

Leaflet trichome micromorphology in the *Dolentes-Brevipedes* taxonomic complex (*Mimosa* L., Mimosoideae)

Mariana GROHAR^{1*}, Sonia ROSENFELDT¹, Matías MORALES^{2,3,4}

¹Department of Biodiversity and Experimental Biology, Faculty of Applied and Natural Sciences, University of Buenos Aires, Buenos Aires, Argentina

²Institute of Biological Resources, National Institute of Agricultural Technology, Buenos Aires, Argentina

³National Council of Scientific and Technological Research (CONICET), Buenos Aires, Argentina

⁴Faculty of Agronomy and Agrifood Sciences, University of Morón, Buenos Aires, Argentina

Received: 24.12.2014 • Accepted/Published Online: 16.05.2015 • Final Version: 01.01.2016

Abstract: We studied leaflets of 18 taxa of the genus *Mimosa*, specifically the *Dolentes-Brevipedes* taxonomic complex, focusing on micromorphology, type, shape, and density of trichomes. Within the nonglandular trichomes we described 3 types of nonbranched and 2 types of branched trichomes. We also described 2 types of stalked glandular trichomes, extending the recent classifications of trichomes in *Mimosa*. Multiseriate trichomes with lateral projections, which resemble incipient branching, are described here for many taxa. Most studied taxa have pubescent leaflets and show great variability in trichome density values. We also found 3 different types of blade margins with variation in type, alignment, and orientation of trichomes. Results show that the micromorphology of blade trichomes allows distinguishing groups of taxa in this complex, which could be a new tool for performing taxonomic treatment of both subseries.

Key words: Nonglandular, glandular, branched, blade margin, Leguminosae, Mimosoideae, *Mimosa dolens*, leaflet micromorphology, taxonomic complex

1. Introduction

Taxonomic position of the genera in the Leguminosae family and the Mimosoideae subfamily is clear due to the uniformity of their diagnostic features. In *Mimosa*, however, a great number of infrageneric categories were proposed whose boundaries were not clear. These groups are summarized by high morphological variability, leading to the formation of complexes of taxa that consequently are not adequately circumscribed (Bentham, 1875, 1876; Barneby, 1991) and are in continuous revision (Grether, 2000; Morales and Fortunato, 2010; Santos-Silva et al., 2013a; Borges et al., 2014; Morales et al., 2014a).

The detection of specimens with intermediate characteristics (Morales and Fortunato, 2010; Luna-Castro et al., 2012; Morales et al., 2014a) represents a difficulty in the delimitation of infrageneric taxa, which could be related to the existence of polyploidy and interspecific hybridization (Seijo, 1993; Morales et al., 2007, 2010; Dahmer et al., 2011; Simon et al., 2011). The presence of highly related taxa has led to the definition of "taxonomic complexes", including groups of taxa that are still not clearly separated (Morales et al., 2010).

Mimosa subser. *Dolentes* Barneby and *M.* subser. *Brevipedes* Barneby comprise species native to the subtropical and warm temperate areas of South America (southern Brazil, Paraguay, northeastern Argentina, Uruguay, and eastern Bolivia). In general, they can be easily differentiated by their high morphological affinity in habit, inflorescence, and leaf morphology. Their representatives are caespitose erect shrubs with 1-jugate pinnae and efoliate exerted pseudoracemes (Barneby, 1991).

The subseries *Dolentes* is monotypic, comprising only *M. dolens* Vell., which is defined by the presence of determinate inflorescence and valvately dehiscent pods grouped in dense spheroid clusters. Five subspecies were recognized by Barneby (1991) in this taxon: *M. dolens* subsp. *callosa* (Benth.) Barneby, *M. dolens* subsp. *rigida* (Benth.) Barneby with 5 varieties (var. *rigida*, var. *rigescens* (Benth.) Barneby, var. *anisitsii* (Lindm.) Barneby, var. *foliolosa* (Benth.) Barneby, var. *deterior* Barneby), *M. dolens* subsp. *acerba* (Benth.) Barneby with 3 varieties (var. *acerba*, var. *latifolia* (Benth.) Barneby, var. *rudis* (Benth.) Barneby), *M. dolens* subsp. *eriophylla* (Benth.) Barneby, and *M. dolens* subsp. *dolens* with 2 varieties (var. *dolens* and var. *pangloea* Barneby).

* Correspondence: mariana.grohar@gmail.com

The subseries *Brevipedes* and *Dolentes* differ in type of inflorescence (indeterminate and determinate, respectively), and type of dehiscence of pods (craspedial and valvar, respectively). The subseries *Brevipedes* comprises 9 species: *M. brevipes* Benth., *M. diversipila* Micheli with 2 varieties (var. *diversipila* and var. *subglabriseta* Barneby & Fortunato emend. M.Morales), *M. fernandez-casasii* Barneby & Fortunato, *M. cryptogloea* Barneby, *M. custodis* Barneby, *M. sceptrum* Barneby, *M. longiracemosa* (Burkart) Barneby, *M. pseudopetiolaris* Barneby, and *M. nitidula* Barneby.

Although Barneby (1991) revised the subseries *Dolentes* and *Brevipedes* extensively, it was recently reported that the presence of specimens with intermediate characteristics between the taxa of both subseries (Luna-Castro et al., 2012; Morales et al., 2014a) leads them to be considered as one of the taxonomic complexes of the genus.

Different types of trichomes (glandular and nonglandular, simple and branched) have been described in leaflets of the subfamily Mimosoideae (Freire de Carvalho, 1970; Hickey, 1979; Leelavathi and Ramayya, 1982). In *Mimosa*, a remarkable variety of trichomes have also been distinguished in different organs, such as stems, leaflets, flowers, fruits, and seeds (Santos-Silva et al., 2013b). Santos-Silva et al. (2013b) suggested that glandular and branched trichomes derived from simple trichomes and probably evolved independently along the evolution of the genus by convergence. Trichome morphology was also used in the revision of many groups in the genus and the description of new taxa, especially in the section *Mimosa* (Morales et al., 2012, 2013; Morales and Fortunato, 2013).

The aim of this work is the classification and description of the micromorphology, shape, and density of leaflet trichomes in the *Dolentes-Brevipedes* complex, in order to differentiate between groups of taxa and clarify its taxonomy.

2. Materials and methods

2.1. Plant material

We used 42 herbarium specimens from 18 different taxa of the subseries *Dolentes* and *Brevipedes*, deposited in different institutions of Argentina (BAB, CTES, LIL, SI) and Brazil (MBM, SPF). They were all identified using the work of Barneby (1991) (Appendix). Four taxa of the complex were not included in this study due to lack of suitable material to perform the anatomic studies. These were *M. dolens* var. *rigida*, *M. longiracemosa*, *M. nitidula*, and *M. fernandez-casasii*.

We sampled 10 leaflets from the middle of the mature leaves of each specimen. We used the modified Dizeo (1973) procedure to clear the leaflets. The main modifications were: rehydration of leaves in water at 37 °C, treatment with 5% KOH until the leaves were white

(10–40 min, depending on the material) and with sodium hypochlorite until translucent (30 min to 36 h, depending on the material), and 10 min of treatment with 80% ethanol and 96% ethanol to remove mesophyll bubbles. The leaflets were stained with a solution of safranin and 80% ethanol and mounted in gelatin-glycerin.

Material was observed and drawn with a Wild M20 light microscope and an Arcano Bino Arcano ZTX ¼ stereoscopic microscope. Images and measurements were made with InfinityAnalyze. Reduction percentage of trichomes was calculated as the ratio between the base and the apex of the trichome.

We followed the terminology used by Barneby (1991) and Santos-Silva et al. (2013b) for the description and classification of trichomes. For the treatment of margin characteristics, we generally followed the classification of Barneby (1991), although we proposed some new categories.

Trichome density was evaluated using a camera lucida attached to the microscope and was then calculated as the number of trichomes/mm². Trichome density of each taxa was analyzed by means of basic statistics (median, minimum, and maximum values). Differences between taxa were examined with a Kruskal–Wallis test (Kruskal and Wallis, 1952). A multiple comparison post hoc test (Zar, 2010) was used to learn which groups differed significantly.

2.2. Multivariate analysis

Principal coordinate analysis (PCoA) was conducted using PAST software (Hammer et al., 2001). This technique is appropriate for the analysis of different types of variables (Henderson, 2006). PCoA was performed with a dataset of taxa having trichomes on the blades. For each taxon, 13 quantitative (continuous), ordinal, and qualitative (nominal and binomial) characters of trichomes were measured or observed (Table 1). We used mixed distances, Jaccard for the binary data, Gower for continuous and ordinal data, and Hamming/p-distance for nominal data.

2.3. Scanning electron microscopy

Two dehydrated leaflets per taxon were sampled in squares of 1 × 1 cm and sputter-coated with gold-palladium for 3 min. They were then examined with a Zeiss Supra 40 FESEM scanning electron microscope at the Center of Advanced Microscopy of the University of Buenos Aires and a Philips SEM 505 at the Museum of Natural Sciences Bernardino Rivadavia (Buenos Aires, Argentina).

2.4. Light microscopy

Ten mature leaflets of one specimen per taxon were included in paraffin. Sections were cut on a rotary microtome at a thickness of 8–10 µm. Sections were stained with safranin-fast green combination for 2 h and washed in ethanol 96% and distilled water for 10 min each (D'Ambrogio, 1986). Slides were mounted in PMyR synthetic resin, analyzed

Table 1. Characters used for principal coordinate analysis (PCoA) in studied taxa of *Mimosa* subser. *Dolentes* and *M.* subser. *Brevipedes*.

