Rachis length (mm)	43.29 ± 9.64	40.67 ± 8.68	48.43 ± 10.86
Leaflet pairs per pinna	17 (9−23)	15 (11−17)	9 (8−15)
Leaflet length (mm)	6.63 ± 1.52	6.80 ± 1.93	11.00 ± 2.15
Leaflet width (mm)	2.78 ± 0.68	2.78 ± 0.87	4.79 ± 0.93
Ratio length: width of leaflets	2.33 ± 0.24	2.51 ± 0.53	2.45 ± 0.27
Primary nerves per leaflet	2 (1−4)	2 (1−3)	2 (1−4)
Interfoliolar segment length (mm)	2.44 ± 0.64	2.68 ± 1.00	5.32 ± 1.45
Ratio interfoliolar segment: rachis length	0.06 ± 0.02	0.06 ± 0.02	0.11 ± 0.02
Stipule length (mm)	5.04 ± 1.04	4.88 ± 1.38	6.06 ± 2.34
Peduncle length (mm)	5.35 ± 3.21	5.71 ± 3.16	9.41 ± 2.92
Heads per axis	15 (2−27)	18 (2−52)	18 (4−65)
Head longest diameter (mm)	5.19 ± 0.73	5.13 ± 0.58	6.27 ± 0.93
Head shortest diameter (mm)	4.86 ± .061	4.97 ± 0.58	5.54 ± 0.68
Ratio length: width of head	1.08 ± 0.09	1.03 ± 0.04	1.15 ± 0.15
Articles per craspedium	2.00 ± 0.50	2.60 ± 0.65	2.25 ± 0.53
Craspedium length (mm)	8.10 ± 1.67	9.18 ± 2.51	9.41 ± 1.31
Craspedium width (mm)	3.34 ± 0.71	3.64 ± 0.44	3.93 ± 0.76
Ratio length: width of craspedium	2.50 ± 0.29	2.56 ± 0.43	2.47 ± 0.43
Habit	Erect subshrub	Erect subshrub	Erect subshrub
Stem pubescence	Generally scaberulous setae	Scaberulous to plumose setae	Scaberulous to plumose setae
Leaflet pubescence	Scaberulous setae and trichomes	Scaberulous to plumose setae and trichomes	Plumose setae and stellate trichomes
Calyx	Campanulate	Campanulate	Campanulate
Corolla pubescence	Glabrous to puberulent	Glabrous to puberulent	Glabrous to puberulent
Pod dehiscence	Craspedia	Craspedia	Craspedia

