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Cytogeography and morphological characterisation of a taxonomic, polyploid complex of *Mimosa* (Leguminosae) from subtropical South America

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Abstract. *Mimosa* subseries *Dolentes* Barneby and *Brevipedes* Barneby are ecologically and morphologically high-diversified infrageneric taxa of this genus in southern South America. We performed a cytogenetical and morphological analysis of both subseries. Chromosome numbers from accessions throughout the area of distribution were studied. The chromosome numbers $2n = 8x = 104$ for *M. dolens* subsp. *callosa* (Benth.) Barneby, *M. dolens* subsp. *acerba* varieties *acerba* (Benth.) Barneby, *latifolia* (Benth.) Barneby and *rudis* (Benth.) Barneby, *M. dolens* subsp. *rigida* var. *rigescens* (Benth.) Barneby, var. *anisitsii* (Lindm.) Barneby and var. *foliolosa* (Benth.) Barneby; and $2n = 4x = 52$ for *M. sceptrum* Barneby, *M.* aff. *custodis* Barneby and *M. dolens* var. *pangloea* Barneby are presented for the first time. Their karyotypes were symmetric, with small chromosomes. There were several areas with taxa growing in sympatry, occasionally with intermediate forms. No diploids were found, which suggests the presence of either a declining polyploid complex or ancient polyploidy in the clade of southernmost representatives of *M.* series *Mimosa*, the most derived of the genus. Some vegetative, inflorescence and carpological characters seem to be associated with chromosome duplication. The distribution pattern of the cytotypes suggests events of chromosome duplication in centres of diversity and expansion of octoploids to the southernmost areas of distribution. Our findings support the importance of polyploidy in the morphological diversity, distribution and speciation of this complex.

Additional keywords: hybrid zones, octoploid, plant speciation.

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Introduction

The genus *Mimosa* L. is composed of more than 530 species and it is one of the most diversified genera in the family Leguminosae (Bessega and Fortunato 2011; Simon *et al.* 2011). *Mimosa* is more abundant in the Neotropics, where ~90% of the species occur. This genus has the following two centres of diversification: (1) South America, including Amazonas Basin and adjacent regions of Brazil, Argentina, Paraguay and Uruguay; and (2) Mesoamerica and southern Mexico. Other minor centres of diversification include Cuba, La Hispaniola, Orinoco Basin and Madagascar (Barneby 1991; Morales and Fortunato 2016).

This megadiverse genus was studied extensively by Bentham (1841, 1842, 1875, 1876), who proposed the following two sections: *Habbasia* DC. and *Mimosa*, on the basis of the number of stamens, namely, haplostemonous *v.* diplostemonous flowers respectively. Later, Barneby (1991) revised the Neotropical species and proposed five sections. *Mimosa* section *Mimosa* was considered by this author the most evolved and diversified; he also divided it into three series, *Mimosa*, *Myriophyllae* Benth. and *Modestae* Benth. *Mimosa* series *Mimosa* is the most diversified among them, including 37 subseries and ~159 species (Barneby 1991).

In *Mimosa*, it was postulated that the basic chromosome number is $x = 13$ (Goldblatt 1981; Isely 1971). This basic chromosome number is predominant in tribes Acacieae, Ingeae and Mimoseae, and it was apparently derived via disploidy from $x = 14$, which is the basic chromosome number of the genus *Dinizia* Ducke (Poggio *et al.* 2008), an early divergent genus of the 'Mimosoideae' clade (Lewis *et al.* 2005; Azani *et al.* 2017). Some genera of Mimoseae have $x = 14$, such as, for example, *Prosopis* L. and *Neptunia* Lour. (Lewis *et al.* 2005; Poggio *et al.* 2008). Successive and extensive cytological studies performed on representatives from the Paleotropics (Bir and Kumari 1975; Nazeer and Madhusoodanan 1983) and Neotropics (Seijo 1993, 1999, 2000; Seijo and Fernández 2001; Morales *et al.* 2010, 2011, 2012, 2014*a*, 2014*b*, 2015; Dahmer *et al.* 2011) support $x = 13$ as the basic chromosome number of the genus. In these works, several ploidy levels were postulated, including $2x = 26$, $3x = 39$, $4x = 52$, $6x = 78$ and $8x = 104$.

The most derived infrageneric groups are polyploid or exhibit different ploidy levels, suggesting that polyploidy is an important evolutionary force that can lead to speciation (Seijo and Fernández 2001; Morales *et al.* 2010, 2014*a*, 2014*b*; Dahmer *et al.* 2011). Presence of polyploids of different origin has been hypothesised on the basis of morphological data (Morales *et al.* 2010, 2014*a*, 2014*b*).

Polyploidy in *Mimosa* has been frequently associated with taxonomic complexes that have high morphological variation and are, therefore, difficult to circumscribe, and this has been interpreted as indicating that polyploids are of hybrid origin (Morales *et al.* 2010, 2014*a*, 2014*b*). Polyploidy seems to be a decisive phenomenon in the diversification of the genus, especially in the southern area of distribution in America, where polyploids are especially concentrated (Seijo 1999; Seijo and Fernández 2001; Morales *et al.* 2010, 2014*a*; Dahmer *et al.* 2011).

The section *Mimosa* series *Mimosa* subseries *Dolentes* Barneby and *Brevipedes* Barneby comprise subshrubs from subtropical grasslands of Brazil, Argentina, Paraguay and Uruguay, with conjugate pinnae and exerted inflorescences (Barneby 1991, pp. 578–581, 705–706). Both subseries appear to be close morphologically (Barneby 1991, p. 706; Morales 2011). Subseries *Dolentes* includes the species of *Mimosa* with virgate erect stems, mostly one-jugate pinnae, determinate (cymose) inflorescences, fertile haplostemonous flowers and pods with valvate dehiscence grouped in spheroid clusters. Subseries *Brevipedes* includes subshrubs with one-jugate pinnae, indeterminate (racemose) inflorescences, haplostemonous fertile flowers, and either clustered pods (i.e. fruit with only valvate dehiscence, i.e. valves separating entire from replum) or unclustered typical craspedia (i.e. fruit breaking up into free-falling articles) (Barneby 1991, pp. 705–706).

Mimosa subseries *Dolentes* is monospecific, with *M. dolens* Vell., divided in five subspecies and eleven varieties, including *M. dolens* subsp. *dolens* var. *dolens*, *M. dolens* var. *pangloea* Barneby; *M. dolens* subsp. *eriophylla* (Benth.) Barneby; *M. dolens* subsp. *callosa* (Benth.) Barneby; *M. dolens* subsp. *acerba* (Benth.) Barneby var. *acerba*, *M. dolens* subsp. *acerba* var. *latifolia* (Benth.) Barneby, *M. dolens* subsp. *acerba* var. *rudis* (Benth.) Barneby and *M. dolens* subsp. *acerba* var. *pseudorigida* (Benth.) Barneby; *M. dolens* subsp. *rigida* (Benth.) Barneby var. *rigida*, *M. dolens* subsp. *rigida* var. *rigescens* (Benth.)

Barneby, *M. dolens* subsp. *rigida* var. *deterior* Barneby and *M. dolens* subsp. *rigida* var. *foliolosa* (Benth.) Barneby (Barneby 1991, p. 581). Subseries *Brevipedes* comprises nine species, including *M. brevipes* Benth., *M. longiracemosa* (Burkart) Barneby, *M. fernandez-casasii* Barneby & Fortunato, *M. sceptrum* Barneby, *M. custodis* Barneby, *M. diversipila* M.Micheli, *M. pseudopetiolaris* Barneby, *M. cryptogloea* Barneby and *M. nitidula* Barneby. *Mimosa sceptrum*, *M. custodis* and *M. longiracemosa* have pods with valvate dehiscence grouped in relatively dense spheroid clusters (Burkart 1948, pp. 192–196; Barneby 1991, pp. 706–707 ; Morales 2011); the remainder of species have craspedia not grouped in spheroid clusters, with the exception of *M. nitidula* and *M. cryptogloea*, whose fruit have not been studied because specimens in herbaria are lacking these organs (Barneby 1991, pp. 711–712, 714–715; Morales 2011). *Mimosa sceptrum* and *M. custodis* have intermediate morphology between the subseries *Dolentes* and *Brevipedes* and their taxonomic position and circumscription are currently in question (Morales 2011).

These subseries have high morphological and ecological diversity. Although the subseries *Dolentes* is monotypic according to Barneby (1991, pp. 578–580), the majority of its infraspecific taxa, subspecies and varieties, were previously considered to be species (Bentham 1842, 1846). Some characters used to distinguish infraspecific taxa in subseries *Dolentes* and *Brevipedes* have also been used to distinguish species in other series or subseries of the section *Mimosa* (Barneby 1991, pp. 506–515, 533–537).

Although subseries *Dolentes* and *Brevipedes* comprise only few species, their infraspecific morphological variability is remarkable and there is some difficulty in adequately distinguishing their taxa. These groups are widely distributed in different ecosystems of subtropical and warm-temperate South America (Barneby 1991, pp. 581, 706).

In the present study, we compared the chromosome number and karyotype of representatives of subseries *Dolentes* and *Brevipedes* with morphological variability, so as to generate hypotheses about the evolution of their composite species; we also provide a model for their future taxonomic revision.

Materials and methods

Plant material

Specimens and material for cytogenetic studies were collected during different field trips in Argentina (2006), Paraguay (2005 and 2008) and Brazil (2009), in expeditions with colleagues from these countries (Table 1). The specimens were deposited in BAB, CTES, FCQ, HUCS, MBM and SI. They were identified according to Barneby (1991) and later nomenclatural modifications (Fortunato *et al.* 2008; Azani *et al.* 2017), and compared with the nomenclatural types of the taxa.

Chromosome studies

For each accession, chromosomes of 3–10 individuals were studied. The seeds collected during the field trips were germinated in Petri dishes at 25°C. Roots with ~1-cm length were pretreated with 0.0002 M 8-hydroxyquinoline for 4–7 h and fixed with 3 : 1 ethanol : glacial acetic acid solution. The material was preserved at 5°C until its use.