Type	Characters
Qualitative, nominal	Trichome type (e-glandular type a/b/glandular/subbarbellate/plumose); projection apex (rounded, intermediate, tapered); trichome base; secretory head (absent; clavate; globose)
Qualitative, binary	Projections (presence/absence); branched (presence/absence)
Quantitative, continuous	Trichome length; trichome density on adaxial face; trichome density on abaxial face
Ordinal	Leaflet margin (not differentiated; differentiated, one row/differentiated, irregular/differentiated, many rows); relative projection length (one-third, half, three-thirds, entire length of trichome)

with a Wild M20 microscope, and photographed with a Nikon Labophot AFX-II microscope at the University of Buenos Aires (Laboratory of Reproductive Biology and Experimental Systems on Vascular Plants, Department of Biodiversity and Experimental Biology).

3. Results

Seven different types of trichomes were observed on the blade and margin of the selected taxa. Trichomes are highly variable in their texture (from rigid to soft), size (50–1000 μm), location in the case of blade trichomes (present on one or both faces of the leaflets), and alignment in the case of margin trichomes.

3.1. Blade trichomes

3.1.1. Types of trichomes

There are different types of blade trichomes in the studied species of the complex: simple and branched nonglandular trichomes and glandular trichomes.

1. Nonglandular nonbranched (simple) trichomes. (Figure 1). This type of trichome is the most frequent among the studied taxa. They exhibit great variation in length (95–1226 μm) and location and appear on both sides of the leaflet. Within this group of trichomes, we detected 3 subtypes:

1A. Conical. Trichomes 285.7–1260.8 μm long, with a 17–69 μm multiseriate basal dilatation and a narrowing of more than 70% towards the apex (Table 2; Figures 1A–1C). Epidermic cells form an irregular podium surrounding the base of the trichome (Figure 1A). This type of trichome was found on both sides of the leaflets in *M. dolens* var. *acerba*, *M. sceptrum*, *M. dolens* var. *rudis*, *M. dolens* var. *latifolia*, *M. dolens* subsp. *callosa*, *M. dolens* subsp. *eriphylla*, *M. custodis*, and *M. pseudopetiolaris*.

In *M. dolens* subsp. *eriphylla*, this type of trichome is curved and forms a densely lanate indumentum, which clearly distinguishes this taxon from the others.

1B. Cylindrical. Thin, uniseriate trichomes, 92.9–312.5 μm long, which present the same width along the whole extension, with the exception of a minute narrowing ca. 40% towards the apex (Table 2), and frequently appears

curved. There is also a minute widening at the point of insertion of the trichome in the epidermis, which is also radially surrounded with epidermal cells (Figure 1D). This type of trichome was found in both faces of the leaflet in *M. cryptogloea* and *M. pseudopetiolaris*.

Regardless of trichome types, we can observe a positive correlation trend between trichome length and percentage of decrease, except for *M. dolens* var. *acerba* and *M. pseudopetiolaris*.

In *M. pseudopetiolaris*, *M. dolens* var. *latifolia*, and *M. brevipes* we observed a concurrence of this trichome type with simple conical trichomes.

2. Nonglandular branched trichomes. Multiseriate trichomes 100–300 μm long with 6–57 pluricellular lateral branches are found in both faces of the leaflet only in *M. diversipila* (Figure 2; Table 3). The distribution of the branches varies among varieties, covering the whole extension of the trichome or only a portion.

2A. Plumose. *M. diversipila* var. *diversipila* shows branches on its whole extension. On the blade, most trichomes are plumose, with a plain central axis 100–164.3 μm long. There are 21–57 branches with a length of 46.4–107.1 μm , which diverge radially from the central axis (Figures 2A and 2B).

2B. Basally barbellate. In *M. diversipila* var. *subglabriseta*, trichomes are branched only in the basal third, and are 189.3–300 μm long with 6–12 branches and a length of 21.3–53.6 μm (Figures 2C and 2D; Table 3). The central axis shows oblique furrows along the whole extension of the trichome. However, some individuals of this taxon show scarce plumose trichomes in an indumentum with most basally barbellate trichomes. This trichome type concurs with plumose trichomes in some individuals of this taxon (Figure 2C) and with simple conical trichomes in others (Figure 1D).

Particular cases. Intermediate stages of branching have also been observed. Those that appear to be simple trichomes, observed with light microscopy, show small lateral projections with great magnification (SEM). This feature was observed in all taxa with conical simple trichomes (Figures 3A–3H). As this characteristic is not

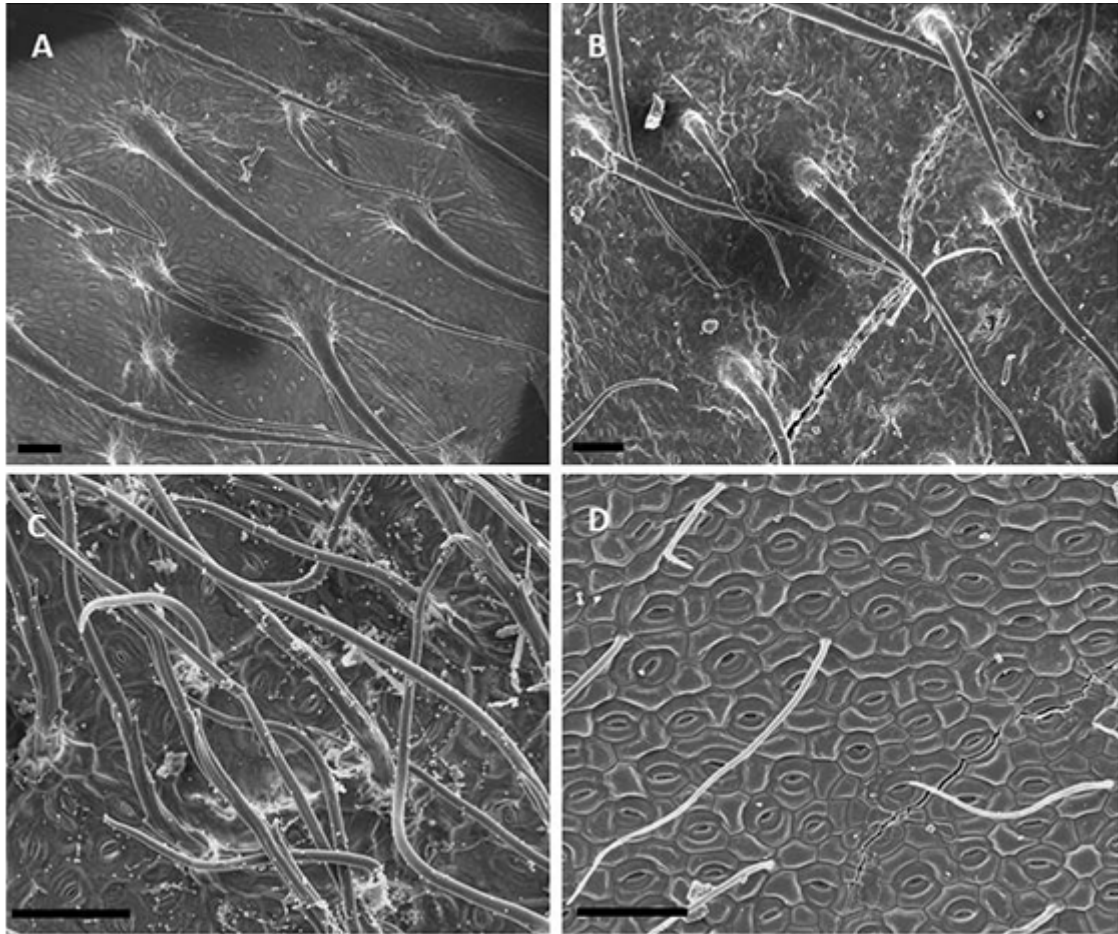


Figure 1. SEM micrographs of nonglandular and nonbranched trichomes of leaflets in *Mimosa* subseries *Dolentes* and subseries *Brevipedes*. **A-** *M. sceptrum*, abaxial face. **B-** *M. custodis*, abaxial face. **C-** *M. dolens* subsp. *eriophylla*, abaxial face. **D-** *M. cryptogloea*, adaxial face. Scale bar indicates 100 μm .

Table 2. Nonglandular, nonbranched trichome measurements: length, base, and apex width, and percentage of width decrease between base and apex in *Dolentes-Brevipedes* complex. Mean and standard deviation are indicated. (I) and (II) correspond to different types of trichomes concurring in the same taxon.