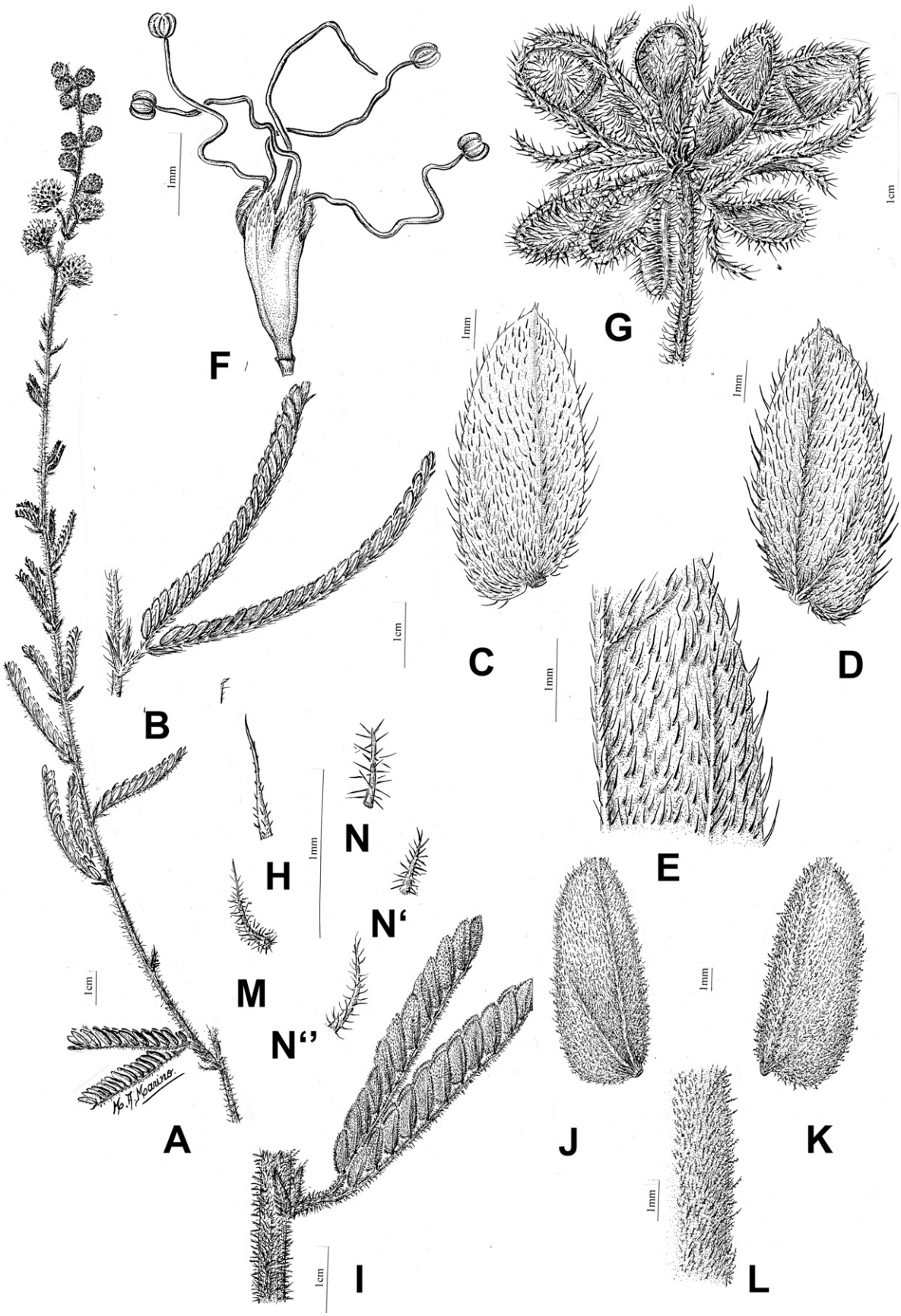

Fig. 1. *Mimosa diversipila* var. *subglabriseta.* A. Flowering branch. B. Leaf with one pair of pinnae. C. Leaflet, adaxial face. D. Leaflet, abaxial face. E. Leaflet, margin detail. F. Flower. G. Craspedia. H. Scaberulous setae. *M. diversipila* var. *diversipila.* I. Leaf with one pair of pinnae. J. Leaflet, abaxial face. K. Leaflet, adaxial face. L. Leaflet, margin detail. M, N, N′, N′′. *M. diversipila* var. *diversipila,* types of plumose setae. (A, B, F, G: *Morales 637* (BAB); C, D, E, H: *Martínez Crovetto 8874* (BAB); I: *Fortunato et al. 8810* (BAB); J, K, L, M: *Zardini 53394* (BAB); N, N′, N′′: *Soria 6468* (MO).

between accessions were tested using Fisher's least significant difference (LSD) test.

Results

Morphological Analysis—Except for the pubescence of leaflets and other vegetative organs, the qualitative characters did not show variation among individuals. Most of these characters appeared relatively constant in the species. By contrast, the pubescence of stems and leaflets showed high variation, from individuals with minutely scaberulous setae to individuals with plumose setae and/or stellate trichomes (Table 2; Fig. 1A–E, M–N′′).

The floral and carpological characters, such as length and pubescence of corolla, type of calyx, length, width, and pubescence of pods, did not show much variation (Table 2; Fig. 1F–G). By contrast, vegetative characters were variable between individuals (Table 2; Fig. 1A–E, H-N′′).

Variables that lacked data from many specimens, such as the height of plants and carpological characters (size of pods, ratio of pod length: width, and number of articles), were not included in the multivariate analysis. The height of plants is not frequently recorded in the field, and, in many cases, it is not possible to infer. Carpological characters were absent in specimens collected at flowering stage, whereas floral characters were not included because they did not exhibit variation at the infraspecific level.

The multivariate analysis included the following characters: petiole length, pinna rachis length, leaflet length, leaflet width and their ratio, leaflet pairs per pinna, primary nerves per leaflet, interfoliolar segment length, ratio of length of interfoliolar segment to length of pinna, stipule length, heads and nodes per axis, longest and shortest head diameters, ratio of longest diameter to shortest head diameter, and peduncle length (Table 1).

The first three principal coordinates (PCo) of principal coordinate analysis (PCoA) accounted for about 50% of total variance. PCo1 accounted for nearly 35.4%, whereas PCo2 and PCo3 explained about 8.2% and 6.0% respectively (Fig. 2). We plotted PCo1 vs. PCo2, and PCo1 vs. PCo3 to visualize the relationships among individuals (Fig. 2A–B). One cluster was comprised of specimens identified a priori as *M. diversipila* var. *diversipila*, and the other cluster included those individuals that had been identified as *M. diversipila* var. *subglabriseta*. Intermediate specimens clustered with *M. diversipila* var. *subglabriseta* in the biplot of the first two axes. The PCoA showed a division between both varieties. Although the group with individuals of *M. diversipila* var. *subglabriseta* and intermediate specimens seems more heterogeneous in pubescence, the group of var. *diversipila* individuals occupies an ample space in the biplot, because they have higher variation in the quantitative characters than the other cluster.

Canonical variates analysis (CVA) maximized the differences between groups and showed a spatial distribution similar to that of PCoA in the first two axes. The specimens of *M. diversipila* var. *subglabriseta* and the intermediate specimens clustered together, while var. *diversipila* appeared as a separate group. The main variables that discriminated the individuals were: leaflet pairs per pinna, interfoliolar segment length, the ratio of interfoliolar segment length to rachis length, leaflet length and leaflet width (Fig. 3). Other less important variables were: nodes per raceme, heads per raceme, pinna rachis length, stipule length, and the ratio of leaflet length to leaflet width (not represented in the biplot).

In the cluster analysis (CA), the UPGMA clustering method produced five reasonably well-balanced and well-delimited groups. The analysis indicated that the classification did not differentiate between specimens of *M. diversipila* var. *diversipila* and specimens of *M. diversipila* var. *subglabriseta* if a partition with less than five groups was used; with a partition of more than five groups, only successive subdivisions of specimens of *M. diversipila* var. *diversipila* were obtained and the average silhouette width was significantly smaller. Thus, the final partition selected was five groups, with a reasonable average silhouette width = 0.23. The CA clustered mainly specimens of *M. diversipila* var. *diversipila* in a large group, and specimens of *M. diversipila* var. *subglabriseta* and intermediate specimens in another large group, with few exceptions. The three remaining groups included four specimens of *M. diversipila* var. *diversipila* that were excluded from the main cluster (Fig. 4).