Table 1.   Voucher specimens and chromosome numbers of members of *Mimosa* subseries *Dolentes* and *Brevipedes* complex

Taxon	2*n*	Voucher specimen	Altitude (m ASL)	Ecosystem or ecoregion	Previous records
M. dolens subsp. *dolens* var. *pangloea* Barneby	52	Brazil. Paraná: Colônia Quero-Quero, 25 Apr. 2009, *R. H. Fortunato et al. 9580* (BAB).	900	Highland grassland	*
	52	Brazil. Paraná: road to Porto Amazonas, 6 Apr. 2014, *Morales et al. 1609* (BAB, MBM).	912	Highland grassland	
M. sceptrum Barneby	52	Paraguay. Amambay: Colonia Fortuna Guazú, 9 Mar. 2008, *R. H. Fortunato et al. 9282* (BAB).	600	Cerrado	*
M. aff. *custodis* Barneby	52	Brazil. Santa Catarina: Serra do Quiriri, 23 Apr. 2009, *R. H. Fortunato et al. 9555a,c,d, e* (BAB).	1200	Higland grassland	
	52	Brazil. Santa Catarina: Quiriri, 23 Apr. 2014, *M. Morales & O. S. Ribas 1624, 1625, 1626, 16430* (BAB, MBM).			
M. dolens Vell. subsp. *acerba* (Benth.) Barneby var. *rudis* (Benth.) Barneby	52	Paraguay. Amambay, Colonia Fortuna Guazú, 9 Mar. 2008, *R. H. Fortunato et al. 9279a* (BAB).	600	Cerrado	*
Intermediate between *M. dolens* subsp. *acerba* var. *rudis* and *M. sceptrum*	52	Paraguay. Amambay: Colonia Fortuna Guazú, 9 Mar. 2008, *R. H. Fortunato 9279b* (BAB).	600	Cerrado	*
M. dolens Vell. subsp. *acerba* (Benth.) Barneby var. aff. *rudis* (Benth.) Barneby	104	Brazil. Paraná: Recanto dos Papagaios, 6 Apr. 2014, *M. Morales & O. S. Ribas 1593* (BAB, MBM).	1030	Highland grassland	*
	104	Brazil. Paraná: Recanto dos Papagaios, 6 Apr. 2014, *M. Morales & O. S. Ribas 1594* (BAB, MBM).	1030	Highland grassland	*
	104	Brazil. Paraná: Recanto dos Papagaios, 18 Apr. 2009, *R. H. Fortunato et al. 9514* (BAB, MBM).	1030	Highland grassland	*
M. dolens subsp. *rigida* (Benth.) Barneby var. *anisitsii* (Lindman) Barneby	104	Paraguay. San Pedro, 1 km al N de Barrio San Pedro, 7 Mar. 2008, *R. H. Fortunato et al. 9217* (BAB).	240	Grassland	*
	104	Paraguay. Amambay: Parque Nacional Cerro Corá, 8 Mar. 2008, *R. H. Fortunato et al. 9253* (BAB).	320	Cerrado	*
	104	Paraguay. Amambay: Porteira Ortiz, 11 Mar. 2008, *R. H. Fortunato et al. 9350* (BAB).	660	Cerrado	*
	104	Brazil. Mato Grosso do Sul: Amambai, Fazenda Laguna Vera, 6 May 2009, *M. Morales et al. 728* (BAB, MBM).	385	Cerrado	
M. dolens subsp. *acerba* (Benth.) Barneby var. *latifolia* (Benth.) Barneby	104	Argentina. Misiones: Oberá, 8 Mar. 2006, *R. H. Fortunato et al. 9027* (BAB).	440	Grassland	2*n* = 104
	104	Paraguay. Caaguazú: 3–4 km al N del Arroyo Tacurú, 8 Mar. 2005, *R. H. Fortunato 8548* (BAB).	280	Grassland	
	104	Argentina. Misiones: San Javier, 8 Mar. 2006, *R. H. Fortunato et al. 9029* (BAB).	100	Grassland	2*n* = 104
	104	Argentina. Misiones: Apóstoles, 9 Mar. 2006, *R. H. Fortunato et al. 9052* (BAB).	94	Grassland	2*n* = 104
	104	Brazil. Rio Grande do Sul: Porto Alegre, *F. Schmidt s.n.* (ICN).	n.d.	Grassland	2*n* = 104
	104	Brazil. Rio Grande do Sul: São Marcos, 24 Feb. 2013, *M. Morales et al. 1062* b (BAB, HUCS).	680	Grassland	2*n* = 104
M. dolens subsp. *rigida* var. *foliolosa* (Benth.) Barneby	104	Argentina. Corrientes: Santo Tomé, 10 Mar. 2006, *R. H. Fortunato et al. 9077* (BAB).	119	Grassland	2*n* = 104
	104	Argentina. Corrientes: Mercedes, 13 Mar. 2013, *M. Morales & G. Cardoso 1153* (BAB)	111	Grassland	2*n* = 104

Table 1.   (*continued*)

Taxon	2n	Voucher specimen	Altitude (m ASL)	Ecosystem or ecoregion	Previous records
	104	Paraguay. Amambay: Porteira Ortiz, 11 Mar. 2008, *R. H. Fortunato et al. 9347* (BAB).	660	Cerrado	2n = 104
	104	Brazil. Mato Grosso do Sul: Alrededores de Bella Vista, 9 May 2009, *M. Morales et al. 819* (BAB).	200	Cerrado	2n = 104
	104	Brazil. Rio Grande do Sul: São Marcos, 24 Feb. 2013, *M. Morales et al. 1062 g* (BAB, HUCS).	680	Grassland	2n = 104
M. dolens subsp. *callosa* (Benth.) Barneby	104	Paraguay. Amambay: Porteira Ortiz, 11 Mar. 2008, *R. H. Fortunato et al. 9347* (BAB).	660	Cerrado	*
M. dolens subsp. *acerba* var. *acerba*	104	Brazil. Paraná: Recanto Botuquara, May 2009, *O. S. Ribas et al. 8218* (BAB).	1030	Highland grassland	*
	104	Brazil. Paraná: Laranjeiras do Sul, BR277, 20 Apr. 2009, *R. H. Fortunato et al. 9551* (BAB).	950	Highland grassland	*
Intermediate individuals between *M. dolens* var. *acerba* and *M. dolens* subsp. *rigida* var. *foliolosa*	104	Brazil. Paraná: Recanto dos Papagaios, 18 Arp. 2009, *R. H. Fortunato et al. 9517* (BAB).	1030	Highland grassland	*

Fixed roots were washed in 0.01 M citric acid–sodium citrate, pH 4.6, buffer to remove the fixative, and transferred to an enzyme solution containing 2 mL of 2% cellulase (w/v) and 20% (v/v) pectinase. The softened material was washed again in the buffer solution mentioned above. To analyse the chromosome number and chromosome size, the material was stained with a drop of haematoxylin or acetic carmine, and the chromosome slides were made by 'squash' technique (Egozcue 1971).

Chromosome length by haploid genome (CLHG) and chromosome area by haploid genome (CAHG) were measured by the means Micromeasure (University of Colorado State, Fort Collins, CO, USA, ver. 3.3, Reeves 2001). CLHG and CAHG were calculated by dividing the total chromosome length and total chromosome area by the ploidy level. To study the distribution of both variables, a normality test of Shapiro–Wilks with modifications (Mahibbur Rahman and Govindarajulu 1997) was used. Homoscedasticity was analysed by means Levene's test (Levene 1960). ANOVA was used to compare the mean values of CLHG and CAHG between ploidy levels. Parametric one-way ANOVA was conducted when variables were normally distributed and homoscedastic, whereas a non-parametric Kruskall–Wallis test was conducted when variables were not normally distributed and heteroscedastic. Where variables exhibited statistically significant differences, a *post hoc* pairwise comparison (Tukey or Mann–Whiney test) was undertaken to identify group pairs involved.

The inter-chromosomal asymmetry index (A_2), which describes variation in chromosome length in a complement, was calculated on the basis of Romero Zarco (1986), as follows:

$$A_2 = SX^{-1}$$

where S represents standard deviation and X the mean of chromosome length.

Morphological analysis of voucher specimens

We scored 25 quantitative and qualitative vegetative, floral and carpological characters (Table 2) from voucher specimens of the subseries *Dolentes* and *Brevipedes*. Cluster analysis was conducted using PAST (Hammer *et al.* 2001). Some morphological characters, especially floral characters, were not included in the cluster analysis because they were absent from the majority of accessions. Cluster analysis was conducted using the following three algorithms: single linkage, unweighted pair-group method with arithmetic mean (UPGMA), and Ward's method. Results from UPGMA had the highest cophenetic correlation (0.8425) and are presented here. We used mixed similarity measures, namely, Gower for continuous and ordinal variables, Hamming/p-distance for nominal variables and Jaccard for the binary variables.

Several univariate analyses were conducted in quantitative characters to detect significant differences between the following two groupings: (1) among groups visualised by the means of the UPGMA analysis; and (2) between ploidy levels. As for analysis of chromosomes, we checked the normality and homoscedasticity of variables to decide whether to use parametric or non-parametric tests. Normality and homocedasticity were evaluated by the means the modified Shapiro–Wilk's test (mentioned above) and Levene's test (Levene 1960) respectively.

We checked whether there were significant differences among groups from UPGMA analysis by using tests of ANOVA, either parametric or non-parametric (Kruskall–Wallis test), depending on the properties (normal distribution and homoscedasticity) of each variable. Comparisons among groups were performed on variables in which statistically significant differences were found using parametric (Tukey) or non-parametric (Mann and Whitney 1947) *post hoc* pairwise tests (Appendix 1, Table A3). All these univariate analyses were performed with INFOSTAT (Di Rienzo *et al.* 2009) and PAST software.

Table 2.   Variables used in the morphological analysis of *Mimosa* subseries *Dolentes* and *Brevipedes*

Type of variable	Variables
Quantitative, continuous	Petiole length (mm); rachis length (mm); petiole : rachis ratio; leaflet length (mm); leaflet width (mm); leaflet length : width ratio; peduncle length (mm); head diameter (mm); legume cluster diameter (mm).
Quantitative, discrete	Leaflet pairs per pinna; visible primary nerves per leaflet; heads per inflorescence axis; nodes per inflorescence axis.
Qualitative, ordinal	Number of pinna pairs (one-jugate, two-jugate); peduncles per node (solitary, paired).
Qualitative, nominal	Leaflet pubescence (strigose, glabrous, intermediate, glandular); leaflet secondary venation (brochidodrome, not visible, anastomosed with pallid band, intermediate between them); leaflet margin (glandular, setose uniseriate, setose pluriseriate, intermediate); indumentum (glabrous, strigose-hispid, glandular); calyx type (campanulate to irregularly palaceous, pappiform); inflorescence type (determinate, indeterminate, both types).
Qualitative, binomial	Glandular trichomes (presence or absence); stem armament (armed or unarmed); marginal pallid band on leaflet (presence or absence); corolla pubescence (glabrous or puberulent).