	Trichome length (μm)	Base width [B] (μm)	Apex width [A] (μm)	Decrease B – A (%)
<i>M. cryptogloea</i>	125.6 \pm 31	7.1 \pm 1	4 \pm 1	44
<i>M. pseudopetiolaris</i> (I)	268.6 \pm 69	5.5 \pm 1	3.4 \pm 1	38
<i>M. dolens</i> subsp. <i>eriophylla</i>	447.1 \pm 108	18.6 \pm 1	5.2 \pm 1	72
<i>M. dolens</i> var. <i>acerba</i>	395 \pm 101	29.9 \pm 7	5 \pm 1	83
<i>M. brevipes</i>	510.6 \pm 100	35.4 \pm 8	5.8 \pm 2	83
<i>M. pseudopetiolaris</i> (II)	482.2 \pm 78	25.6 \pm 3	5 \pm 1	81
<i>M. dolens</i> subsp. <i>callosa</i>	636.7 \pm 134	43.2 \pm 9	5.8 \pm 1	87
<i>M. custodis</i>	688.2 \pm 113	48.7 \pm 12	7.7 \pm 2	84
<i>M. dolens</i> var. <i>rudis</i>	768.7 \pm 204	40.8 \pm 6	7.2 \pm 2	81
<i>M. sceptrum</i>	966.3 \pm 232	52.2 \pm 16	6.3 \pm 2	88
<i>M. dolens</i> var. <i>latifolia</i>	1019 \pm 206	36.3 \pm 5	7.2 \pm 1	80

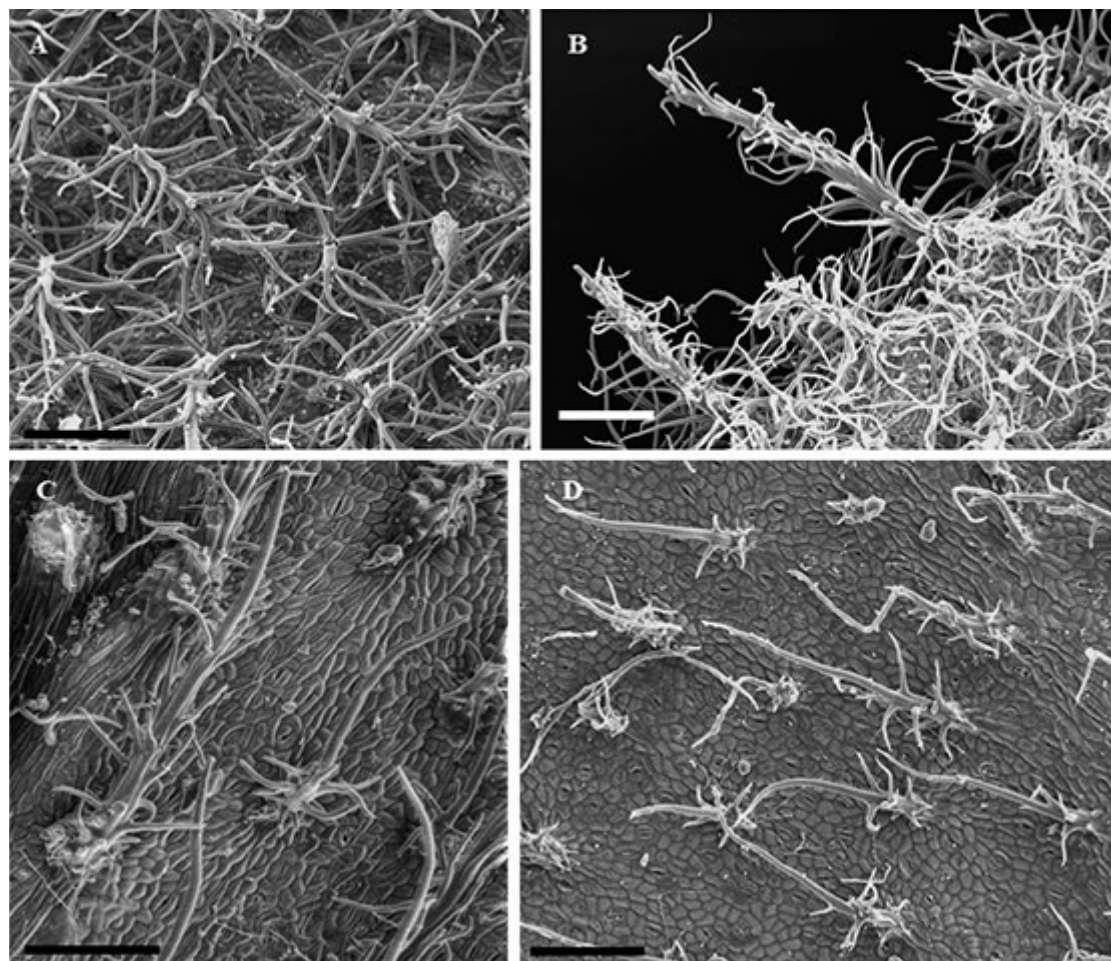


Figure 2. SEM micrographs of nonglandular branched trichomes of leaflets in *Mimosa Dolentes-Brevipedes* complex. **A and B-** *M. diversipila* var. *diversipila*, adaxial face. **C and D-** *M. diversipila* var. *subglabriseta*, adaxial face. Scale bar indicates 100 μm .

Table 3. Nonglandular branched trichome measurements on blades in both varieties of *Mimosa diversipila*: trichome length, branch length, and number of branches. Mean and standard deviation are indicated.

	Trichome length (μm)	Branch length (μm)	Number of branches
<i>M. diversipila</i> var. <i>diversipila</i>	164.6 \pm 73	77.9 \pm 15	33.2 \pm 14
<i>M. diversipila</i> var. <i>subglabriseta</i>	229.6 \pm 35	35.6 \pm 12	9 \pm 2

observed in light microscopy, we considered this type of trichome as simple.

In general, projections have a variable distribution in simple conical trichomes. In some taxa they are scarce, such as *M. dolens* var. *acerba*, *M. pseudopetiolaris*, *M. brevipes*, and *M. dolens* var. *rudis*. These trichomes have a multiseriate base (Figures 3G and 3H), and projection apex separates a little from the rest in the first third or half. At this point, projections have rounded (Figures 3B, 3C, and 3E) or tapered (Figures 3A and 3D) ends. At the

trichome apex only the central axis is observed, which frequently shows longitudinal oblique furrows.

In *M. dolens* subsp. *callosa*, *M. dolens* var. *latifolia*, and *M. custodis*, projections are scarce and present only in a few trichomes of the leaflets; in that case they cover three-quarters of the trichome length. In *M. sceptrum*, however, they cover the entire trichome length.

Trichome projections are most evident in *M. dolens* subsp. *eriophylla*. In this taxon they are easily visible and very abundant (Figure 3F). In fact, in some trichomes,

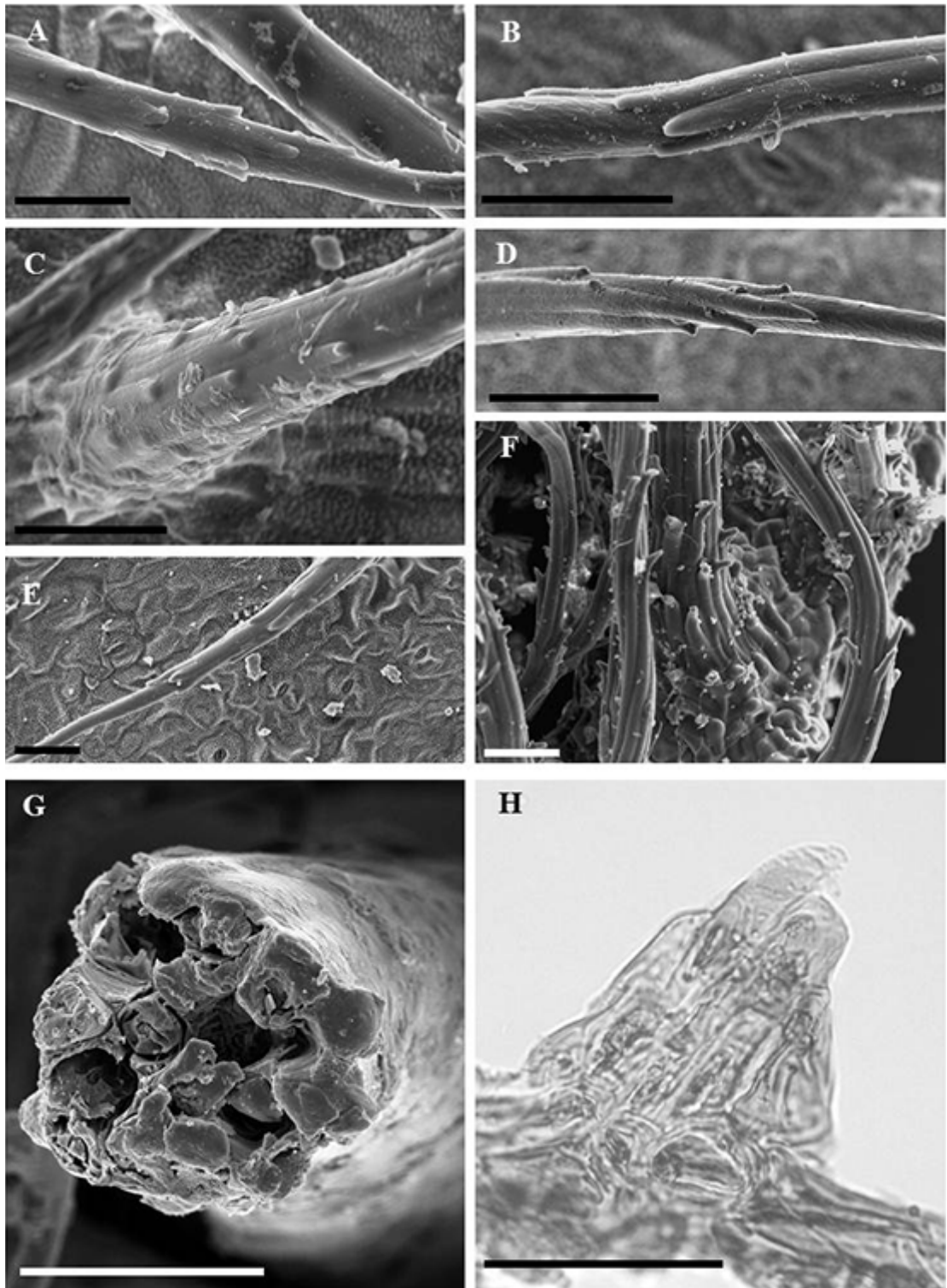


Figure 3. SEM and LM micrographs of leaflet trichome projections in *Mimosa* subseries *Dolentes* and subseries *Brevipedes*. **A-** *M. sceptrum*, abaxial face. **B-** *M. dolens* var. *acerba*, abaxial face. **C-** *M. dolens* var. *rudis*, adaxial face. **D-** *M. brevipes*, adaxial face. **E-** *M. dolens* var. *acerba*, adaxial face. **F-** *M. dolens* subsp. *eriophylla*, abaxial face. **G-** trichome cross-section in *M. brevipes*, abaxial face. **H-** Cleared trichome base in *M. dolens* var. *latifolia*, abaxial face. Scale bar in figures A, B, C, D, E, F, and H indicates a length of 50 μm and in figure G it indicates 20 μm.

projections protrude so much from the central axis that the apex shows a tapered morphology that resembles a real branch.