The non-parametric multivariate analysis of variance (np-MANOVA) showed significant differences between the varieties (F = 20.31, p = 0.0001). Pairwise comparisons

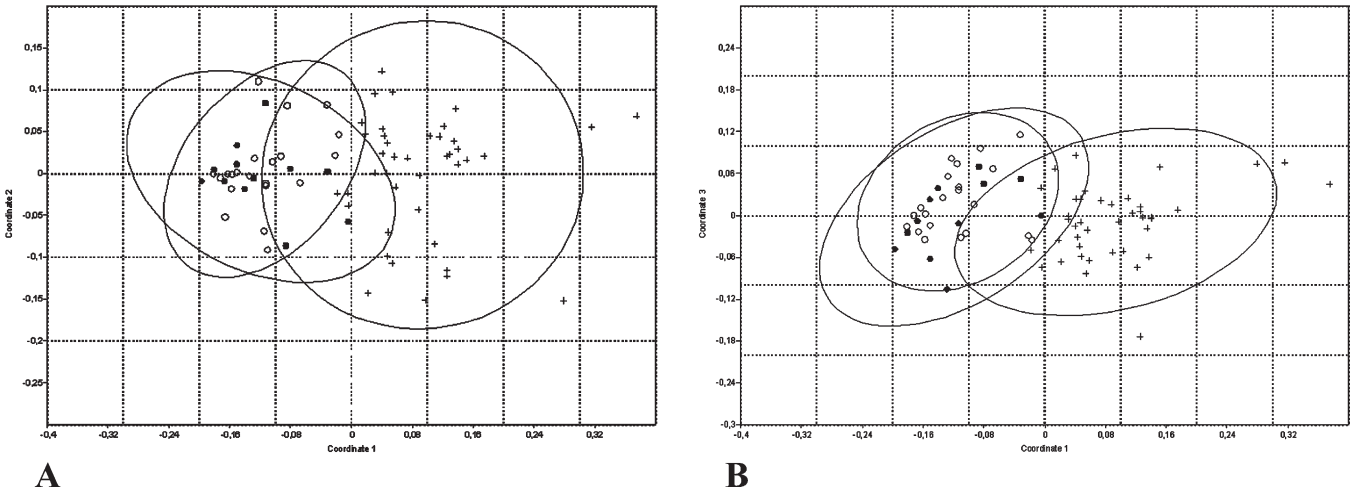

Fig. 2. Biplot showing the first three axes of the principal coordinates analysis. Open circles: *M. diversipila* var. *subglabriseta;* crosses: *M. diversipila* var. *diversipila;* closed circles: intermediate individuals between varieties. A. Biplot showing the axes 1 and 2. B. Biplot showing the axes 1 and 3. Ellipses include 95% of individuals in each group.

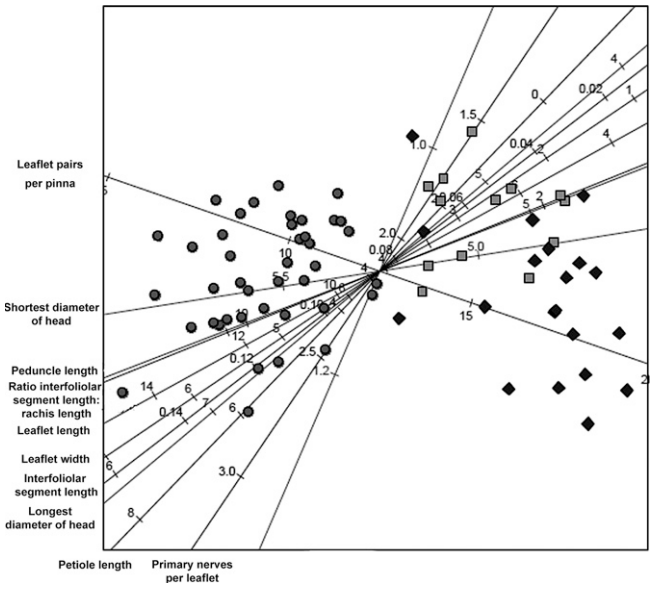

FIG. 3. Biplot showing the first two axes of the canonical variate analysis. Circles: *M. diversipila* var. *diversipila;* squares: intermediate individuals between the varieties; black diamonds: *M. diversipila* var. *subglabriseta.*

demonstrated that *M. diversipila* var. *subglabriseta* and the intermediate individuals did not differ ($F = 0.3973$, $p = 0.935$), but that both differed from *M. diversipila* var. *diversipila* ($F = 29.25$, $p = 0.0001$, $F = 20.27$, $p = 0.0001$ respectively).

Geographic Distribution*—Mimosa diversipila* var. *diversipila* is distributed in Central and northern Paraguay and adjacent areas of Brazil (Mato Grosso do Sul State), whereas *M. diversipila* var. *subglabriseta* occurs in southern Paraguay and Northeastern Argentina. The general geographic distribution of intermediate specimens coincides with that of *M. diversipila* var. *subglabriseta*. We also found *M. diversipila* var. *subglabriseta* in a locality at latitude 24°S in Canendiyú Department, Paraguay, not coinciding with the general pattern of distribution of this taxon (Fig. 5A).

According to the specimen labels and field observations, *M. diversipila* var. *diversipila* occurs generally in cerrado or cerradão areas (scrubs with red sandy soils), whereas *M. diversipila* var. *subglabriseta* is more frequent in subtropical savannas and grasslands or campos. The tetraploid cytotype of *M. diversipila* var. *diversipila* occurs in the southern area of distribution of this taxon (the middle area of distribution of the species), while the diploid cytotype occurs in its northern area of distribution. The diploid cytotype of *M. diversipila* var. *subglabriseta* was collected in the southern extreme of distribution of *M. diversipila* (Fig. 5B).