To detect differences between ploidy levels and morphological characters, after examining normality and homoscedasticity, we conducted a non-parametric Mann–Whitney (U) test for each quantitative character examined. In the case of qualitative characters, a contingency table was performed and absolute and relative frequencies were calculated. There were calculated Chi-Square, V-Crámer and contigency coefficient statistic indexes. The Chi-Square statistic allows inference of whether variables are dependent or independent. We performed these analyses in PAST; in this program, the significance of association between two variables is given, with P-values from the Chi-Square distribution and a permutation test with 9999 replicates (Hammer *et al.* 2001); however, Chi-Square does not provide information about magnitude of the association. For this reason, we included two tests, V-Crámer and contingency coefficient, which allowed evaluation of the degree of the association, with values between 0 (no association) and 1 (perfect association).

Results

Plant identification

The characterisation of voucher specimens allowed identification of the following taxa: *M. sceptrum*, *M. dolens* subsp. *dolens* var. *pangloea*, *M. dolens* subsp. *callosa*, *M. dolens* subsp. *rigida* varieties *rigescens*, *anisitsii* and *foliolosa* and *M. dolens* subsp. *acerba* varieties *rudis*, *acerba* and *latifolia*. There were some intermediate specimens between the following taxa: (1) *M. dolens* subsp. *rigida* var. *foliolosa* and *M. dolens* subsp. *acerba* var. *acerba*; and (2) *M. sceptrum* and *M. dolens* subsp. *acerba* var. *rudis*. Some specimens were identified as *M.* aff. *custodis*, because they were morphologically similar to *M. custodis*, but they differed by presence of a pallid band on glabrous (or almost) leaflets (Table 1).

Chromosome numbers

The following chromosome numbers were found: $2n = 4x = 52$, for *M. sceptrum*, *M.* aff. *custodis*, *M. dolens* subsp. *acerba* var. *rudis* and *M. dolens* subsp. *dolens* var. *pangloea*; $2n = 8x = 104$, for *M. dolens* subsp. *callosa*, *M. dolens* subsp. *rigida* var. *foliolosa*, *M. dolens* subsp. *rigida* var. *anisitsii*, *M. dolens* subsp. *rigida* var. *rigescens*, *M. dolens* subsp. *acerba* var. aff. *rudis*, *M. dolens* subsp. *acerba* var. *acerba*, *M. dolens* subsp. *acerba* var. *latifolia* and the intermediate

specimens between *M. dolens* var. *acerba* and *M. dolens* subsp. *rigida* (Table 1, Fig. 1).

In several cases, we found taxa with the same ploidy level growing in sympatry, sometimes with individuals exhibiting intermediate morphology (Table 3, Fig. 2). Tetraploids occur in the areas of Cerrado ecoregion from Paraguay and adjacent Brazil (generally below 900 m ASL), as well as in highlands with grasslands or savannas from southern Brazil (campos or campos de altitude, at 900–1200 m ASL). Octoploids are distributed throughout the studied area, i.e. southern Brazil, Paraguay and north-eastern Argentina, mainly below 900 m ASL (Table 1, Fig. 2).

Karyotype parameters

Karyotypes observed were symmetric with small chromosomes, generally shorter than than 3 µm (Fig. 3). CLHG ranged from 11.21 µm in the intermediate individuals between *M. dolens* subsp. *rigida* and *acerba* to 25.14 µm in *M. dolens* var. *pangloea*. CLHG was normally distributed according to the results of Shapiro–Wilk's test ($W = 0.92$; $P = 0.1311$) and variances were homogeneous (Levene's test: $P = 0.05$). ANOVA showed that there were statistically significant differences among taxa (Table 4). CAHG ranged from 6.56 µm in some accessions of *M. dolens* subsp. *acerba* var. *latifolia* to 27.94 µm in *M. dolens* var. *pangloea*. CAHG was not normally distributed according to the results of Shapiro–Wilk's test ($W = 0.84$; $P < 0.05$). Variances were not homogeneous (Levene's test: $P < 0.05$). Non-parametric ANOVA did not detect statistically significant differences among accessions ($P = 0.05$). The A_2 index ranged from 0.16 in some accessions of *M. dolens* subsp. *acerba* var. *latifolia* to 0.30 in accessions of *M. dolens* var. *pangloea* (Table 4).

Morphological analysis

Although the majority of specimens could be identified satisfactorily by using the Barneby's (1991) classification, some showed morphological variation differing from the original description or from the morphology of nomenclatural types. Specimens from highland grasslands of southern Brazil (*Fortunato 9555a,e,d,e*; *Morales 1624*, *1625*, *1626*, *1630*) exhibited long and indeterminate inflorescences and a leaf formula similar to that of *M. custodis*, but they differed from its type specimen by the presence of a pallid band in the leaflet margin and few and sparse glandular trichomes. The specimens
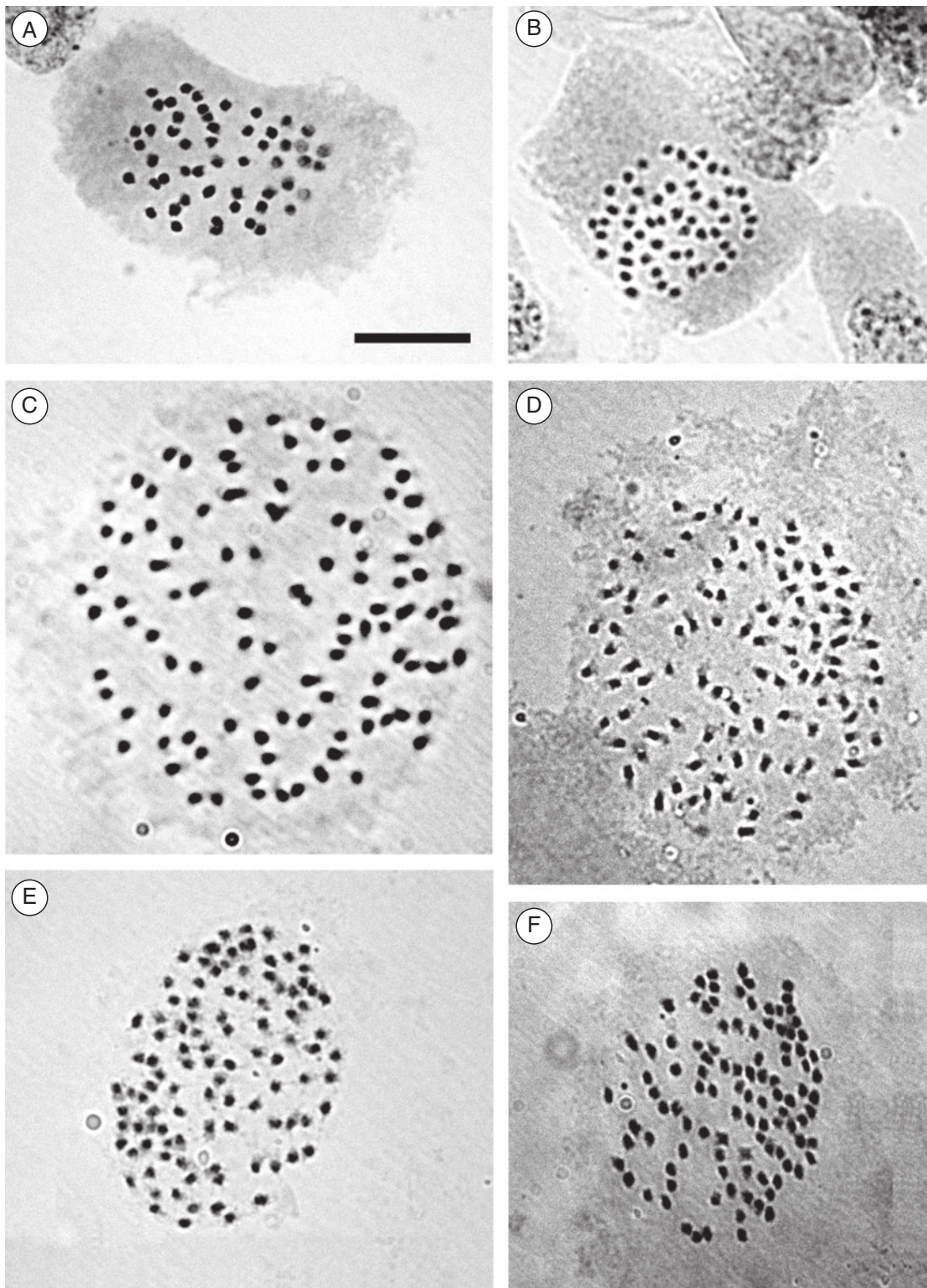
Fig. 1. Mitotic metaphases of *Mimosa* subseries *Dolentes* and *Brevipedes*. A. *M. dolens* subsp. *acerba* var. *rudis*, $4x = 52$. B. *M. sceptrum*, $4x = 52$. C. *M. dolens* subsp. *rigida* var. *anisitsii*, $8x = 104$. D. *M. dolens* subsp. *acerba* var. *latifolia*, $8x = 104$. E. *M. dolens* subsp. *rigida* var. *foliolosa*, $8x = 104$. F. *M. dolens* subsp. *callosa*, $8x = 104$. Scale bar: 10 μm.