3. Glandular trichomes. We found stalked glandular trichomes with 3–7-celled stalks and a multicellular, globose, or ovoid head. They were found in the blades of 2 taxa of the *Dolentes-Brevipedes* complex, *M. dolens* var. *pangloea*, and *M. cryptogloea*. We distinguished 2 different subtypes among them:

3A. Globose. Glandular trichomes with uniseriate stalks of 3 cells and a multicellular, 10–12-celled globose head (Figures 4A and 4B). They cover the entire blade and the margin of leaflets in *M. dolens* var. *pangloea*.

3B. Clavate. Glandular trichomes with uniseriate stalks with 3–7 cells, with a multicellular, generally 4-celled, ovoid, or elongated head (Figures 4C and 4D). They were observed at the base and the primary vein of leaflets in *M. cryptogloea* in concurrence with simple cylindrical trichomes.

3.1.2. Trichome density

In *Dolentes-Brevipedes* complex, trichome density is highly variable in pubescent taxa, ranging from 10 to 176 trichomes/mm² on the adaxial face and from 14 to 242 trichomes/mm² on the abaxial face (Table 4).

In most taxa, trichome density seems to be similar on both faces of the leaflet. However, in other taxa it seems to differ between the adaxial and abaxial face. For example, *M. dolens* var. *pangloea*, *M. dolens* var. *rudis*, and *M. custodis* exhibit higher density of trichomes in the abaxial face, whereas *M. dolens* subsp. *erriophylla* and *M. sceptrum* have higher density in the adaxial face.

In *Dolentes-Brevipedes*, the group of taxa included in *M. dolens* subsp. *rigida* (*M. dolens* var. *foliolosa*, *M. dolens* var. *deterior*, *M. dolens* var. *anisitsii*, and *M. dolens* var. *rigescens*) is characterized by absence of trichomes.

Although we analyzed the median values of trichome density of each leaflet face, we observed variability in location of trichomes among individuals of the same taxa,

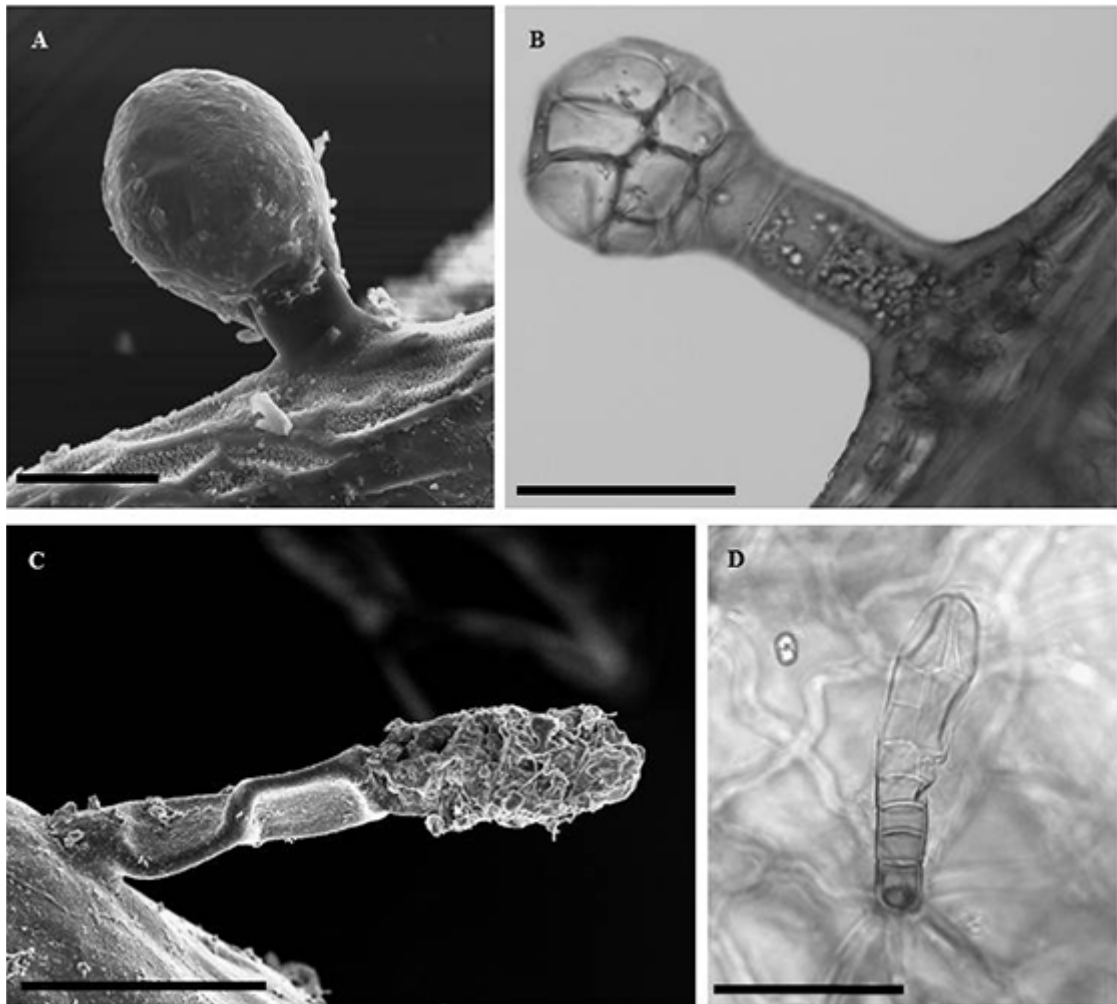


Figure 4. SEM and LM micrographs of glandular trichomes of leaflets in *Mimosa Dolentes-Brevipedes* complex. **A** and **B**- *M. dolens* var. *pangloea*, abaxial face. **C** and **D**- *M. cryptogloea*, adaxial face. Scale bar indicates 50 μ m.

Table 4. Median of trichome (t) density in *Dolentes-Brevipedes* complex. Different letters (a, b, c, d) mean statistical differences between taxa on one face of leaflet ($P < 0.05$).

	Trichome type	Adaxial face		Abaxial face	
		Density (t/mm ²)	Min-max	Density (t/mm ²)	Min-max
<i>M. dolens</i> var. <i>foliolosa</i>	-	0	0	0	0
<i>M. dolens</i> var. <i>deterior</i>	-	0	0	0	0
<i>M. dolens</i> var. <i>anisitsii</i>	-	0	0	0	0
<i>M. dolens</i> var. <i>rigescens</i>	-	0	0	0	0
<i>M. dolens</i> var. <i>dolens</i>	-	0	0	0	0
<i>M. dolens</i> var. <i>pangloea</i>	3A	19.5 ^a	18–23	30.5 ^{a,b,c}	30–31
<i>M. dolens</i> var. <i>rudis</i>	1A	20 ^{a,b}	19–21	21 ^{a,b}	20–22
<i>M. custodis</i>	1A	20.5 ^{a,b}	18–23	32.5 ^{a,b,c}	25–43
<i>M. dolens</i> var. <i>latifolia</i>	1A + 1B	21 ^{a,b}	19–23	24.5 ^{a,b}	21–28
<i>M. dolens</i> subsp. <i>callosa</i>	1A	23.5 ^{a,b,c}	19–31	32 ^{a,b,c}	29–35
<i>M. dolens</i> var. <i>acerba</i>	1A	25 ^{a,b}	10–32	19 ^{a,b}	14–45
<i>M. dolens</i> subsp. <i>erriophylla</i>	1A	34 ^{a,b,c,d}	33–35	28.1 ^{a,b}	27–29
<i>M. diversipila</i> var. <i>subglabriseta</i>	2B + 1A or 2A	35.5 ^{a,b,c,d}	30–41	44.5 ^{b,c}	36–53
<i>M. sceptrum</i>	1A	38.5 ^{b,c,d}	38–39	34.5 ^{a,b,c}	34–35
<i>M. brevipes</i>	1A + 1B	33 ^{a,b,c,d}	23–72	33.5 ^{a,b,c}	18–75
<i>M. cryptogloea</i>	1B + 3B	55.5 ^{c,d}	52–59	59 ^{b,c}	52–66
<i>M. diversipila</i> var. <i>diversipila</i>	2A	82 ^d	72–94	76.5 ^c	73–80
<i>M. pseudopetiolaris</i>	1A + 1B	122.5 ^d	69–176	174.5 ^c	107–242

as some of them are pubescent in both faces and others only in the abaxial face, as in *M. brevipes* and *M. custodis*.

We described a great inter- and intraspecific variability in absolute values of density. Although these values form a continuum, we can differentiate trends of taxa to show low or high density. Excluding glabrous taxa, almost all taxa show low density values. Only a few taxa have high density values, such as *M. dolens* subsp. *erriophylla*, *M. diversipila* var. *diversipila*, and *M. pseudopetiolaris* (Table 4; Figures 1A and 1B).

Regarding trichome density, there are statistically significant differences between groups of taxa. Some infraspecific taxa of *M. dolens* have differences in trichome density compared to species of the subseries *Brevipedes*: *M. cryptogloea*, *M. pseudopetiolaris*, and *M. diversipila* var. *diversipila*, which exhibit the highest values of trichome density.