***Cytogenetic Studies*—**The cytogenetic studies suggest that *M. diversipila* var. *diversipila* has variable ploidy levels: $2n = 2x = 26$ and $2n = 4x = 52$, while *M. diversipila* var. *subglabriseta* is diploid: $2n = 2x = 26$ (Fig. 6A–B). CLHG ranged from 12.9 μm in tetraploid accessions of *M. diversipila* var. *diversipila* to 21.62 μm in diploid accessions of the same variety. There are significant differences in CLHG between accessions ($F = 11.14$, $p = 0.0192$), especially between diploid accessions of *M. diversipila* var. *diversipila* and the diploid accessions of *M. diversipila* var. *subglabriseta* and tetraploid accessions of *M. diversipila* var. *diversipila*. The morphological characters of diploid and tetraploid individuals do not differ amply (Fig. 6C).

Discussion

The specimens included in this study were all readily identified as *Mimosa diversipila,* because all these individuals exhibited a similar habit (erect, virgate subshrubs), an indumentum of branched trichomes or setae on vegetative organs, indeterminate and elongate inflorescences, haplostemonous flowers, and typical craspedia. We conclude that this species is easily distinguishable by these characters from allied species, and

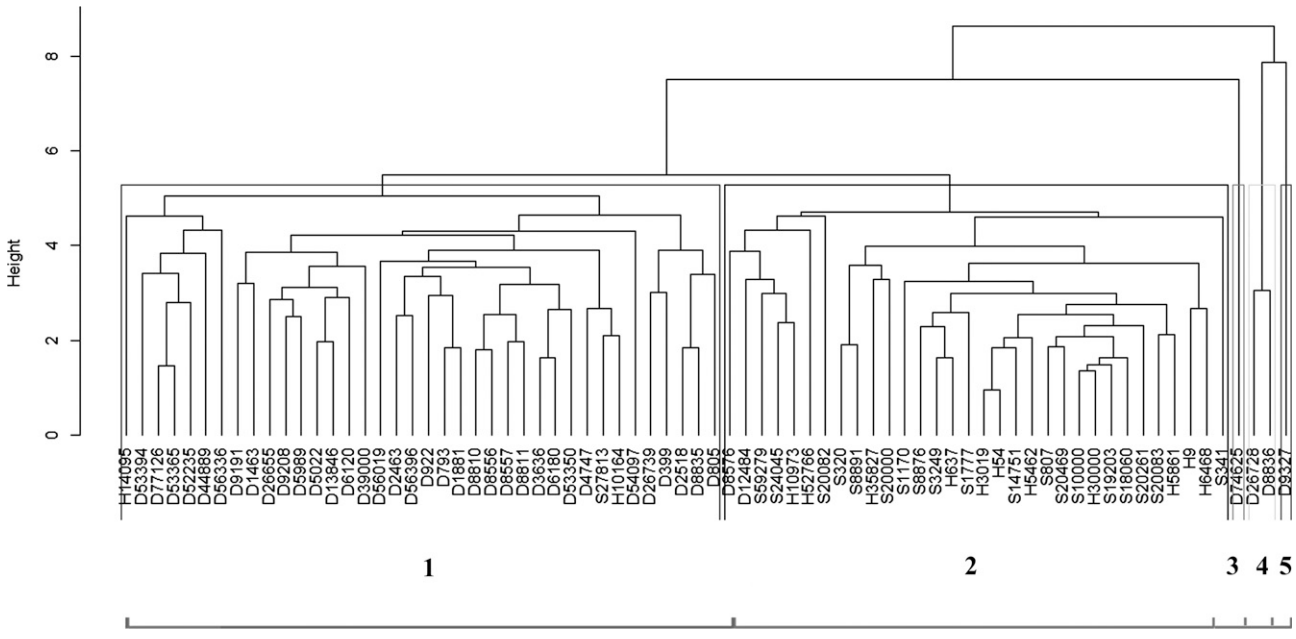

FIG. 4. Hierarchical cluster analysis in *M. diversipila,* with five groups, unweighted pair-group method using arithmetic averages (UPGMA) based on Euclidean distances. Numbers represent groups. Codes indicate the classification of each individual (S = *M. diversipila* var. *subglabriseta;* H = intermediate specimens between the varieties; D = *M. diversipila* var. *diversipila*) followed by the individual accession number.

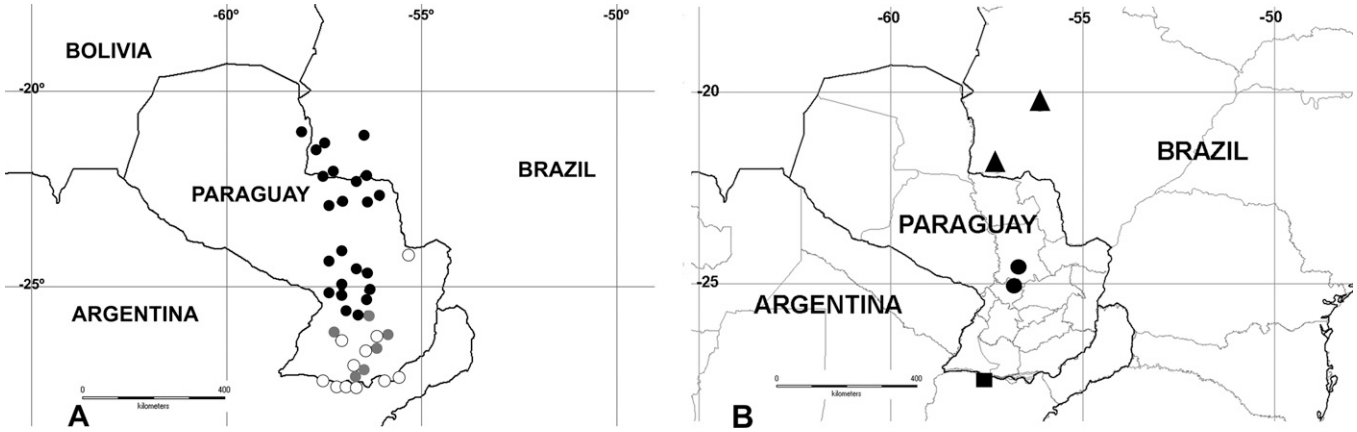

FIG. 5. A. The geographic distribution of *M. diversipila*. Open circles: *M. diversipila* var. *subglabriseta*; closed circles: *M. diversipila* var. *diversipila*; grey circles: intermediate specimens between the varieties. B. Geographic distribution of accessions of *M. diversipila* included in the present study. Squares: *M. diversipila* var. *subglabriseta*, $2n = 2x = 26$; circles: *M. diversipila* var. *diversipila*, $2n = 4x = 52$; triangles: *M. diversipila* var. *diversipila*, $2n = 2x = 26$.