Table 3.   Areas of sympatry between taxa in the *Mimosa* subseries *Dolentes–Brevipedes* complex and description of the taxa and intermediate forms

| Population	Taxon	Ploidy level	Main distinctive morphological characters between taxa and intermediate forms
Pedro Caballero, Paraguay	*M. dolens* subsp. *rigida* var. *rigescens*	8*x*	Leaf formula i/12–21; leaflets 16 × 6 mm; calyx paleaceous 0.5-mm length.
	M. dolens subsp. *callosa*		Leaf formula i/18–32; leaflet size 10 × 3.5 mm; calyx campanulate <0.5-mm length.
	M. dolens subsp. *rigida* var. *anisitsii*		Leaf formula i/7–10; leaflet size 29 × 14 mm; calyx campanulate <0.5-mm length.
Fortuna Guazú, Paraguay	*M. dolens* subsp. *acerba* var. *rudis*	4*x*	Only basally foliate stems; leaf formula 3–7; leaflets 72 × 36 mm, 6-nerved.
	M. sceptrum		Normally foliate stems; petiole 10–20 mm length; leaf formula i/7–20, leaflets 2–3-nerved;
	Intermediate specimens between *M. sceptrum* and *M. dolens* subsp. *acerba* var. *rudis*		Only basally foliate stems; petiole 80–83 mm long; leaf formula i/6–7; leaflets 38 × 14 mm, 4–5-nerved,
Recanto dos Papagaios, Brazil	*M. dolens* subsp. *acerba* var. aff. *rudis*	8*x*	Only basally foliate stems; petiole 40–102 mm long; leaf formula i/9–16; leaflets 22–26 × 7–8 mm, 3-nerved.
	Intermediate between *M. dolens* subsp. *acerba* var. *acerba* and *M. dolens* subsp. *rigida* var. *foliolosa*.		Leaflets glabrous, with a pallid marginal band, secondary venation anastomosed with the margin and continuous, pluriseriate margin.
São Marcos, Brazil	*M. dolens* subsp. *rigida* var. *foliolosa*	8*x*	Leaflets glabrous, 3-nerved, with visible brochidodrome secondary venation.
	M. dolens subsp. *acerba* var. *latifolia*		Leaflet strigose to hirsute, 1–2-nerved, with not visible secondary venation.
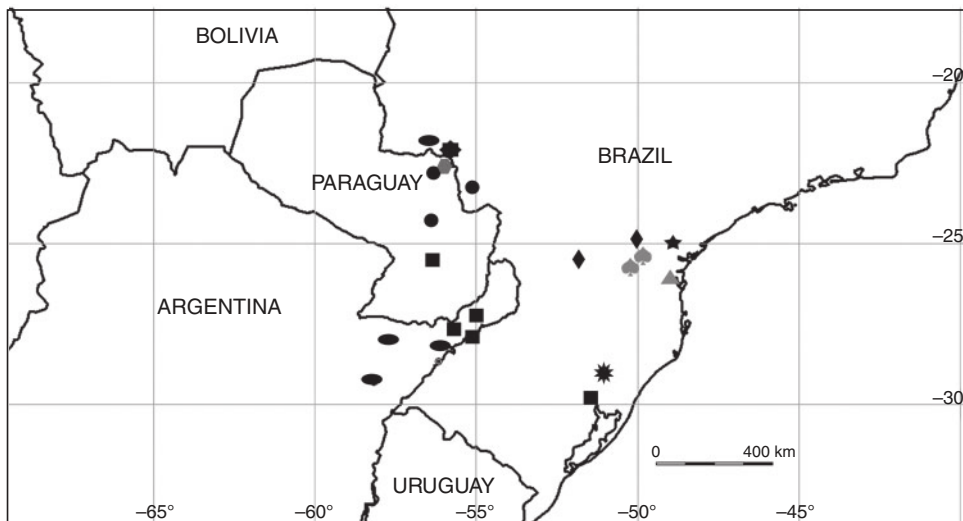
Fig. 2.   Collecting sites of voucher specimens. *Mimosa dolens* subsp. *rigida* var. *foliolosa* (ellipses). *M. dolens* subsp. *rigida* var. *anisitsii* (circle). *M. dolens* var. *pangloea* (suit). *M.* aff. *custodis* (triangle). *M. dolens* subsp. *acerba* var. *latifolia* (square). *M. dolens* var. *acerba* (rhombus). Sympatry areas with *M. dolens* subsp. *acerba* var. *rudis*, *M. sceptrum* and intermediate between them (hexagon). Area of sympatry between *M. dolens* subsp. *acerba* vars. *latifolia* and *rudis*, and intermediate between *M. dolens* var. *acerba* and *M. dolens* subsp. *rigida* var. *foliolosa* (five-point star). Area of sympatry between *M. dolens* subsp. *rigida* vars. *rigescens* and *anisitsii* and *M. dolens* subsp. *callosa* (eight-point star). Area of sympatry between *M. dolens* subsp. *acerba* var. *latifolia* and *M. dolens* subsp. *rigida* var. *foliolosa* (ten-point star). Grey represents the tetraploid accessions and black represents the octoploid accessions.

Morales 1593 and *1594* were very close to *M. dolens* subsp. *acerba* var. *rudis*, and differed from it only by their petiole length, which was similar to *M. dolens* subsp. *acerba* var. *latifolia* (Tables 3, 5). We also found intermediate specimens between (1) *M. dolens* subsp. *acerba* var. *rudis* and *M. sceptrum*, distinguished from both taxa by the intermediate leaf formula
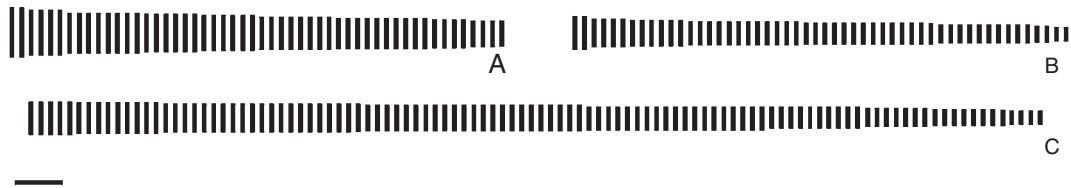
Fig. 3. Idiograms of some taxa of the subseries *Dolentes* and *Brevipedes*. Entire chromosome set. A. *M. dolens* subsp. *dolens* var. *pangloea*, $4x$ = 52. B. *M. sceptrum*, $4x$ = 52. C. *M dolens* subsp. *callosa*, $8x$ = 104. Scale bar: 1 μm.

Table 4. Karyotype parameters in species of subseries *Dolentes* and *Brevipedes*: chromosome length per haploid genome (CLHG), chromosome area per haploid genome (CAHG) and asymmetry index (A$_2$)

CLGH was analysed by means parametric ANOVA and *post hoc* pairwise comparison Tukey's test; CAHG was analysed by means non-parametric ANOVA (Kruskal–Wallis test) and pairwise comparison Mann–Whitney test. Values within a column followed by the same lower-case, superscript letter are not statistically significantly different (P = 0.05)

Taxon	n	Accession	Ploidy level	CLHG (mean ± standard deviation; μm)	CAHG (mean ± standard deviation; μm^2)	A$_2$
M. dolens var. *acerba*–*M. dolens* subsp. *rigida* intermediates	5	RHF9517	$8x$	11.21 ± 1.39[a]	8.55 ± 3.92[a]	0.17
M. dolens subsp. *acerba* var. *latifólia*	5	RHF9029	$8x$	11.90 ± 3.34[a]	6.56 ± 2.37[a]	0.16
M. sceptrum	5	RHF9282	$4x$	12.35 ± 0.18[a]	8.69 ± 1.32[a]	0.17
M. dolens subsp. *rígida* var. *anisitsii*	5	RHF9209	$8x$	15.49 ± 3.97[a,b]	13.98 ± 8.05[a]	0.16
M. dolens subsp. *rigida* var. *foliolosa*	5	RHF9077	$8x$	15.64 ± 3.93[a,b]	12.79 ± 5.85[a]	0.19
M. dolens subsp. *acerba* var. *acerba*	5	OR8212	$8x$	13.42 ± 0.09[a,b]	8.66 ± 1.00[a]	0.24
M. dolens subsp. *acerba* var. *rudis*	5	RHF9279	$4x$	13.94 ± 0.90[a,b]	9.88 ± 1.07[a]	0.18
M. dolens subsp. *rigida* var. *rigescens*	5	RHF9347	$8x$	14.93 ± 1.14[a,b]	8.43 ± 0.27[a]	0.17
M. dolens subsp. *acerba* var. *latifolia*	5	RHF8548	$8x$	17.53 ± 3.59[a,b]	16.12 ± 7.15[a]	0.29
M. dolens subsp. *acerba* var. *latifolia*	5	RHF9006	$8x$	17.89 ± 3.00[a,b]	14.44 ± 4.26[a]	0.24
M. custodis	5	RHF9555	$4x$	21.76 ± 3.17[a,b]	13.28 ± 1.49[a]	0.28
M. dolens var. *pangloea*	5	MM1657	$4x$	21.13 ± 2.44[a,b]	17.73 ± 3.68[a]	0.30
M. dolens var. *pangloea*	5	RHF9597	$4x$	25.14 ± 1.80[b]	27.94 ± 2.98[a]	0.20

and intermediate leaflet size, and (2) *M. dolens* var. *acerba* and *M. dolens* subsp. *rigida* var. *foliolosa*, distinguished from both taxa by the intermediate pubescence (Tables 3, 5). Many specimens of *M. dolens* exhibited indeterminate inflorescences but they can be assigned to subseries *Dolentes* because of their general morphology (Table 5, Fig. 4).

Five groups were distinguished at 0.75 similarity (Fig. 4) and their morphology is compared in Tables 5 and 6. Selected univariate statistics are given in Tables 6 and A3).

Non parametric tests were performed based on the properties of quantitative variables, such as normality and homocedasticity revealed by Shapiro–Wilk and Levene's tests (Tables A1, A2). The main differences among groups were mostly in qualitative characters, but also in some quantitative characters, which allowed taxa or groups with statistically significant differences (Tables 6, A3) to be distinguished. The only quantitative character that did not differ significantly among taxa was the number of nodes per inflorescence (Table 6).

The Mann–Whitney pairwise comparison test showed significant differences among several groups. Pod-cluster diameter showed the most significant differences between Groups 1 and 2 and the remainder. Group 2 exhibited significant differences in peduncle length with all groups. Group 3 could be distinguished from the remainders especially by its number of leaflets per pinna, number of visible primary veins, and petiole : rachis ratio. Group 4 exhibited the largest differences, especially with Groups 1 and 5, in capitula per

inflorescence. Both Group 3 and Group 4 showed most significant differences in visible primary veins per leaflet. Group 5 exhibited the largest differences from remainder of taxa in petiole length, pinnae rachis length, size of leaflets (width and length; Tables 6, A3).

The five groups observed in the UPGMA cluster analysis (Fig. 4, Table 5) are characterised as follows.