Multivariate analysis. The first 3 principal coordinates of PCoA accounted for about 79% of total variance. PCo1 accounted for nearly 55%, whereas PCo2 and PCo3

explained about 13% and 11%, respectively (Figure 5). We plotted PCo1 vs. PCo2, and PCo1 vs. PCo2 and PCo3 to visualize the relationships among individuals (Figure 5). It was possible to observe different clusters. The most conspicuous comprised *M. dolens* subsp. *callosa*, *M. custodis*, *M. sceptrum*, *M. dolens* var. *acerba*, *M. dolens* var. *latifolia*, and *M. dolens* subsp. *erriophylla*.

There are also other clusters, though less evident. One comprises *M. brevipes*, *M. pseudopetiolaris*, and *M. diversipila* var. *diversipila*, and the other *M. diversipila* var. *subglabriseta*, *M. dolens* var. *pangloea*, and *M. cryptogloea*. It is interesting to point out that *M. dolens* var. *rudis* appears isolated, though quite near the first cluster.

3.1.3. Margin type and margin trichomes

In the *Dolentes-Brevipedes* complex there are 3 types of margin (Figure 6; Table 5):

1. Differentiated. This is a corneous margin whose texture differs clearly from the blade. It is covered with thick, stiff, and basally dilated trichomes (Figures 6A–6D). This margin type is found in all representatives of *M.*

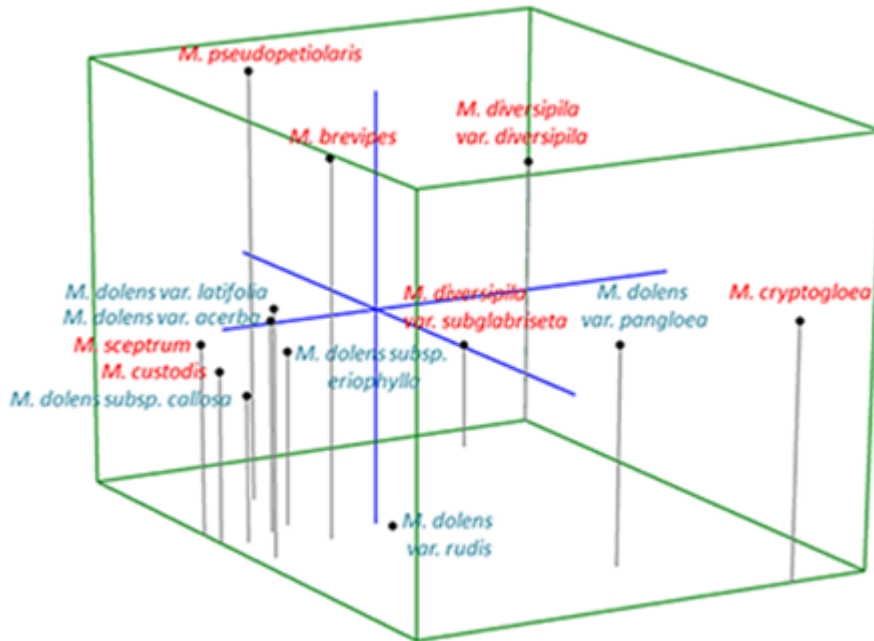


Figure 5. Principal coordinate analysis based on trichome characters in pubescent taxa of *Mimosa* subser. *Dolentes* (blue) and *M.* subser. *Brevipedes* (red).

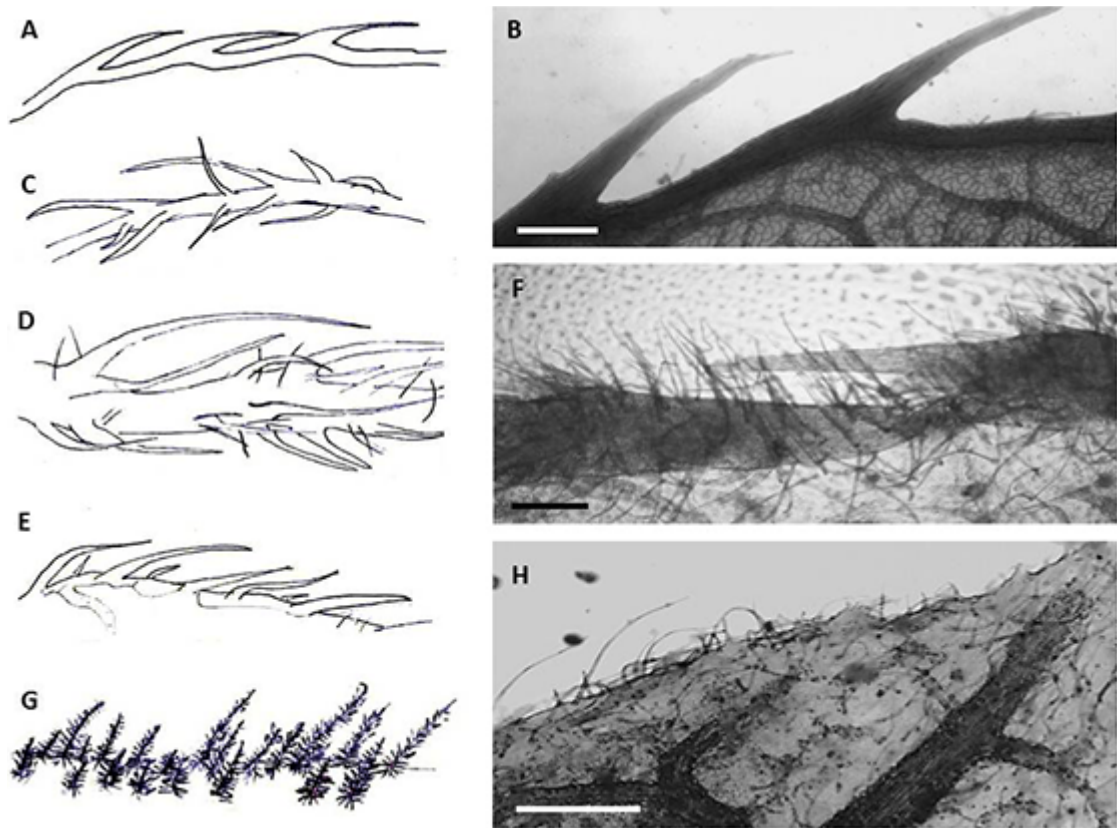


Figure 6. Drawings and LM micrographs of margin types in *Mimosa* subseries *Dolentes* and subseries *Brevipedes*. A and B- *M. dolens* var. *deterior*, differentiated margin with trichomes in one row. C- *M. dolens* var. *acerba*, differentiated margin with trichomes in many rows. D- *M. dolens* var. *rudis*, differentiated margin with irregularly disposed trichomes. E and F- intermediate margin. E, *M. brevipes*. F, *M. pseudopetiolaris*. G and H- nondifferentiated margin. G, *M. diversipila* var. *diversipila*. H, *M. diversipila* var. *subglabriseta*. Scale bar indicates 200 µm.

Table 5. Margin characteristics in *Dolentes-Brevipedes* complex. Margin type, trichome type, and disposition are indicated.

	Margin type	Disposition of stiff trichomes	Type of trichomes
<i>M. dolens</i> var. <i>foliolosa</i>	Differentiated	One row	Simple stiff
<i>M. dolens</i> var. <i>deterior</i>	Differentiated	One row	Simple stiff + 1C
<i>M. dolens</i> var. <i>anisitsii</i>	Differentiated	One row	Simple stiff
<i>M. dolens</i> var. <i>rigescens</i>	Differentiated	One row	Simple stiff
<i>M. dolens</i> var. <i>pangloea</i>	Differentiated	One row	Simple stiff + 3A
<i>M. dolens</i> var. <i>dolens</i>	Differentiated	One row	Simple stiff + 3A + 1B
<i>M. cryptogloea</i>	Differentiated	One row	Simple stiff + 1B
<i>M. dolens</i> subsp. <i>callosa</i>	Differentiated	One row	Simple stiff
<i>M. dolens</i> subsp. <i>eriophylla</i>	Differentiated	Many rows	Simple stiff
<i>M. dolens</i> var. <i>acerba</i>	Differentiated	Many rows	Simple stiff
<i>M. sceptrum</i>	Differentiated	Many rows	Simple stiff + 1B
<i>M. custodis</i>	Differentiated	Many rows	Simple stiff + 1B
<i>M. dolens</i> subsp. <i>callosa</i>	Differentiated	Many rows	Simple stiff
<i>M. pseudopetiolaris</i>	Differentiated	Many rows	Simple stiff + 1B
<i>M. dolens</i> var. <i>latifolia</i>	Differentiated	Irregular	Simple stiff + 1B
<i>M. dolens</i> var. <i>rudis</i>	Differentiated	Irregular	Simple stiff + 1B
<i>M. brevipes</i>	Intermediate	One row	Simple stiff + 1B
<i>M. pseudopetiolaris</i>	Intermediate	One row	Simple stiff + 1B
<i>M. diversipila</i> var. <i>diversipila</i>	Not differentiated	-	2A
<i>M. diversipila</i> var. <i>subglabriseta</i>	Not differentiated	-	2B + 1B
<i>M. brevipes</i>	Not differentiated	-	1A + 1B

dolens, *M. pseudopetiolaris*, *M. custodis*, *M. sceptrum*, and *M. cryptogloea*.

2. Intermediate. On this margin type, there are simple stiff trichomes, different from blade trichomes, which resemble trichomes of differentiated margins. Their bases are expanded, but not completely fused between them in a continuous margin (Figures 6E and 6F). This margin type appears infrequently in *M. pseudopetiolaris* and *M. brevipes*.