it is possible to classify it in *Mimosa* subseries *Brevipedes* (Barneby 1991). In concordance with our results, the taxonomic status of the species has not been modified in two sucessive revisions (Burkart 1948; Barneby 1991). We agree with other authors that the specific rank of *M. diversipila* should not be modified.

However, this species showed high variation in pubescence (especially in the vegetative organs) and quantitative characters. The results of the multivariate analyses that simultaneously assessed data of different types (measurement, ratio, ordinal) show that *M. diversipila* varieties *diversipila* and *subglabriseta* comprise two different groups, while the intermediate specimens are grouped with *M. diversipila* var. *subglabriseta* (Figs. 2–3).

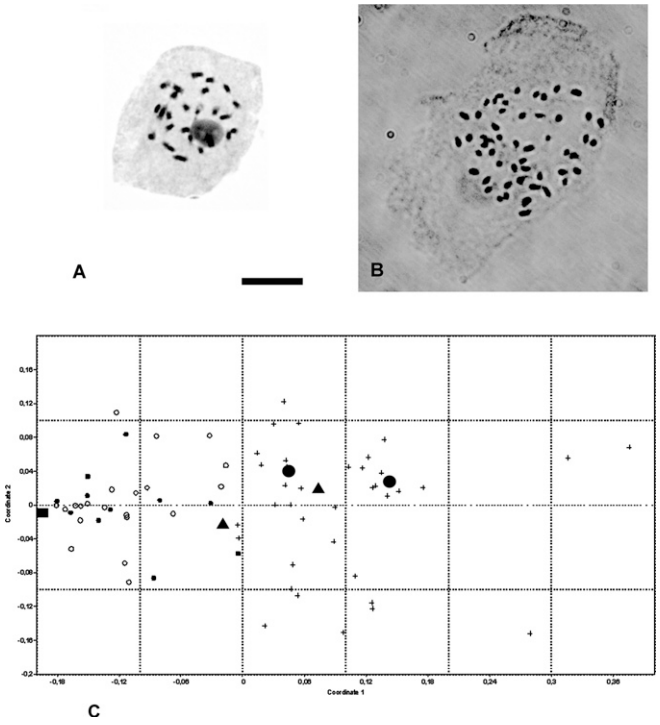

FIG. 6. Cytogenetics of *Mimosa diversipila*. A. Chromosomes of *M. diversipila* var. *subglabriseta*, $2n = 2x = 26$. B. Chromosomes of *M. diversipila* var. *diversipila*, $2n = 4x = 52$. Scale Bar: 10 μm. C. Distribution of the diploid and tetraploid accessions in the biplot of the first axes of PCoA. Open circles: *M. diversipila* var. *subglabriseta;* crosses: *M. diversipila* var. *diversipila;* closed circles: intermediate individuals between varieties; squares: *M. diversipila* var. *subglabriseta*, $2n = 2x = 26$; large circles: *M. diversipila* var. *diversipila*, $2n = 4x = 52$; large triangles: *M. diversipila* var. *diversipila*, $2n = 2x = 26$.

The main characters that contribute to the morphological variation between the varieties are vegetative: number of leaflet pairs, interfoliolar segment length, and length and width of leaflets (Table 3). None of the remaining quantitative characters were able to discriminate the varieties. On the contrary, other quantitative floral and carpological characters appeared relatively stable within the species. The height of plants differed between both varieties and intermediate specimens; *M.diversipila* var. *subglabriseta* is generally shorter than *M. diversipila* var. *diversipila* (Table 1). Nonetheless, since this character is frequently lacking on herbarium labels and is difficult to evaluate, it was not included in the multivariate analyses.

Chromosome numbers $2n = 2x = 26$ in *M. diversipila* var. *subglabriseta*, and $2n = 2x = 26$ and $2n = 4x = 52$ in *M. diversipila* var. *diversipila* are new reports. These results coincide with previous work that indicated $x = 13$ as the base chromosome number in the genus (Isely 1971; Goldblatt 1981; Seijo 1993, 1999, 2000; Seijo and Fernández 2001; Morales et al. 2010, 2011, 2012; Dahmer et al. 2011; Olkoski and Schifino Whitman 2011).

Tetraploid individuals of *M. diversipila* var. *diversipila* are morphologically indistinguishable from diploids of the same variety (Fig. 6C), and both have the same indumentum on leaflets and other vegetative organs and size of organs, but differ in these characters from diploid *M. diversipila* var. *subglabriseta.* Although more evidence is still necessary, given the lack of morphological variation between tetraploids and diploids, autopolyploidy appears to be the most likely cause of polyploidy in *M. diversipila* var. *diversipila*. Autopolyploidy has also been hypothesized in other polyploid complexes of *Mimosa*, such as the *M. debilis* Humb. & Bonpl. ex Willd. complex (Morales et al. 2010).