Group 1

Mimosa dolens var. *pangloea*: presence of armed stems, indumentum with densely glandular trichomes, leaflets with glandular margin and glabrous surfaces and paired inflorescences, with pod clusters generally very small (mean cluster diameter: 13.47–13.87 mm). This group of individuals was tetraploid ($2n = 4x = 52$).

Group 2

Mimosa aff. *custodis*, *M. dolens* var. *acerba* and intermediate forms with *M. dolens* subsp. *rigida*: unarmed stems, eglandular indumentum and leaflets with variable pubescence and venation, but constantly with presence of a pallid marginal band on the leaflets. This group exhibited very short peduncles (mean length: 8.13 mm) and small pod clusters (arithmetic mean of cluster diameters: 13.19–14.16 mm). This set of individuals were tetraploid ($2n = 4x = 52$) and octoploid ($2n = 8x = 104$).

Table 5. Comparison of selected qualitative morphological characters in *Mimosa* subseries *Dolentes–Brevipedes*

Taxon (group from cluster analysis)	Voucher	Habit	Stems Armament and pubescence	Pubescence	Leaflet Venation	Margin	Calyx	Inflorescence
M. dolens subsp. *dolens* var. *pangloea* (1)	MM1654, 1655, 1656, RHF9580	Erect and virgate subshrubs	Hispid and glandular, armed with infranodal aculei	Glabrous to glandular	Secondary venation brochidodrome or poorly visible	Continuous, uniseriate, generally glandular	Campanulate	Indeterminate
M. aff. custodis (2)	RHF9555a,c,d,e	Erect and virgate subshrubs	Hispid or strigose, unarmed	Scarcely hirsute on both faces, with a pallid and coriaceous marginal band	Secondary venation poorly visible	Continuous uniseriate to pluriseriate	Campanulate	Indeterminate
M. dolens subsp. *acerba* var. *acerba* (2)	RHF9551a,b, OSR8212	Erect and virgate subshrubs	Hispid or strigose, unarmed	Hirsute on both faces, with a pallid and coriaceous marginal band	Secondary venation poorly visible	Continuous, pluriseriate	Campanulate	Determinate
Intermediate specimen between *M. dolens* var. *acerba* and *M. dolens* subsp. *rigida* (2)	RHF9517	Erect and virgate subshrubs	Hispid or strigose to glabrous, unarmed	Glabrous or almost on both faces, with a pallid and coriaceous marginal band	Secondary venation anastomosed with the pallid band	Continuous uniseriate	Campanulate	Determinate
M. dolens subsp. *acerba* var. *latifolia* (3)	RHF9027,9029, 8548, 9052	Erect and virgate subshrubs with leaves throughout the stems	Hispid or strigose, unarmed	Hirsute or strigose on both faces	Poorly visible	Continuous, pluriseriate	Campanulate, sometimes pappiform	Determinate
M. sceptrum (3)	RHF9282	Erect and virgate subshrubs with leaves throughout the stems	Hispid or strigose, unarmed	Hirsute on both faces	Poorly visible	Continuous, pluriseriate	Campanulate	Indeterminate
M. dolens subsp. *rigida* var. *anisitsii* (4)	RHF9217,9253, 9350; MM728	Erect and virgate subshrubs, generally more than 1 m tall	Loosely hispid to glabrous, unarmed	Glabrous, sometimes hirsute in three first pairs	Secondary venation visibly brochidodrome	Continuous, uniseriate	Campanulate	Determinate to indeterminate.
M. dolens subsp. *rigida* var. *rigescens* (4)	RH9347a	Erect and virgate subshrubs	Loosely hispid to glabrous, unarmed	Glabrous, sometimes hirsute in the three first pairs	Secondary venation visibly brochidodrome	Continuous, uniseriate	Pappiform	Determinate
M. dolens subsp. *rigida* var. *foliolosa* (4)	MM819, 1153, 1062 g, RHF9077	Erect and virgate subshrubs	Loosely hispid to glabrous, unarmed	Glabrous	Secondary venation visibly brochidodrome	Continuous, uniseriate	Campanulate	Mainly determinate.
M. dolens subsp. *callosa* (4)	RHF9347c	Erect and virgate subshrubs	Loosely hispid to glabrous, unarmed	Glabrous, sometimes hirsute in three first pairs	Secondary venation visibly brochidodrome	Continuous, uniseriate	Campanulate	Determinate
M. dolens subsp. *acerba* var. *rudis* (5)	RHF9279a	Erect, virgate subshrubs with only basal leaves	Hispid or strigose, unarmed	Hirsute on both faces	Poorly visible	Continuous, pluriseriate	Campanulate	Indeterminate
Intermediate between *M. dolens* subsp. *acerba* var. *rudis* and *M. dolens* subsp. *sceptrum* (5)	RHF9279b	Erect and virgate subshrubs with leaves throughout the stems	Hispid or strigose, unarmed	Hirsute on both faces	Poorly visible	Continuous, pluriseriate	Campanulate	Indeterminate
M. dolens subsp. *acerba* var. *aff. rudis* (5)	MM1593, 1594; RHF 9514	Erect and virgate subshrubs with leaves throughout the stems	Hispid or strigose, unarmed, sometimes with setaculei	Hirsute or strigose on both faces	Poorly visible	Continuous, pluriseriate	Campanulate	Determinate

Group 3

Mimosa dolens subsp. *acerba* var. *latifolia* and *M. sceptrum*: presence of regularly foliated stems, leaflets with hirsute or strigose pubescence and secondary venation not visible and absence of a pallid marginal band. This group exhibited the largest number of leaflets per pinna (9–24), relatively short petioles in relation to the pinna rachis (mean petiole : rachis of ratio 6 : 1) as well as few visible primary veins (1–3) per leaflet. Individuals studied were tetraploid ($2n = 4x = 52$) and octoploid ($2n = 8x = 104$).

Group 4

Mimosa dolens subsp. *rigida* and subsp. *callosa*: glabrous leaflets with visibly brochidodrome venation and continuous, uniseriate margin. In addition, Group 4 exhibited the largest number of visible primary veins per leaflet (2–5) and the smallest number of nodes and capitula per inflorescence (mainly 1–7, in one specimen up to 25). This group was octoploid ($2n = 8x = 104$).

Group 5

Mimosa dolens subsp. *acerba* var. *rudis* and close specimens: presence of only basally foliated stems, leaflets with hirsute or strigose pubescence and pluriseriate margin and no visible secondary venation. In addition, this group can be distinguished by having the largest leaves, with petioles and pinnae rachis very long (mean petiole length: 44.94 mm and rachis length: 86.23 mm) and large leaflets (mean length: 22.76 mm, and

width: 7.59 mm). Individuals were tetraploid ($2n = 2x = 52$) or octoploid ($2n = 8x = 104$).

The *U*-test was chosed based on Levene's test (Table A4), detecting statistically significant differences between tetraploid and octoploid accessions in the number of leaflet pairs per pinna, number of capitula and nodes per inflorescence, peduncle length and legume clusters diameter, whereas the remaining characters did not show statistically significant differences (Table 7, Fig. 4). The analysis of qualitative characters showed that indumentum, pubescence of leaflets, armament of stems, inflorescence type and presence of glandular trichomes were associated differently from ploidy levels, whereas the remaining qualitative characters did not show an association (Tables 5, 8).

Discussion

Chromosome numbers and karyotype parameters

The present results in the *Mimosa* subseries *Dolentes–Brevipedes* complex supported that $x = 13$ is the basic chromosome number of the genus, as was previously postulated (Isely 1971), and supported in more recent works (Goldblatt 1981; Seijo 1993, 1999, 2000; Seijo and Fernández 2001; Morales *et al.* 2010, 2011, 2012, 2014*a*, 2014*b*; Dahmer *et al.* 2011). The accessions studied here were all polyploid, namely, tetraploid and octoploid. Polysomaty appears in the species of *Mimosa* subseries *Dolentes* and *Brevipedes*, as has also been reported frequently for other members of *Mimosa* (Seijo 1993, 1999; Seijo and Fernández 2001; Morales *et al.* 2010, 2014*a*; Dahmer *et al.* 2011; Olkoski and Schifino-Wittmann 2011).
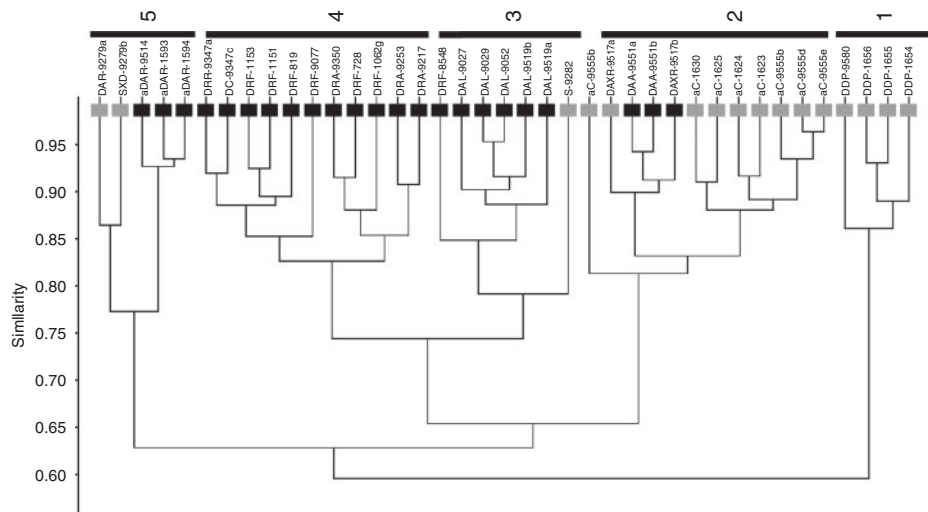
Fig. 4. Hierarchical cluster analysis in *Mimosa* subseries *Dolentes* and *Brevipedes*, unweighted pair-group method using arithmetic averages (UPGMA) based on mixed similarity measures. Alphanumerical code indicates the taxon and (contained within parentheses) collection initials and collection number: DAR, *Mimosa dolens* subsp. *acerba* var. *rudis*; aDAR, *Mimosa dolens* subsp. *acerba* var. aff. *rudis*; SxD, Intermediate between *M. sceptrum* and *M. dolens* subsp. *acerba* var. *rudis*; DRR, *Mimosa dolens* subsp. *rigida* var. *rigescens*; DRF, *M. dolens* subsp. *rigida* var. *foliolosa*; DRA, *M. dolens* subsp. *rigida* var. *anisitsii*; DAL, *Mimosa dolens* subsp. *acerba* var. *latifolia*; S, *M. sceptrum*; aC, *M.* aff. *custodis*; DAA, *Mimosa dolens* subsp. *acerba* var. *acerba*; DAXR, intermediate between *M. dolens* var. *acerba* and *M. dolens* subsp. *rigida*; DDP, *M. dolens* subsp. *dolens* var. *pangloea*. Grey squares indicate tetraploid accessions; dark squares indicate octoploid accessions. Bars and numbers represent clusters.