3. Not differentiated. The blade texture continues on the margin without any difference in trichome types or texture (Figures 6G and 6H). This margin type is characteristic of *M. diversipila* and *M. brevipes*.

In differentiated and intermediate margins, some variability in trichome location is observed. In some taxa, stiff trichomes are aligned in one row, yet in one or more directions (Figures 6A and 6B; *M. dolens* var. *foliolosa*, *M. dolens* var. *deterior*, *M. dolens* var. *anisitsii*, *M. dolens* var. *rigescens*, *M. dolens* var. *pangloea*, *M. dolens* var. *dolens*, and *M. cryptogloea*). In other taxa, trichomes are aligned

in a few rows or irregularly around the margin (Figures 6C and 6D; *M. dolens* subsp. *eriophylla*, *M. dolens* var. *acerba*, *M. sceptrum*, *M. custodis*, *M. dolens* var. *latifolia*, and *M. dolens* var. *rudis*). In *M. dolens* subsp. *callosa* and *M. pseudopetiolaris* we found individuals with both differentiated coriaceous and nondifferentiated margins (Table 5).

In some taxa, there is only one type of trichome on the margin: simple trichomes in *M. dolens* var. *foliolosa*, *M. dolens* var. *anisitsii*, *M. dolens* var. *rigescens*, *M. dolens* subsp. *eriophylla*, and *M. dolens* var. *acerba* and branched trichomes in *M. diversipila* var. *diversipila*.

In some taxa of this taxonomic complex, it is possible to observe more than one type of trichome on the margin; in many cases, several are similar to blade trichomes (Tables 4 and 5). The most frequent concurrence is between simple conical trichomes and simple cylindrical trichomes. However, there are also other combinations: in both varieties of *M. dolens* subsp. *dolens*, there are simple

conical and glandular globose trichomes, although in *M. dolens* var. *dolens* simple cylindrical trichomes are also present. In *M. diversipila* var. *subglabriseta*, branched trichomes are accompanied by simple cylindrical trichomes, and in *M. brevipes* we observed simple conical trichomes with shorter and frequently curved simple cylindrical trichomes.

In *M. dolens* var. *rigescens*, *M. dolens* var. *latifolia*, *M. dolens* var. *deterior*, *M. dolens* var. *pangloea*, and *M. pseudopetiolaris* we observed projections similar to those recorded on the blade trichomes. However, in this case, projections are minute and cover the whole trichome surface.

4. Discussion

As was described for the genus *Mimosa* (Barneby, 1991; Santos-Silva et al., 2013b), in the *Dolentes-Brevipedes* complex there are also many trichome types on the blades, including simple and branched nonglandular trichomes and 2 glandular trichomes. Within each subseries, and frequently among individuals of the same taxon, there is a remarkable trichome heterogeneity. Therefore, it is difficult to set an infrageneric classification system using only this feature.

Simple conical trichomes are found in most taxa of both subseries (*Dolentes* and *Brevipedes*), which limits its taxonomic value, as they do not group taxa. Simple cylindrical trichomes are, however, distinctive of *M. pseudopetiolaris* and *M. cryptogloea*.

Simple conical trichomes of *M. dolens* subsp. *eriophylla* are also distinctive, because they form a dense lanate indumentum that clearly distinguishes this subspecies from all the other taxa of *M. dolens* (Barneby, 1991). The percentage of thickness reduction and external topology of the trichome, with dense lateral projections in its basal half, supports this classification and distinguishes this type of trichome from simple cylindrical trichomes.

Plumose trichomes represent a particular case. *M. diversipila* has been described as one of the few species in the series *Mimosa* with this type of trichome, and the only one in the complex that we studied (Barneby, 1991; Santos-Silva et al., 2013b). Both varieties of this species, *M. diversipila* var. *subglabriseta* and *M. diversipila* var. *diversipila*, differ from each other in indumentum type. According to Barneby (1991), this difference is based on the presence of short plumose (almost substellate) trichomes on the blades of *M. diversipila* var. *diversipila* and simple, smooth trichomes on *M. diversipila* var. *subglabriseta*. Later, this statement was rectified, describing minutely scabrous trichomes in *M. diversipila* var. *subglabriseta* and plumose in *M. diversipila* var. *diversipila* (Luna-Castro et al., 2012), although there were some intermediate cases (Morales et al., 2014a). With high-resolution techniques, we observed

that trichomes of *M. diversipila* var. *diversipila* are indeed branched along the whole extension. Branched trichomes also occur in *M. diversipila* var. *subglabriseta*, although branches are only in the basal third of the trichome and are half the length of those of *M. diversipila* var. *diversipila*. This could correspond with the description of “minutely scabrous” trichomes mentioned by Morales et al. (2014a) for this variety. Our results support the distinction of *M. diversipila* because of the presence of branched trichomes on the leaflets, and also the distinction of both varieties, as they were separated in recent taxonomic treatments of this species (Barneby, 1991; Morales et al., 2014a).

Glandular trichomes appear to have considerable taxonomic value. They have been described in other species of the genus *Mimosa* (Barneby, 1991; Santos-Silva et al., 2013b). However, in the *Dolentes-Brevipedes* complex, they characterize particularly *M. dolens* var. *pangloea* (subser. *Dolentes*) and *M. cryptogloea* (subser. *Brevipedes*) (Barneby, 1991). Our study supports the distinction of these taxa according to the presence of glandular trichomes, although morphological analysis shows 2 different subtypes of glandular trichomes: globose in *M. dolens* var. *pangloea* (type 3A) and clavate in *M. cryptogloea* (type 3B). This last type should correspond to glandular trichomes with clavate heads, described in vegetative and reproductive organs, but not in leaflets of other species of the genus (Santos-Silva et al., 2013b). However, additional studies are still necessary to make comparisons between glandular trichomes of different species in different organs.

Trichome density is one of the most environmentally variable of all vegetative traits (Metcalf and Chalk, 1950). For this reason, it is important to be cautious with taxonomic conclusions derived from the analysis of this trait. In other complexes of *Mimosa*, such as *Mimosa debilis* Humb. & Bonpl. ex Willd., the presence and absence of indumentum has been described as a diagnostic character (Burkart, 1948), although the variability of trichome density does not allow the separation of the taxa (Barneby, 1991; Morales et al., 2010). However, in the genus *Mimosa* it is still not clear whether the observed differences in leaflet pubescence are related to ecological or genetic variability (Morales et al., 2010, 2013).

In the *Dolentes-Brevipedes* complex, trichome density shows great variability, ranging from 10 to 176 trichomes/mm² on the adaxial face and from 14 to 242 trichomes/mm² on the abaxial face. This wide range of variation might be due to the diverse geographic origin of the material. As environmental implication exceeds this work, we describe only the absolute values. The trends we observed reveal that, in general, low-density values correspond to the subseries *Dolentes* (e.g., *M. dolens* var. *pangloea*, *M. dolens* var. *rudis*) and high-density values are related to subseries *Brevipedes* (e.g., *M. diversipila* and *M. pseudopetiolaris*).

Regarding the leaflet margin, in *Mimosa* section *Batocaulon* series *Quadrivalves* Barneby, this feature has been described focusing on the number of cells instead of external morphology (Flores-Cruz et al., 2004). However, the analysis mentioned above did not show taxonomically relevant results to separate species. According to our results, a differentiated margin with stiff trichomes would be a common characteristic in many representatives of the *Dolentes-Brevipedes* complex, although it is not exclusive. In fact, the presence of nondifferentiated leaflet margin allows us to distinguish *M. brevipes*, *M. pseudopetiolaris*, and *M. diversipila* from the rest of the taxa of the complex. This trend was also observed by Barneby (1991) and was especially remarked to separate *M. dolens* subsp. *rigida* from *M. dolens* subsp. *acerba*, and *M. custodis* from *M. brevipes*.

There are particular cases observed on trichomes that appear to be simple, but with great magnification (1500×) they show small lateral projections in the basal half. This morphology has scarcely been observed in other families; in Melastomataceae (*Tibouchina* Aubl.), it was described as “roughened hairs” (Guimarães et al., 1999). Given the uncertainty of descriptions and categorizations, we considered them as simple trichomes, as they appear to be under a stereoscopic microscope.

Evolutionary implications of trichome morphology in *Mimosa* have recently been studied by Santos-Silva et al. (2013b). In this study, an ancestral character state reconstruction was made using a densely sampled phylogeny of the genus in order to test Barneby’s hypothesis about evolution of trichome types in *Mimosa*. It was hypothesized that branched and glandular trichomes, with all their variants, derived from nonglandular and nonbranched trichomes (Barneby, 1991; Santos-Silva et al., 2013b). In the present study, we observed, under high magnification, the intermediate stages of branching between simple and branched trichomes in conical simple trichomes. Multiseriate bases could be thought of as incipient branches; however, it is difficult to locate them in the evolutionary scheme originally proposed by Barneby (1991) and lately completed by Santos-Silva et al. (2013b). Projections could be located at 3 points in the evolutionary scheme: 1) reduction from plumose trichomes, 2) reduction from medusiform trichomes, or 3) an intermediate stage between simple and branched trichomes (Santos-Silva et al., 2013b).

The interpretation of the role of the new trichome types in the evolutionary scheme of trichomes in *Mimosa* could enlighten the evolution of branching in *M. diversipila*. As branched trichomes are considered a derived feature in the mentioned study, subbarbellate trichomes in *M. diversipila* var. *subglabriseta* would be an ancestral trait in relation to plumose trichomes of *M. diversipila* var. *diversipila* when this evolutionary scheme is assumed. The presence of

polyploids among taxa with plumose trichomes (Morales et al., 2014a, 2014b), as well as optimizations of trichomes on molecular trees (Simon et al., 2011; Santos-Silva et al., 2013b), would support the idea that branching in trichomes is a derived character, given their presence in individuals with high ploidy levels or from derived clades. Following this hypothesis of evolutionary increasing of trichome branching, intermediate stages with inconspicuous projections observed in simple trichomes could be the intermediate step between simple and plumose trichomes.