Morphometric studies have been shown to be an adequate tool to infer the origin of polyploids, especially when combined with molecular or cytogenetic studies. For example, in *Solidago altissima* L. (Richardson and Hanks 2011) and *Veronica chamaedrys* L. (Bardy et al. 2010), autopolyploidy was reported as the most probable origin of polyploid taxa due to sympatry of cytotypes, an inability to distinguish the cytotypes using multivariate analysis of

TABLE 3. Characters that distinguish varieties of *M. diversipila.*

Character	Taxon	Minimum	Percentile (10%)	Percentile (90%)	Maximum
Petiole length (mm)	*M. diversipila* var. *diversipila*	0.1	2	13	17
	M. diversipila var. *subglabriseta*	0.5	2	5	14
Rachis length (mm)	*M. diversipila* var. *diversipila*	42	45	82	97
	M. diversipila var. *subglabriseta*	30	30.5	67	71
Leaflet pairs per pinna	*M. diversipila* var. *diversipila*	9	9	14	17
	M. diversipila var. *subglabriseta*	12	15	22	26
Leaflet length (mm)	*M. diversipila* var. *diversipila*	7.5	10.5	18	20.5
	M. diversipila var. *subglabriseta*	4.5	6	11	16
Leaflet width (mm)	*M. diversipila* var. *diversipila*	3.5	4.5	7.5	9
	M. diversipila var. *subglabriseta*	2	2.1	7	10
Ratio leaflet length: width	*M. diversipila* var. *diversipila*	2.17	2.33	3.5	3.67
	M. diversipila var. *subglabriseta*	1.83	2.38	3.67	5.5
Interfoliolar segment length (mm)	*M. diversipila* var. *diversipila*	4	5	10	12
	M. diversipila var. *subglabriseta*	1.3	2	4.5	6
Ratio interfoliolar segment: rachis length	*M. diversipila* var. *diversipila*	0.07	0.08	0.15	0.19
	M. diversipila var. *subglabriseta*	0.03	0.05	0.10	0.14

morphological variation, and the lack of other species with which to hybridize.

In other species, morphometric techniques supported the allopolyploid origin of certain taxa. For example, in *Cardamine silana* Marhold & Perný, principal component analysis of both quantitative and qualitative morphological characters differentiated hexaploid and diploid taxa, suggesting that the hexaploids may be of hybrid origin and providing a hypothesis to the putative parents (Perný et al. 2005). Thórsson et al. (2007) also found evidence of hybridization in the polyploid complex *Betula nana* L.–*B. pubescens* Ehrh., given that cytotypes were clearly distinguished by their morphological variation.

Chromosome size data are in concordance with previous studies on *Mimosa* (Morales et al. 2011; unpublished data). Chromosomes are small, and the CLHG is similar to that of species with the smallest chromosomes in the genus, such as *M. debilis* and *M. xanthocentra* Mart. (Morales 2011). The index of interchromosomal asymmetry (A_2) is also similar to that of the other species previously evaluated and reveals that chromosome size is relatively uniform (Table 4).

In *Mimosa diversipila,* tetraploids of var. *diversipila* have smaller chromosomes than their related diploids; this may be a trend in some *Mimosa* species groups (Morales unpublished data). In higher plants, polyploidy usually appears to be associated with a decrease in genome size in terms of DNA content in haploid genomes (Soltis et al. 2003; Kellogg and Bennetzen 2004).

Barneby (1991) used quantitative characters to distinguish taxa in different groups of *Mimosa* by measuring the extreme values of the organs. In some cases, he was successful, but in other cases these characters failed to differentiate species or infraspecific taxa, for example, *M. debilis* (Morales and Fortunato 2010; Morales et al. 2010). In *M. diversipila,* although there is some overlap in several characters between the varieties, the quantitative characters distinguish them.

Based on morphology, we conclude that both varieties should be retained as recognized taxonomic entities, although their circumscription should be modified, including quantitative characters to identify them. The number of leaflet pairs, length and width of leaflets, and length of interfoliolar segments appear to be the main characters that contribute to the morphological variation, and together with the pubescence (especially of leaflets), are diagnostic characters (Table 3).

Although chromosome number and size do not distinguish the varieties, it is interesting to note that *M. diversipila* var. *diversipila* has higher morphological variation, than *M. diversipila* var. *subglabriseta*. The presence of different ploidy levels could contribute to this phenomenon, because polyploidy can generate morphological variation especially in the size of organs (Grant 1971; Balao et al. 2011).

In Mimosoideae, and especially in *Mimosa,* the presence of complexes with high morphological variation (especially in quantitative characters) is frequent and creates difficulties for the identification and circumscription of taxa. In this context, multivariate techniques can be decisive in resolving these problems, especially when combined with cytogenetics and molecular markers. For example, multivariate approaches proved useful to infer the natural classification of the Argentinean *Acacia* and clarify the circumscription of varieties in the infraspecific taxonomic complex *A. caven* (Mol.) Mol. (Pometti et al. 2007), as well as the presence of interspecific hybrids between representatives of *Prosopis* L. in the Argentinean Chaco region (Ferreyra et al. 2013).

In this first study of the infraspecific *M. diversipila* complex, a combination of different multivariate techniques allows us to visualize the presence of two infraspecific entities, which also appear to be characterized by their geographic

TABLE 4. Chromosome number ($2n$), ploidy (x), chromosome length by haploid genome (CLHG), Asymmetry Index (A_2) in *M. diversipila.* Different superscripts for CLHG indicate that the values are significantly different with Fisher's LSD test, $p <= 0.05$. $F = 11.14$, $p = 0.0192$.