Table 6.　Comparison of selected quantitative morphological characters in *Mimosa* subseries *Dolentes–Brevipedes* complex
Groups from unweighted pair-group method with arithmetic mean (UPGMA) analysis and Kruskall–Wallis test values

Group	Taxon	n	Continuous characters (mean ± standard deviation)										Discrete characters (median)		
			Petiole (mm)	Rachis (mm)	Petiole: rachis ratio	Leaflet length (mm)	Leaflet width (mm)	Leaflet length : width ratio	Peduncle length (mm)	Pod clusters major diameter (mm)	Pod clusters minor diameter (mm)	Leaflet pairs per pinna	Visible primary nerves per leaflet	Nodes per inflorescence	Capitula per inflorescence
H-statistic			22.21	27.60	13.93	27.4	26.83	9.081	15.24	25.33	26.65	18.99	20.08	9.309	20.08
P-value			0.0001807	0.000015	0.007506	0.00001646	0.00002147	0.05906	0.000423	0.00004285	0.00002321	0.0007445	0.0002897	0.05088	0.0003822
1	*M. dolens* subsp. *dolens* var. *pangloea*	4	19.91 ± 6.63	28.06 ± 11.91	1.41 ± 0.40	9.92 ± 1.66	3.63 ± 0.80	2.80 ± 0.19	13.29 ± 4.88	13.81 ± 0.80	13.47 ± 0.62	7 (7–13)	3 (3–4)	7 (5–13)	21 (5–25)
2	*M. dolens* var. *acerba* and intermediate forms with *M. dolens* subsp. *rigida*	11	11.35 ± 4.36	29.95 ± 3.86	3.24 ± 1.66	6.24 ± 1.34	2.45 ± 0.51	2.60 ± 0.40	8.13 ± 4.98	13.19 ± 3.65	14.16 ± 1.47	13 (11–19)	2 (1–3)	5 (1–12)	9 (3–32)
3	*M. dolens* subsp. *acerba* var. *latifolia*, *M. sceptrum*	7	13.83 ± 8.40	49.69 ± 19.89	6.16 ± 2.31	11.79 ± 6.57	4.61 ± 2.60	2.54 ± 0.38	14.49 ± 7.86	18.54 ± 6.53	18.97 ± 4.35	16 (9–24)	1 (1–3)	3 (3–12)	6 (3–43)
4	*M. dolens* subsp. *rigida* vars. *foliolosa*, *rigescens* and *anisitsii*; *M. dolens* subsp. *callosa*.	11	25.85 ± 14.62	54.68 ± 26.24	4.96 ± 3.40	12.77 ± 5.28	5.58 ± 2–75	2.432 ± 0.30	15.54 ± 6.87	21.80 ± 8.73	20.98 ± 8.51	12 (7–30)	3 (2–5)	3 (1–13)	6 (1–32)
5	*M. dolens* subsp. *acerba* var. *rudis*	5	44.94 ± 27–79	86.23 ± 41.72	0.97 ± 0.92	22.76 ± 13.68	7.59 ± 5.36	3.18 ± 0.35	15.72 ± 6.12	21.39 ± 4.45	20.55 ± 4.25	9 (6–13)	3 (2–4)	10 (2–15)	15 (13–18)

Table 7. Differences between ploidy levels in quantitative morphological characters in *Mimosa* subseries *Dolentes* and *Brevipedes*

Mann–Whitney test statistically significant differences are: *, $P < 0.05$

Character type	Character	4x	8x	U-value	P-value
Quantitative, continuous		Mean $\pm$ standard deviation			
	Petiole length (mm)	25.94 ± 25.41	18.93 ± 15.38	145.00	0.3191
	Rachis, length (mm)	42.66 ± 27.76	68.72 ± 32.61	84.00	0.0058*
	Leaflet length (mm)	15.27 ± 17.78	14.31 ± 7.67	142.00	0.2789
	Leaflet width (mm)	6.60 ± 8.82	5.91 ± 3.53	138.00	0.2385
	Leaflet, ratio length : width	2.53 ± 0.36	2.52 ± 0.43	168.00	0.7399
	Peduncle length (mm)	11.78 ± 4.1	20.14 ± 12.81	88.50	0.0087
	Legume cluster largest diameter (mm)	15.66 ± 2.65	23.99 ± 6.51	40.50	5.9800×10^{-5}*
	Legume cluster smallest diameter (mm)	14.60 ± 2.77	23.04 ± 6.18	26.50	9.9380×10^{-6}*
		Median (minimum–maximum range)			
Quantitative, discrete	Number of leaflet pairs per pinna	11 (5–16)	13 (8–30)	87.5	7.70×10^{-3}*
	Visible primary nerves per leaflet	2.5 (1–4)	3 (1–6)	170.5	0.7895
	Heads or fruit clusters per inflorescence axis	7 (13–2)	3 (1–15)	69.0	0.0124*
	Nodes per inflorescence axis	17 (4–43)	5 (1–25)	52.0	2.0929×10^{-4}*

Table 8. Association measures between qualitative morphological characters and cytotypes in *Mimosa* subseries *Dolentes* and *Brevipedes*

Type of character	Character (states)	Chi-Square (χ^2)	V-Crámer	Contingency coefficient
Qualitative, nominal	Habit (virgate efoliate stems; normally foliate stems)	0.8947	0.0255	0.0255
	Leaflet pubescence (strigose, glabrous, intermediate, glandular)	0.0012	0.7687	0.6094
	Leaflet margin	0.4102	0.1585	0.1565
	Leaflet secondary venation (brochidodrome, not visible, anastomosed with pallid band, intermediate between them)	0.1875	0.4114	0.3883
	Indumentum (glabrous, strigose–hispid, glandular)	0.0005	1.0000	0.7071
	Inflorescence type (determinate, indeterminate)	0.0002	0.8058	0.6275
Qualitative, binomial	Glandular trichomes (presence or absence)	0.0002	0.7416	0.5957
	Stem armament (armed or unarmed)	0.0020	0.5933	0.5102
	Marginal pallid band of leaflet (presence or absence)	0.4835	0.1348	0.1336

The following chromosome numbers are new reports: $2n = 4x = 52$, for *M. dolens* var. *pangloea*, *M. sceptrum*, *M.* aff. *custodis*, *M. dolens* subsp. *acerba* var. *rudis*; $2n = 8x = 104$, for *M. dolens* subsp. *callosa*, *M. dolens* subsp. *rigida* var. *foliolosa*, *M. dolens* subsp. *rigida* var. *rigescens*, *M. dolens* subsp. *acerba* var. aff. *rudis* and *M. dolens* subsp. *acerba* var. *latifolia*. The present results confirmed the chromosome number $2n = 8x = 104$ for *M. dolens* subsp. *acerba* and subsp. *rigida* (Seijo 1993).

The chromosomes studied were very small (CLGH generally <18 μm), in concordance with previous results in other taxa of *Mimosa* (Morales *et al*. 2010, 2011, 2014*a*, 2014*b*). There is a slight difference in chromosome size between tetraploid *M. dolens* subsp. *dolens* var. *pangloea*, which had the largest chromosomes, and the rest of the taxa examined (Table 4).

In this complex, tetraploids do not differ significantly from octoploids in chromosome size, with the exception of a few accessions. Our data suggested that some tetraploids from campos de altitude, i.e. highland grasslands from 900 to 1200 m ASL, seem to have larger chromosomes than do tetraploids from Cerrado and all octoploids analysed. Differences in chromosome size could be related to differences in genome size. Variations in chromosome size (and, eventually, in genome size) occur in distant clades or taxonomic groups of

Mimosa, and a decreasing chromosome size seems to be frequently associated with increasing ploidy levels (Morales 2011; Morales *et al*. 2011). Variations in chromosome size, as well as polyploidy (Dahmer *et al*. 2011; Morales *et al*. 2014*a*, 2014*b*), have arisen via independent events throughout the evolutionary history of the genus and in different events within infrageneric groups.

The A_2 index is very similar in all taxa studied, which indicates that the chromosome size in the same complement is uniform, consistent with all species of *Mimosa* that we studied previously (Morales *et al*. 2011, 2014*a*, 2014*b*). Karyotype appears to be relatively symmetric in all studied taxa, with little variation in size among chromosome pairs (Table 4).

Morphology and cytogenetics

The combination of a typical habit (erect subshrubs with efoliate pseudoracemes), mainly one–jugate pinnae, espiculate leaves, indeterminate inflorescence, and tetramerous, diplostemonous flowers, allows identification of the specimens as members of either subseries *Dolentes* or *Brevipedes* (Barneby 1991). Sometimes it is difficult to distinguish between both these subseries. Barneby (1991, pp. 510–515) suggested a differentiation of *Dolentes* and *Brevipedes* by their

inflorescences, which are determinate and indeterminate respectively, but we found some intermediate forms between them; critical individuals were observed previously either in herbarium specimens or in the field (Morales 2011).

We found some specimens with indeterminate inflorescences but resembling *Dolentes* in habit, leaf morphology, and in having dense clusters of pods; the analysis of these specimens and their comparison with the nomenclatural types of taxa prevented us from classifying them effectively using to the scheme of Barneby (1991, pp. 510–515).

The individuals with indeterminate inflorescences and glabrous leaflets with uniseriate margins should strictly be identified as members of subseries *Brevipedes*. However, those specimens cannot be included in that subseries because their morphology is not consistent with any of the described members of subseries *Brevipedes*, according to Barneby (1991, pp. 705–716). The overall morphology resembles *M. dolens* subsp. *rigida* and they should be considered as members of that taxon. The individuals with indeterminate inflorescences but strigose or hirsute leaflets with pluriseriate, setose margins and no visible secondary venation could be classified in different taxa, according to Barneby (1991, pp. 510–515, 582–583, 706–707), as *M. sceptrum*, *M.* aff. *custodis* or *M. dolens* subsp. *acerba* var. *rudis.* In addition, all the specimens classified as *M. dolens* var. *pangloea* differ from it only by having indeterminate inflorescences.