Taxonomic significance. Considering the characteristics of blade trichomes, it is difficult to separate both subseries as proposed by Barneby (1991). However, some taxa do show clear differences. For example, *M. dolens* subsp. *eriophylla* can be easily differentiated from all other studied taxa by simple conical trichomes with pronounced projections at higher magnifications. This is consistent with Barneby’s (1991) separation of the taxon as a different subspecies of subseries *Dolentes*, based on indumentum type. *M. diversipila* is also easily separated from other species of subseries *Brevipedes*, as it is the only taxon with branched trichomes, which was also remarked as a taxonomically important trait for this taxa in Barneby’s (1991) classification. Within these species, both varieties are also clearly differentiated by trichomes with branches along the whole extension in *M. diversipila* var. *diversipila* and only in the basal third in *M. diversipila* var. *subglabriseta*.

Glandular trichomes separate *M. dolens* var. *pangloea* with the presence of globose multicellular heads and *M. dolens* var. *dolens* and *M. cryptogloea* by their elongated multicellular heads.

These differences in trichome morphology between *M. dolens* var. *dolens* and *M. dolens* var. *pangloea* were unexpected, since both are included in the same subspecies (*M. dolens* subsp. *dolens*), which forms a well-defined entity in the complex by its armed stems, glandular indumentum, and inflorescence with paired peduncles (Barneby, 1991; Sabino et al., 2010). The similarity in the morphology of trichomes between *M. dolens* var. *pangloea* and *M. cryptogloea* was also unexpected, since they share few exomorphological characters; in fact, *M. cryptogloea* resembles *M. dolens* subsp. *acerba* more than subsp. *dolens*. In addition, *M. diversipila* var. *diversipila*, *M. pseudopetiolaris*, and *M. dolens* subsp. *eriophylla* differ from the rest because of high trichome density. Finally, *M. dolens* var. *dolens* and all taxa of *M. dolens* subsp. *rigida* in Barneby’s (1991) system differ due to the absence of trichomes on the leaflet blades.

On the other hand, differentiated margin type includes all taxa, except for *M. diversipila*, *M. brevipes*, and some specimens of *M. pseudopetiolaris*. Consequently, this feature is stable in subseries *Dolentes*, although not

in subseries *Brevipedes*, as it also comprises taxa with nondifferentiated margin, such as *M. diversipila*, and some taxa with variable margin, such as *M. brevipes* and *M. pseudopetiolaris*.

Comparing all these data with the classification proposed by Barneby (1991), it is clear that no trichome feature clearly differentiates subseries *Dolentes* from subseries *Brevipedes*. However, it is remarkable that in *M. dolens* subsp. *rigida*, all varieties are glabrous, which makes this subspecies very stable. On the other hand, in PCoA analysis, almost all varieties of *M. dolens* form a cluster, except *M. dolens* var. *rudis*. Remarkably, *M. sceptrum* and *M. custodis* appear to be fully included in the *M. dolens* cluster; the affinity among these 3 species was observed by Barneby (1991) and in recent studies (Sabino et al., 2010), but only by exomorphological analyses. It is interesting to observe that the taxa of the subseries *Brevipedes* appear sparsely distributed. As was described, trichomes in these taxa are quite different. Together with PCoA results, this shows that this subseries is morphologically heterogeneous and requires a new detailed taxonomic revision.

References

- Barneby RC (1991). Sensitivae *Censitae*: a description of the genus *Mimosa* Linnaeus (Mimosoideae) in the new world. *Mem New York Bot G* 65: 1–835.
- Bentham G (1875). Revision of the suborder Mimosae. *Trans Linn Soc* 30: 335–664.
- Bentham G (1876). *Mimosa*. In: Martius CFP, Eichler AW, Urban I, editors. *Flora Brasiliensis*, Vol. 15 (2). Leipzig, Germany: Fleischer, pp. 294–390.
- Borges LM, Simon MF, Pirani JR (2014). The census continues: two new montane species of *Mimosa* (Leguminosae Mimosoideae) from southeastern Brazil. *Phytotaxa* 177: 35–48.
- Burkart A (1948). Las especies de *Mimosa* de la flora Argentina. *Darwiniana* 8: 9–231 (in Spanish).
- Dahmer N, Simon MF, Schifino-Wittmann MT, Hughes CE, Miotto STS, Giuliani JC (2011). Chromosome numbers in the genus *Mimosa* L.: cytotaxonomic and evolutionary implications. *Plant Syst Evol* 291: 211–220.
- D'Ambrogio A (1986). Manual de técnicas en histología vegetal. Buenos Aires, Argentina: Hemisferio sur SA (in Spanish).
- Dizeo CG (1973). Nueva técnica de diafanización. *Bol Soc Argent Bot* 15: 126–129 (in Spanish).
- Flores-Cruz M, Santana-Lira HD, Koch SD, Grether R (2004). Taxonomic significance of leaflet anatomy in *Mimosa* series *Quadrivalves* (Leguminosae, Mimosoideae). *Syst Bot* 29: 892–902.
- Freire de Carvalho L (1970). Contribuição estudo da nervação foliar das leguminosas dos cerrados ii. Mimosoideae. *An Acad Bras Cienc* 42: 79–88 (in Portuguese with abstract in English).
- The analysis of trichome micromorphology shows high variation in the *Dolentes-Brevipedes* complex. However, trichomes have taxonomic value as they differentiate taxa and their groups. In some cases, as in *M. diversipila*, the distinctions support differences found in morphometric data (Morales et al., 2014a); in other cases, they differ from previous work, as in the division of taxa in subseries *Dolentes* and *Brevipedes* (Barneby, 1991). Since the presence of this variability in the genus makes the taxonomic treatment very complex, it is necessary to start further multidisciplinary studies in this complex, including micromorphological, molecular, and anatomical data, focusing especially on evolutionary relationships between its taxa.

Acknowledgments

We are grateful to Dr Renée H Fortunato and all staff of the Instituto de Recursos Biológicos (CIRN, INTA) for their assistance during our visits to the herbarium. We acknowledge the financial support of UBA (Grant 20020120100056BA UBACyT 2013-2016 GC).

Grether R (2000). Nomenclatural changes in the genus *Mimosa* (Fabaceae, Mimosoideae) in southern Mexico and central America. *Novon* 10: 29–37.

Guimarães PJ, Fernandes NTR, Borges Martins A (1999). Morfologia dos tricomas em *Tibouchina* sect. *Pleroma* (D. Don) Cogn. (Melastomataceae). *Braz Arch Biol Technol [Online]* 42: 4 (in Portuguese).

Hammer O, Harper DAT, Ryan PD (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontol Electron* 4: 1–9.

Henderson A (2006). Traditional morphometrics in plant systematics and its role in palm systematics. *Bot J Linn Soc* 151: 103–111.

Hickey LJ (1979). A revised classification of the architecture of dicotyledonous leaves. In: Metcalfe CR, Chalk L, editors. *Anatomy of the Dicotyledons*, Vol. 1. 2nd ed. Oxford, UK: Oxford University Press, pp. 25–39.

Kruskal WH, Wallis WA (1952). Use of ranks on one-criterion variance analysis. *J Am Stat Assoc* 47: 583–621.

Leelavathi P, Ramayya N (1982). Trichomes in relation to taxonomy I. Mimosoideae. *Geophytology* 12: 6–21.

Luna-Castro J, Morales M, Fortunato RH (2012). *Mimosa diversipila* var. *subglabriseta* (Fabaceae), a new record for the flora of Paraguay. *Bol Soc Argent Bot* 47: 457–460.

Metcalfe CR, Chalk L (1950). *Anatomy of the Dicotyledons: Leaves, Stem and Wood in Relation to Taxonomy with Notes on Economic Uses*. 2nd ed. Oxford, UK: Clarendon Press.