Taxon	Locality	Accession	Chromosome number	CLHG (mm)	A_2
M. diversipila var. *diversipila*	Mato Grosso do Sul, Brazil	*M. Morales 922*	$2n = 2x = 26$	17.64 ± 1.40^{b}	0.21
	Mato Grosso do Sul, Brazil	*M. Morales 793*	$2n = 2x = 26$	21.62 ± 0.50^{c}	0.16
	Paraguay	*R. H. Fortunato 8810*	$2n = 4x = 52$	12.90 ± 0.30^{a}	0.21
	Paraguay	*R. H. Fortunato 9191*	$2n = 4x = 52$	$15.79 \pm 1.05^{a,b}$	0.20
M. diversipila var. *subglabriseta*	Northeastern Argentina	*M. Morales 637*	$2n = 2x = 26$	16.84 ± 0.50^{b}	0.25

distribution. In addition, polyploidy, a phenomenon occurring in at least 22% of the cytologically studied species of *Mimosa* (Dahmer et al. 2011; Morales et al. 2011), could notably contribute to the infraspecific morphological variation in *Mimosa diversipila*.

Taxonomic Treatment

Mimosa diversipila var. subglabriseta Barneby & Fortunato emend. M. Morales & Fortunato—TYPE: ARGENTINA. Misiones: Capital, Arroyo Itaembé, 17 Jan 1966, *Krapovickas 12094* (holotype: US; isotype: CTES!).

Erect subshrubs to 1 m high; stems unarmed, with indumentum of scaberulous setae. Petioles 0.2–1.7 cm long, terete or subterete. Leaves 1-jugate. Longer pinna rachis 3–7.1 cm long. Longer leaflets (12–)14–26–jugate, 0.45–1.6 × 0.2–0.7 cm; length:width ratio 2.16–3.67, pubescence composed of scaberulous setae that partially cover the surface, finely 1–3–nerved dorsally from pulvinule, secondary venation barely visible (if clearly visible then brochidodromous), subcoriaceous, brownish to olivaceous, more rarely green in herbarium specimens. Inflorescences indeterminate (racemes) with 18–46 heads; longer peduncles 0.5–1.6 cm long. Heads (without stamens) 4.5–7.5 × 3–5 mm in diameter, globose, ellipsoid or moriform and shortly hispid previous to anthesis. Calyx 0.1–0.4 mm, campanulate or tubulose, membranaceous, rarely long-aristate; corolla 2–2.5(–3.5) mm, puberulent, androecium with pink filaments, free or shortly monadelphous around the base of ovary; gynoecium with pubescent ovary. Craspedia 0.65–1.7 × 0.3–0.55 cm, hispid to villose, with scaberulous or plumose setae, with 1–3 articles. Fig. 1A–H.

Distribution and Habitat—*Mimosa diversipila* var. *subglabriseta* occurs in northeastern Argentina (Misiones and Corrientes provinces) and southern Paraguay (in the departments of Itapúa, Caazapá, Paraguarí, Guairá, and Alto Paraná, but with a population more distant in Canendiyú), generally in a range from 25°S to 27°S. This taxon grows in subtropical savanna and open campos.

Representative Specimens Examined—PARAGUAY. Alto Paraná: In regione fluminis Alto Paraná, *Fiebrig 6180* (SI). Caaguazú: Ruta 3, 47 km al N de Coronel Oviedo, 17 Apr 1995, *Schinini et al. 29247* (CTES). Caazapá: Ayo. Pindapoy, Yutí, 19 Jan 1949, *Rosengurt 5462* (SI); Ea. Tapytá of Shell Forestry Ltd. on road to Tayá-i Creek, 13 Dec 1999, *Zardini and Brítez 52766* (BAB); same locality, on trail to Yuquerí Creek, 14 Dec 1999, *Zardini and Brítez 52885* (BAB); Santa Úrsula, 55 km NE de Yuty, 23 Mar 1993, *Schinini et al. 27813* (BAB). Canendiyú: Mbaracayú National Reserve, administered by Foundation Moisés Bertoni, 14 Jan 1988, *Zardini and Vera 47883* (BAB). Guairá: Azucarera de Tebicuary, Arroyo Ihacá, 21 Jan 1973, *Schinini 5861* (SI); Tebicuary, 06 Feb 1945, *Rojas 12484* (SI). Itapúa: Ayolas, Refugio, 01 Feb 1982, *Bordas 3019* (CTES); Isla Yaciretá, 19 Feb 2004, *Peña et al. 1777* (CTES); Pirapó (Punta Fierro), 05 Dec 1890, *Sellow s. n.* (LP); Posta-Cué, dans les práiries humides, 15 Mar 1884, *Balansa 9* (G); Salitre Cué, 25 Jan 1944, *Pavetti and Rojas 10973* (SI); Yacyretá Dam Island Reserve, 05 Dec 2002, *Zardini and Gamarra 59279* (BAB). Misiones. Ea. "La Soledad," Santiago, 03 Feb 1955, *Pedersen 3249* (BAB); Junction Road Ruta 1 and camino to Montiel Potrero, 4 km N of Villa Florida, 30 Jan 1996, *Brooks and Zardini 341* (MO). Paraguarí: 1 km N of Villa Florida on Tebicuary River, 25 May 1993, *Zardini and Guerrero 35827* (BAB); Caapucú, Ea. Barrerito, *Rojas and Ramírez 13152* (SI).

ARGENTINA. Corrientes: Entre Itá Ibaté e Ituzaingó, 16 Jan 1961, *Nicora and Cámara Hernández 320* (SI); Dpto. Berón de Astrada, 15 km W de Itá Baté, Arroyo Santa Isabel, 16 Jan 1977, *Schinini 14095* (CTES); Dpto. Berón de Astrada 2 km al S de Valencia, por Ruta 12, 05 Mar 2006, *Fortunato et al. 8891* (BAB, SI); Dpto. Ituzaingó, Ruta 38, 10 km de Ruta Nacional 12, 28 Jan 1987, *Pozner 54* (BAB); Dpto. Ituzaingó, Camino a la Represa Yaciretá, 15 Feb 2008, *Morales et al. 637* (BAB); Dpto. Ituzaingó, Isla Apipé Grande, Puerto San Antonio, 09 Dec 1973, *Krapovickas et al. 24045* (SI). Misiones: Dpto. Candelaria, Loreto, 28 Jan 1907, *Spegazzini s. n.* (BAB); Dpto. Candelaria, Santa Ana, 16 Mar 1943, *Burkart 14773* (SI); Dpto. Capital, Itaimbé, 29 Dec 1934, *Rodríguez 1170* (BAF); Dpto. Capital, Posadas, 14 Jan 1907, *Spegazzini s. n.* (BAB), 15 Jan 1907, *Spegazzini s. n.* (BAB); 01 Feb 1960, *Martínez Crovetto 8876* (BAB), 02 Feb. 1919, *Bertoni 807* (LP).