Unweighted pair-group method with arithmetic mean grouped the individuals in the following five main clusters: a cluster with *M. dolens* var. *pangloea*; a second cluster with members of subseries *Dolentes* and *Brevipedes* with a pallid band in the margin of leaflets; a third cluster with members of both subseries exhibiting hirsute or strigose leaflets; a fourth cluster comprising the members with glabrous leaflets; and a fifth cluster with members with large leaves and largely efoliate stems. This grouping pattern is not concordant with the infrageneric circumscription of Barneby (1991, pp. 510–515) because the members of subseries *Brevipedes* are not distinguished from similar members of subseries *Dolentes* by indeterminate inflorescence.

The groups detected by UPGMA analysis are partially concordant with the infraspecific treatment of Barneby (1991, pp. 582–583) of *M. dolens.* The separation of the subspecies *acerba* and ssp. *rigida* appears to be consistent, but *M. dolens* subsp. *callosa* seems to be grouped together with *M. dolens* subsp. *rigida*. Definition of *M. dolens* subsp. *callosa*, exclusively on the number of leaflet pairs (Barneby 1991, pp. 582–583), should be revised because their individuals do not differentiate into clusters.

There are some intermediate specimens among taxa that were collected in areas where species occur in sympatry. A transitional form, between *M. sceptrum* and *M. dolens* subsp. *acerba* var. *rudis*, was observed. Intermediate individuals were observed growing in biotic sympatry with individuals of both taxa, suggesting a possible hybrid zone. In the UPGMA analysis, it was observed that these intermediates clustered together with *M. dolens* subsp. *acerba* var. *rudis* because of the habit, but other characters are exclusive to *M. sceptrum.*

Intermediate individuals between *M. dolens* subsp. *acerba* var. *acerba* and *M. dolens* subsp. *rigida* were found in several areas of southern Brazil. These specimens had leaflets that are glabrous, but exhibit a pallid marginal band. UPGMA grouped them with *M. dolens* var. *acerba*, suggesting that the presence of the marginal band is a trait with taxonomic value. In addition to the pallid marginal band on leaflets, that set of specimens exhibits short peduncles. All of them (var. *acerba* and intermediate) are octoploid and clustered together, suggesting the circumscription of *M. dolens* var. *acerba* would be different from the circumscription of this taxon according to Barneby (1991, pp. 582–583); it should include individuals with glabrous leaflets exhibiting the marginal band (Fig. 4).

The groups defined by morphology are not directly correlated with the ploidy level, because the clusters can comprise tetraploid, octoploid or both cytotypes. We could infer associations between morphological characters and ploidy levels, especially in indumentum, leaf formula, inflorescence and fruit.

Among the accessions studied, an indumentum with profuse glandular trichomes appears exclusively in the tetraploid *M. dolens* var. *pangloea*, as was previously observed (Barneby 1991, pp. 582–583, 589–590; Morales 2011; Grohar *et al.* 2016), but glands can also be found in *M. dolens* var. *custodis* (also tetraploid). All the octoploids analysed here have an eglandular indumentum. We have hypothesised that evolution of trichomes in *Mimosa* involves unidirectional events of transformation, from eglandular to glandular trichomes (Santos-Silva *et al.* 2013). Thus, in the subseries *Dolentes* and *Brevipedes*, tetraploid taxa with glandular trichomes would be plesiomorphic in comparison to octoploid taxa with eglandular trichomes. We observed that the field populations of tetraploid glandular and octoploid eglandular *M. dolens* that are separated by a few kilometres exhibit gene flow between them and show similar genetic structure (similar genetic groups inferred by means of Bayesian analyses from amplified fragment length polymorphism (AFLP) data, according to Morales *et al.* 2016). We hypothesise that these tetraploids are ancestors of their closest octoploids.

There is also an association between ploidy levels and pubescence of leaflets; all tetraploids had strigose, hirsute or glandular, i.e. non-glabrous, leaflets. We also observed another association between ploidy levels and the pubescence of leaflets; all tetraploids had strigose, hirsute or glandular, i.e. non-glabrous, leaflets; in opposition, octoploids included taxa that mostly have only glabrous leaflets, such as *M. dolens* subsp. *rigida* (Barneby 1991, pp. 582–583).

The combination of indeterminate inflorescences with small clusters of pods on short peduncles appears mainly in tetraploids (Fig. 4, Table 5), whereas the combination of determinate inflorescences and pods grouped in dense and large clusters on relatively longer peduncles (which would correspond to subseries *Dolentes sensu stricto* according to Barneby 1991, pp. 578–580) appears exclusively in octoploids.

Although it is possible to observe a great variation in pair number, size and venation of leaflets in *M.* subseries *Dolentes* and *Brevipedes*, there are mostly no obvious correlations between leaflet morphology and ploidy level. Each of the cytotypes exhibits a broad range of leaflet size and leaf formula, with extremes from few- and large-leaved (*M. dolens* subsp. *rigida* var. *anisitsii*, *M. dolens* var. *rudis*) to many and small-leaved leaflets (*M.* aff. *custodis*, *M. dolens* subsp. *callosa*, *M. dolens*

subsp. *acerba* var. *latifolia*). Nevertheless, tetraploids were almost all few-leaved (Table 5).

A pappiform calyx was observed only in *M. dolens* subsp. *rigida* var. *rigescens*, as well as in some accessions of *M. dolens* subsp. *acerba* (all octoploids), but there are still insufficient data to make inferences about variation of this character with the ploidy level. Likewise, in some populations of *M. dolens* var. *latifolia*, it was possible to observe minute *setaculei* in the stems, resembling a transition to *M. dolens* subsp. *dolens*, but this character did not seem to be associated with changes in the chromosome number.

Cytogeography

The geographic pattern of the cytotypes shows that tetraploids occur mainly at low latitudes from 22 to 26°S, in Cerrado and campos of southern Brazil and Paraguay, whereas octoploids occur in a wider range of distribution, at higher latitudes, from 22 to 29°S, in campos and savannas from southern Brazil and north-eastern Argentina (Fig. 2). This pattern (ploidy levels increasing with latitude) appears to be similar to those seen in other taxa in the genus, including *M. debilis* Humb. and Bonpl. ex Willd. (Morales *et al.* 2010), section *Batocaulon* series *Stipellares* Benth. (Seijo 1993, 1999; Morales *et al.* 2011, 2014*a*), and, in a broader sense, the southernmost area of distribution of *Mimosa* (Seijo and Fernández 2001; Morales *et al.* 2014*a*).

The tetraploid entities, *M. sceptrum*, *M.* aff. *custodis* and *M. dolens* var. *rudis* (although the last also has some octoploids) are native to campos de altitude and Cerrado and they seem to be restricted to these environments. In contrast, almost all octoploid entities, including *M. dolens* subsp. *acerba* var. *latifolia*, *M. dolens* subsp. *acerba* var. *acerba*, *M. dolens* subsp. *callosa* and *M. dolens* subsp. *rigida*, show a larger distributional range than that of tetraploids. The areas where tetraploids and octoploids overlap (*campos de altitude* and *Cerrado* in Amambay slopes) are considered centres of diversification of the *Mimosa* subseries *Dolentes* and *Brevipedes*, which could infer from Barneby (1991, pp. 712–713) and our recent data (Morales 2011), because of the high number of taxa occurring there.

Octoploids appear to be expanding to the southernmost areas of distribution of these subseries, where they could be the predominant cytotype, according to our studies (Fig. 2). We hypothesise that polyploidy plays an important role in the expansion or colonisation and adaptation to new environments, at least in the southernmost diversified groups of *Mimosa*. Our conclusions are supported by a representative number of chromosome counts covering most of the distribution range of this complex, including the greatest centres of diversification at present known, namely, Paraná State (Brazil) and the slopes of Sierra del Amambay, in the Brazil–Paraguay border (Barneby 1991, p. 581; Morales 2011).

A more widespread distribution of polyploids, in comparison with diploids or individuals with lower ploidy levels, was observed in several families of vascular plants in the northern hemisphere (Stebbins 1984; Brochmann *et al.* 2004; Majure *et al.* 2012). The presence or predominance of polyploids at highest latitudes was frequently related to colonisation in inter-glacial

periods of Pliocene and Pleistocene by high-ploidy level individuals (Stebbins 1971). According to molecular data (Simon *et al.* 2011), *M. dolens* and allied species diverged at *c.* 5 million years ago. Consequently, climatic variations in the Quaternary could be a feasible explanation for the present distribution of the cytotypes in the subseries *Dolentes–Brevipedes* complex.

Classification of the polyploid complex

The presence of a set of individuals and populations that are morphologically similar, with intermediate forms among the taxa, high ploidy levels and apparent absence of diploids, suggests the presence of an extensive taxonomic and polyploid complex in the subseries *Dolentes–Brevipedes*, which could be classified as a declining or relictual complex, categories described by Stebbins (1971).

Stebbins (1971) mentioned that relictual polyploid complexes are defined by the absence of the diploid ancestors, which would be extinct, and declining complexes are polyploid complexes that have some related diploid that would not be the direct ancestor. The taxonomic groups including relictual complexes, such as *Sequoia sempervirens* Endl. (Stebbins 1971; Scott *et al.* 2016), *Lactoris fernandeziana* Phil. (Bernardello *et al.* 1999) and *Bischofia javanica* Blume (Hans 1973) are generally monotypic or ditypic genera whose possible ancestors are extinct.

In the case of the *Mimosa* subseries *Dolentes–Brevipedes* complex, the diploid ancestors are not known, but it is possible to find diploids in distant clades of the phylogenetic trees of Simon *et al.* (2011) in *Mimosa*. For this reason, this complex could be catalogued as a declining polyploid complex.

Diploids may exist as relicts at the northern extremes of the distribution (some areas of Cerrado and highland grasslands from São Paulo and Minas Gerais), for which chromosome data are sparse. Nevertheless, the *M.* subseries *Dolentes–Brevipedes* complex would not be a young polyploid complex, because the polyploids are largely distributed throughout the area of distribution of this complex. There are some polyploid complexes in the genus *Mimosa* that should be classified as a young complex or youthful–mature complex according to Stebbins (1971), such as the *M. debilis* complex, which has two cytotypes, diploid and tetraploid; in this group of taxa, both ploidy levels seem to be occupying different ecological niches but they are sympatric in some areas (Morales *et al.* 2010).