- Morales M, Arenas L, Remis MI, Wulff AF, Poggio L, Fortunato RH (2014a). Morphometric and cytogenetic studies in *Mimosa diversipila* (Mimosoideae, Leguminosae) and their taxonomic and evolutionary inferences. *Syst Bot* 39: 875–883.
- Morales M, Arenas L, Remis MI, Wulff AF, Poggio L, Fortunato RH (2014b). Chromosome studies in southern species of *Mimosa* (Fabaceae, Mimosoideae) and their taxonomic and evolutionary inferences. *Plant Syst Evol* 300: 803–817.
- Morales M, Fortunato RH (2010). Taxonomic and nomenclatural novelties in *Mimosa* L. subser. *Mimosa* (Leguminosae) in Austral South America. *Candollea* 65: 169–184 (in Spanish with abstracts in English and French).
- Morales M, Fortunato RH (2013). A new species of *Mimosa* (Mimosoideae, Leguminosae) from the inter-Andean dry valleys. *Phytotaxa* 114: 33–41.
- Morales M, Ribas OS, Santos-Silva J (2012). A new polyploid species of *Mimosa* (Leguminosae, Mimosoideae) from the highlands of southern Brazil. *Syst Bot* 37: 399–403.
- Morales M, Santos-Silva J, Ribas OS (2013). A new species of *Mimosa* sect. *Mimosa* (Leguminosae, Mimosoideae) from southern Brazil. *Brittonia* 65: 148–153.
- Morales M, Wulff AF, Fortunato RH, Poggio L (2007). Chromosome study in species of *Mimosa* (Mimosoideae, Fabaceae) from southern South America. *Bol Soc Argent Bot* 42: 55–56 (in Spanish with abstract in English).
- Morales M, Wulff AF, Fortunato RH, Poggio L (2010). Chromosome and morphological studies in the *Mimosa debilis* complex (Mimosoideae, Fabaceae) from southern South America. *Aust J Bot* 58: 12–22.
- Sabino G, Arenas L, Morales M (2010). Caracterización morfológica en el complejo *Mimosa* Subseries Dolentes-Brevipedes (Mimosoideae, Leguminosae). In: XV Reunión Científica del Grupo Argentino de Biometría (XXV International Biometric Conference); Florianópolis, Brazil (in Spanish).
- Santos-Silva J, Simon MF, Tozzi AMGDA (2013a). A new species of “jurema” (*Mimosa* ser. *Leiocarpae* Benth.) from Bahia, Brazil. *Syst Bot* 38: 127–131.
- Santos-Silva J, Tozzi AMGDA, Fragomeni M, Guedes Urquiza N, Morales M (2013b). Evolution of trichome morphology in *Mimosa* L. (Leguminosae-Mimosoideae). *Phytotaxa* 119: 1–20.
- Seijo G (1993). Chromosome numbers in species of genus *Mimosa* of Argentina. *Bol Soc Argent Bot* 29: 219–223 (in Spanish with abstract in English).
- Simon MF, Grether R, de Queiroz LP, Särkinen TE, Dutra VF, Hughes CE (2011). The evolutionary history of *Mimosa* (Leguminosae): toward a phylogeny of the sensitive plants. *Am J Bot* 98: 1201–1221.
- Zar JH (2010). *Biostatistical Analysis*. 5th ed. Englewood Cliffs, NJ, USA: Prentice Hall.

Appendix. References of herbarium specimens.

- BRAZIL. Mato Grosso do Sul: Nova Andradina do Sul, Dourados, 20.V.2009, M. Morales et al. 1020 (BAB). *M. brevipes* Benth.
- PARAGUAY. Amambay: 2 km de la prop. Del Sr. Abraho, hacia Pta. Porá, 08.V.1989, R. H. Fortunato et al. 1181 (BAB). *M. brevipes* Benth.
- PARAGUAY. Amambay: N. Cerro Corá, 1 km de la Casa del Guardaparque, 04.V.1989, R. H. Fortunato et al. 1059 (BAB). *M. brevipes* Benth.
- BRAZIL. Mato Grosso do Sul: Bonito, Rodovia Bonito a Campo dos Índios, próximo de Três Morros, 10.III.2003, G. Hatschbach et al. 74471 (MBM323491). *M. brevipes* Benth.
- BRAZIL. Mato Grosso: Campo Grande-Rochedo, 13.VII.1969, G. Hatschbach & Guimarães 21845 (MBM11260). *M. brevipes* Benth.
- PARAGUAY. Amambay: P. N. Cerro Corá, centro histórico, ca. Río Aquidabán, 04.V.1989, R. H. Fortunato et al. 1093 (BAB). *M. brevipes* Benth.
- PARAGUAY. Amambay: P. N. Cerro Corá, en el cerrado del Cerro Trébol, 05.V.1989, R. H. Fortunato et al. 1128 (BAB). *M. brevipes* Benth.
- BRAZIL. Mato Grosso do Sul: Rod. BR-267, próximo do trevo para Bela Vista, 11.III.2003, G. Hatschbach et al. 74574 (MBM323494). *M. brevipes* Benth.
- ARGENTINA. Corrientes: Santo Tomé, Ruta Nacional 12, entre Posadas e Ituzaingó, 18.III.2013, M. Morales et al. 1357 (BAB). *M. dolens* subsp. *callosa* (Benth.) Barneby.
- PARAGUAY. Caaguazú: Caaguazú, 12.I.1991, E. Zardini et al. 25853 (BAB). *M. dolens* subsp. *callosa* (Benth.) Barneby.
- PARAGUAY. San Pedro: 36 km N de San Estanislao, 18.V.1969, A. Krapovickas et al. 13955 (CTES). *M. cryptogloea* Barneby.
- PARAGUAY. Amambay: Pedro Juan Caballero, 14.II.1951, Schwarz 11900 (CTES91920). *M. custodis* Barneby.
- PARAGUAY. Amambay: Pedro Juan Caballero, 08.II.1951, Schwarz 11802 (LIL371548). *M. custodis* Barneby.
- BRAZIL. Mato Grosso do Sul: Miranda, Trevo Bodoquena-Miranda, 14.V.2009, Morales 793 (BAB, MBM). *M. diversipila* var. *diversipila* Barneby.
- PARAGUAY. Itapúa: Ayolas, Refugio, 01.II.1982, E. Bordas 3019 (CTES 91820). *M. diversipila* var. *subglabriseta* Barneby.
- BRAZIL. Mato Grosso do Sul: Antonio João, Rodovia Amambay-Antonio João, 07.V.2009, M. Morales et al. 772 (BAB). *M. dolens* subsp. *eriphylla* (Benth.) Barneby.
- BRAZIL. Paraná: Guarapuava, Camino en dirección al Cristo, 18.IV.2009, R. H. Fortunato et al. 9479 (BAB). *M. dolens* var. *acerba* (Benth.) Barneby.
- BRAZIL. São Paulo: São Paulo, Ipiranga, 07.II.1908, Swederwaldt 3 (BAB). *M. dolens* var. *acerba* (Benth.) Barneby.
- BRAZIL. Paraná: Palmeira - São Mateus do Sul, 1 km Del cruce BR277 en dirección a São Joao do Triunfo, 25.IV.2009, R. H. Fortunato et al. 9612 (BAB). *M. dolens* var. *acerba* (Benth.) Barneby.
- BRAZIL. Paraná: Palmeira, 02.II.1975, Pedersen 10953 (CTES). *M. dolens* var. *acerba* (Benth.) Barneby.
- BRAZIL. Rio de Janeiro: Capanema (Jardim Bot. do Rio de Janeiro 5267) (SI). *M. dolens* var. *acerba* (Benth.) Barneby.
- PARAGUAY. San Pedro: 1 km N Del Barrio San Pedro, en dirección a Pedro Caballero por ruta 3, 07.III.2009, R. H. Fortunato et al. 9217 (BAB). *M. dolens* var. *anisitsii* (Lindman) Barneby.
- PARAGUAY. Amambay: Pedro J. Caballero, 1.1 km en dirección a Porteira Ortiz, 11.III.2008, R. H. Fortunato et al. 9350 (BAB).
- BRAZIL. Santa Catarina: Campos Novos, 31.I.1963, P. R. Reitz 6395 (SI). *M. dolens* var. *deterior* Barneby. *M. dolens* var. *anisitsii* (Lindman) Barneby.
- BRAZIL. Santa Catarina: Mafra, 12.XII.1962, R. Klein 3931 (SI). *M. dolens* var. *deterior* Barneby.
- BRAZIL. São Paulo: Itararé, Estrada Itararé-Bom Sucesso, próximo a entrada da Fda. São Nicolau, 21.VI.1993, V. C. Souza et al. 2167 (MBM). *M. dolens* var. *deterior* Barneby.
- BRAZIL. São Paulo: São Bento do Sapucaí, 13.IV.1995, J. Y. Tamashiro et al. 882 (SPF98791). *M. dolens* var. *dolens* Vellozo.
- BRAZIL. São Paulo: São Paulo, 05.II.1968, O. Handro 2013 (SPF83076). *M. dolens* var. *dolens* Vellozo.
- BRAZIL. Parana: Punta Grossa, 05.XI.64, L. Dombrowski et al. 802 (SI). *M. dolens* var. *foliolosa* (Benth.) Barneby.
- ARGENTINA. Corrientes: San Miguel, Ruta Provincial 118, a 54 km de la intersección con Ruta Nacional 12, 07.III.2007, M. Morales y J. G. Seijo 277 (BAB). *M. dolens* var. *foliolosa* (Benth.) Barneby.
- BRAZIL. Paraná: Palmeira, Recanto dos Papagaios, 18.IV.2004, R. H. Fortunato et al. 9514 (BAB). *M. dolens* var. *latifolia* (Benth.) Barneby.
- BRAZIL. Mato Grosso: Bataguçu, 15.II.1970, G. Hatschbach 23569 (BAB). *M. dolens* var. *latifolia* (Benth.) Barneby.
- BRAZIL. Paraná: Palmeira, Recanto dos Papagaios, 18.IV.2009, R. H. Fortunato et al. 9516 (BAB). *M. dolens* var. *latifolia* (Benth.) Barneby.
- BRAZIL. Paraná: Palmeira, BR277, 15 km al W de Colonia Quero-Quero, 25.IV.2009, R. H. Fortunato et al. 9597 (BAB). *M. dolens* var. *pangloea* Barneby.
- BRAZIL. Paraná: Lapa, BR-476, 5 km O de Lapa, 30.XI.1986, O. S. Ribas y J. M. Silva 201 (MBM132263). *M. dolens* var. *rigescens* (Benth.) Barneby.
- PARAGUAY. Amambay: Colonia Fortuna Guazú, 09.III.2008, R. H. Fortunato et al. 9279 (BAB). *M. dolens* var. *rudis* (Benth.) Barneby.
- PARAGUAY. Amambay: Colonia Fortuna Guazú, 09.III.2008, R. H. Fortunato et al. 9282 (BAB). *M. sceptrum* Barneby.
- PARAGUAY. Paraguari: Ybicuí, 14.XII.1989, E. M. Zardini 8688 (MO1518284). *M. pseudopetiolaris*.