Mimosa diversipila M. Micheli var. diversipila— TYPE: PARAGUAY. Mboiacatí, pies de Villa Rica, 23 Feb 1876, *Balansa 1463* (lectotype: G!; isotype: P!).

Erect subshrubs to 2.5 m high; stems unarmed, with indumentum of branched trichomes (scaberulous to plumose setae). Leaves with petioles 0.5–1.4 cm, terete or subterete; pinnae 1–jugate; longer pinna rachis 4.2–9.7 cm long; leaflets 9–17–jugate, 0.75–2.05 × 0.35–0.9 cm, length:width ratio 1.83–5.5, pubescence composed of plumose setae or stellate trichomes on both surfaces, generally densely covering the entire surface, 1–4–nerved dorsally from pulvinule, secondary venation inconspicuous, membranous. Inflorescence indeterminate (racemes) with 5–65 heads; the longest peduncles 0.2–2.7 cm long; heads (without stamens) 4.5–8.5(–11) mm in diameter, globose to elipsoid or moriform, thinly hispid previous the anthesis. Flowers with calyx 0.1–0.5 mm, tubulose or campanulate, membranous, not aristate; corolla 2.5–3.5 mm long; androecium with filaments pink, free or shortly monadelphous around the base of the ovary; gynoecium with ovary pubescent. Craspedia 0.7–1.6 × 0.3–0.6 cm, hispid to villose, with 2–4 articles. Fig. 1I–N''.

Distribution and Habitat—*Mimosa diversipila* var. *diversipila* occurs in northern Paraguay (from departments of Paraguarí and Guairá to Alto Paraguay) and adjacent areas of Brazil (Mato Grosso do Sul state), in a latitudinal range from 20°S to 26°S. This taxon grows in open savanna, cerrado, and cerradão (scrubs with red sandy soils).

Representative Specimens Examined—BRASIL. Mato Grosso do Sul: Mun. Miranda, Trevo Bodoquena-Miranda, 14 May 2009, *Morales et al. 922* (BAB, MBM); mun. Caracol, Rio Perdido, 09 May 2009, *Morales et al. 793* (BAB, MBM).

PARAGUAY. Alto Paraguay: Primavera, 12 Dec 1954, *Woolston 399* (SI). Amambay: Around Bella Vista, 11 Jan 2000, *Zardini and Guerrero 53350* (BAB); Around Bella Vista N, 11 Jan 2000, *Zardini and Guerrero 53365, 53394* (BAB); National Park Cerro Corá, along Aceite-í Creek, 12 Nov 1999, *Zardini and Báez 52627* (BAB); Parque Nacional Cerro Corá 28 Feb 2001, *Zardini and Acosta 56336* (BAB); Trail to Arroyo Estrella, 08 May 2000, *Zardini et al. 54097* (BAB). Caaguazú: 20 km N de Coronel Oviedo, 16 Feb 1968, *A. Krapovickas et al. 13846* (SI). Concepción: Around Yby-Jaú, 01 Mar 2001, *Zardini and Guerrero 56396* (BAB); Ea. San Fernando and Paso Horqueta, 17 Mar 1994, *Zardini 39000* (BAB); Rancho Z. Potrero Plantel, 12 Dec 1991, *Degen 2518* (MO); Villa Sana. Zwischen Rio Apa und Aquidaban, *Fiebrig 5022* (G). Cordillera: 3 km de Arroyos y Esteros, por Ruta 3 en dirección a San Estanislao, margen río Yaghuy, 14 Mar 2005, *Fortunato et al. 8810* (BAB, SI); Tobatí, 31 Jan 1902, *K. Fiebrig 805* (G); Tobatí, Ybytú Silla Mesa, 03 Mar 1991, *Zardini and Velázquez 26739* (BAB); Tobatí, Ybytú Silla Mesa, Northern area, 03 Mar 1991, *Zardini and Velázquez 26729* (BAB); Ybytú Silla mesa, Southern Area, 23 Feb 1991, *Zardini and Vera 26655* (BAB). Guairá: 3 km E del cruce Ruta Nacional 3, Cnel. Oviedo-Villarrica en dirección a Cnia. Independencia, 08 Mar 2005, *Fortunato et al. 8556* (BAB, SI); Cnia. Independencia, región de Villarrica, *Rojas 4747* (SI); Prope Villarrica in campis siccis, *Hassler 8576* (G); Villarrica, *Jorgensen 3636* (LP). Paraguarí: ad marginem silvarum prope Sapucay, *Hassler 1881* (G). San Pedro: 1.7 km al S del Puente del Ayo Tobatiry, 06 Mar 2008, *Fortunato et al. 9191* (BAB); 23 km al W de San Estanislao, camino a Rosario, Mar 1989, *Eskuche 6120* (BAB); 8 km de San Estanislao en dirección a Itacurubí del Rosario, 14 Mar 2005, *Fortunato et al. 8835* (BAB, SI); Desvío a San Pedro, Estancia La Blanca, 01 Mar 1994, *Soria 6468* (MO); in campis prope San Estanislao, *Hassler 5989* (G).

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