Another possible explanation for the lack of diploids would be ancestral polyploidisation, because current members of *M.* subseries *Dolentes* and *Brevipedes* could have originated from tetraploid ancestors within *M.* section *Mimosa*. Clade X, according to the phylogenetic reconstruction of Simon *et al.* (2011), is composed of several members of section *Mimosa*, including *M. dolens*, which are predominantly polyploid (Seijo 1993, 1999; Seijo and Fernández 2001; Morales *et al.* 2010, 2011, 2012, 2014*a*, 2014*b*; Dahmer *et al.* 2011). That clade represents mainly the southernmost representatives of the genus from subtropical and temperate regions of South America.

Tetraploids that are restricted to Cerrado and campos de altitude (*M. sceptrum*, *M. custodis*, *M. dolens* subsp. *acerba* var. *rudis*) from lower latitudes could be ancestors of

octoploids that are highly dispersed throughout campos from southern Brazil, north-eastern Argentina and Paraguay (low and higher latitudes). It is possible that chromosome duplication occurred in the southern areas of Cerrado ecoregion and highland grasslands, with formation of octoploids, which later expanded to the southernmost areas, colonising the campos and savannas of southern extreme of Brazil, Argentina, and Paraguay.

The morphological diversity in both cytotypes may be due to some degree of hybridisation among taxa in *Dolentes–Brevipedes*, given the presence of intermediate specimens among specific and infraspecific taxa. These intermediates occur only in sympatric areas (which are common in the subseries *Dolentes–Brevipedes* overall distributional area), suggesting a hybrid-zone pattern. In all sampling sites, we found only one cytotype, but it is expected that there would be areas of contact between cytotypes, given their vicinity in the southernmost Cerrado and campos from south-eastern Brazil. However, octoploids can be also almost morphologically identical to the tetraploids, which could be explained by the presence of polyploids with different origins.

Octoploids can originate by tetraploid × hexaploid or diploid × hexaploid crosses (Katsiotis and Forsberg 1995). However, we have observed neither hexaploids nor odd polyploids in the *Mimosa* subseries *Dolentes–Brevipedes* complex, suggesting that the origin of the octoploids that we detected could be from unreduced gametes of tetraploid individuals, another mechanism for octoploid genesis (Sanderson 2011). The multidisciplinary study of contact zones between the tetraploid and octoploid cytotypes is a very promising for understanding the evolutionary biology of the genus *Mimosa* in southern South America, because these cytotypes can generate hybrids that could be sterile (Sanderson 2011).

Conclusions

The subseries *Dolentes* and *Brevipedes* form a taxonomic and relictual, polyploid complex. The complex includes several taxa with high morphological variation and intermediate forms among them. This complex is formed by tetraploid taxa, including members of both subseries, and octoploid taxa, which includes exclusively members of subseries *Dolentes*. According to morphological studies, chromosome duplication could be associated with several morphological changes in the vegetative organs, as well as in the inflorescence and fruit; tetraploids show indeterminate inflorescences with small clusters of pods, and octoploids mostly have determinate inflorescences with larger clusters of legumes. Octoploid cytotypes are widely distributed across the distributional area of this complex, whereas tetraploid cyotypes appear to be restricted to certain environments and lower latitudes.

In *Mimosa* subseries *Dolentes* and *Brevipedes*, the presence of intermediate forms suggests the existence of polyploids of different origin. Because of the confusing circumscription of the taxonomic entities, it is necessary to generate a new taxonomic proposal revising the rank of the published infrageneric taxa, from subseries to varieties. This might require analyses of intra- and inter-populational variation.

The comparatively high diversification of octoploids in ecology and morphology suggests that polyploidy, and potentially hybridisation, appear to be relevant in the adaptation, dispersion and evolution of this taxonomic complex of *Mimosa* in their southernmost areas of distribution.

In spite of detailed taxonomic study, further sampling in *M.* subseries *Brevipedes* is needed to confirm the findings and inferences of the present study. The situation has been confounded by a proliferation of infrageneric taxa in *Mimosa*, with most species known only by their nomenclatural types. Future study should also involve sampling of the hypothesised hybrids and parents using next-generation sequencing and associated population-genetics analysis to test our hypotheses.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Appendix 1

Table A1. Shapiro–Wilk's test applied to quantitative variables analysed

Character	W*	p (unilateral D)
Petiole length	0.77	<0.001
Pinna rachis length	0.86	<0.001
Leaflet number of pairs per pinna	0.91	0.02
Leaflet length	0.73	<0.001
Leaflet width	0.67	<0.001
Leaflet ratio length : width	0.90	0.0058
Leaflet visible primary veins per leaflet	0.90	0.0067
Peduncle length	0.77	<0.001
Legume cluster largest diameter	0.88	<0.001
Legume cluster smallest diameter	0.89	0.002
Capitula or fruit clusters per inflorescence axis	0.63	<0.001
Nodes per inflorescence axis	0.84	<0.001

Table A2. Levene's test in *Mimosa* subseries *Dolentes–Brevipedes* complex comparing groups from cluster analysis

Character	Levene's test	Levene's test for medians
Petiole length (mm)	<0.010	<0.001
Rachis, length (mm)	0.130	0.100
Leaflet length (mm)	0.010	0.030
Leaflet width (mm)	<0.010	0.050
Leaflet, ratio length:width	0.05	0.441
Peduncle length (mm)	0.01	0.005
Legume cluster largest diameter (mm)	0.01	0.001
Legume cluster smallest diameter (mm)	0.002	0.008
Number of leaflet pairs per pinna	0.181	0.314
Visible primary nerves per leaflet	0.595	0.691
Heads or fruit clusters per inflorescence axis	0.001	0.01
Nodes per inflorescence axis	0.046	0.544

Table A3. **Non-parametric Mann–Whitney tests in quantitative characters in *Mimosa* subseries *Dolentes–Brevipedes* complex, comparing five groups detected by means unweighted pair-group method with arithmetic mean (UPGMA) cluster analysis**

Character	4	1	5	3	2
Applied to petiole length					
4		0.8446	0.0414*	0.0112*	0.0061*
1	0.8446		0.0365*	0.0294*	0.0062*
5	0.0414*	0.0366*		0.0058*	0.0019*
3	0.0112*	0.0294*	0.0058*		0.3742
2	0.0061*	0.0062*	0.0019*	0.3742	
Applied to rachis of pinnae length					
4		0.01083*	0.4967	0.0701	0.0552
1	0.0108*		0.0194*	0.0467	0.9516
5	0.4967	0.0194*		0.0058	0.0019*
3	0.0701	0.0467*	0.0058*		0.0035*
2	0.0552	0.9516	0.0019*	0.0035	
Applied to leaflet pairs per pinna					
4		0.0058*	0.1109	0.0564	0.1721
1	0.0058*		0.7086	0.0099*	0.0041*
5	0.1109	0.7086		0.0181*	0.0144*
3	0.0564	0.0099*	0.0181*		0.030*
2	0.1721	0.0040*	0.0144*	0.030*	
Applied to leaflet length					
4		0.2145	0.0414*	0.1128	0.0552
1	0.2145		0.0194*	0.6358	0.0043*
5	0.0414*	0.01945*		0.0094*	0.0019*
3	0.1128	0.6358	0.0094*		0.0099*
2	0.0552	0.00432*	0.0019*	0.010*	
Applied to leaflet width					
4		0.0581	0.3079	0.0462*	0.0717*
1	0.0581		0.0194*	0.9244	0.0043*
5	0.3079	0.0194*		0.0227*	0.0019*
3	0.0462*	0.9244	0.0227*		0.0099*
2	0.0717	0.0043*	0.0019*	0.0099*	
Applied to leaflet primary nerves					
4		0.0225*	0.0521	0.0008*	0.0006*
1	0.0225*		0.6056	0.1092	0.6357
5	0.0521	0.6056		0.0228*	0.2397
3	0.0008*	0.1092	0.0228*		0.0424*
2	0.000*	0.6357	0.2397	0.0424*	
Applied to peduncle length					
4		0.4723	0.6504	0.6507	0.0021*
1	0.4723		0.9021	0.7763	0.0179*
5	0.6504	0.9021		1.0000	0.0113*
3	0.6507	0.7763	1.0000		0.0077*
2	0.0021*	0.0179*	0.0113*	0.0077*	
Applied to pod cluster major diameter					
4		0.0074*	0.1923	0.0853	0.0001*
1	0.0074*		0.0194*	0.0467*	0.9032
5	0.1923	0.0194*		0.6261	0.0018*
3	0.0853	0.0467*	0.6261		0.0035*
2	0.0001*	0.9032	0.0018*	0.0035*	
Applied to minor pod cluster diameter					
4		0.00496*	0.2815	0.2048	0.08118
1	0.0050*		0.0194*	0.0179*	0.6261
5	0.2815	0.0194*		1.0000	0.0019*
3	0.2048	0.0179*	1.0000		0.0015*
2	0.0812	0.6261	0.0019*	0.0015*	
Applied to capitula per inflorescence					
4		0.0037*	0.0017*	0.0957	0.0083*
1	0.0037*		0.7972	0.0073*	0.1996
5	0.0016*	0.7972		0.0041*	0.1114
3	0.0957	0.0073*	0.0041*		0.1456
2	0.0083*	0.1996	0.1114	0.1456	

Table A4.   Levene's test in *Mimosa* subseries *Dolentes–Brevipedes* complex comparing ploidy levels

Character	Levene's test	Levene's test for medians
Petiole length (mm)	0.3160	0.8600
Rachis, length (mm)	0.1300	0.1000
Leaflet length (mm)	0.0838	0.4520
Leaflet width (mm)	0.0819	0.4595
Leaflet, ratio length:width	0.1576	0.1902
Peduncle length (mm)	0.0143	0.0448
Legume cluster largest diameter (mm)	0.0029	0.0036
Legume cluster smallest diameter (mm)	0.0122	0.0094
Number of leaflet pairs per pinna	0.1392	0.3229
Visible primary nerves per leaflet	<0.0010	0.0200
Head or fruit clusters per inflorescence axis	0.4924	0.3757
Nodes per inflorescence axis	0.0261	0.0395