

Pollen diversity and its phylogenetic implications in *Mimosa* ser. *Leiocarpae* Benth. (Leguminosae, Mimosoideae)

JULIANA SANTOS-SILVA^{1,3}, MARCELO FRAGOMENI SIMON² & ANA MARIA GOULART DE AZEVEDO TOZZI³

¹Departamento de Ciências Humanas – DCH/Campus VI, Universidade do Estado da Bahia, Caetité, Brazil, ²Embrapa Recursos Genéticos e Biotecnologia, PqEB, Brasília, Brazil, ³Departamento de Biologia Vegetal, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, Brazil

Abstract

With approximately 30 species, *Mimosa* ser. *Leiocarpae* is the largest series of *M.* sect. *Batocaulon* and is particularly abundant in semi-arid environments of South America. Previous studies have shown that the pollen type is remarkably congruent with the plastid gene tree and it presents relatively low levels of homoplasy, having utility for phylogenetic reconstruction within *Mimosa*. Pollen morphology of 29 of the 30 species belonging to *Leiocarpae* was studied using scanning electron microscopy to identify diagnostic characters and to test taxonomic and phylogenetic hypotheses. The pollen grains of *Leiocarpae* are dispersed in tetrads or bitetrads, which are decussate, isopolar or heteropolar, compressed or not, elliptical or ellipsoidal outline. The apertures are porate. The ornamentation is areolate-verrucate, and the size of grains varies from 8.5 to 43.7 μm in the longer and 7.1 to 20 μm in the shorter dimension. The pollen morphology does not support the monophyly of *Leiocarpae*, which is in accordance with evidence from molecular phylogenetic studies.

Keywords: bitetrads, palynology, phylogeny, tetrads, areolate-verrucate

Mimosa L. is a monophyletic genus of Mimosoideae comprising about 540 species, 490 of which are native to the Americas, 31 to Madagascar, and four to east Africa and southwest Asia each (Barneby, 1991; Du Puy et al., 2002; Simon et al., 2011). Species of *Mimosa* are recognised by a set of characters including bipinnate leaves with sessile leaflets, spicate or capitulate inflorescences of trimerous to pentamerous flowers, haplostemonous or diplostemonous androecia with white, pinkish or yellow filaments, and anthers without apical glands. The craspedia fruits predominate across the entire genus, but ‘sacelo’ fruits, which are derived from a craspedium that is reduced to one article and a short deciduous replum, are also present (Barneby, 1991).

The most recent classification of *Mimosa* proposed by Barneby (1991) divided the genus into five sections, 41 series and 37 subseries, substantially improving on the previous classifications

of Bentham (1841, 1875). *Mimosa* ser. *Leiocarpae* Benth., the largest series of *M.* sect. *Batocaulon* DC., is characterised by a highly diverse indumentum with glandular, medusiform, stellate, tector and verruciform trichomes; tetramerous flowers on spicate inflorescences; and craspedium-type fruits. This series originally consisted of around 28 species distributed predominantly in semi-arid environments of South America. After Barneby’s (1991) revision, two new species were described for *Leiocarpae*: *Mimosa craspedisetosa* (Fortunato & Palese, 1999) and *M. ferricola* (Silva & Tozzi, 2011); the section now totals 30 species.

Members of *Leiocarpae* are fast-growing trees and shrubs, and many of them occur in highly disturbed areas such as roadside trails, which makes them ideal candidates for restoration of degraded habitats. Moreover, some species have multiple uses. In north-eastern Brazil, the leaves and fruits of

Correspondence: Juliana Santos Silva, Departamento de Ciências Humanas – DCH/Campus VI, Universidade do Estado da Bahia, 46400-000, Caetité, Bahia, Brazil. E-mail: jullybandeira@hotmail.com

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Mimosa ophthalmocentra and *M. tenuiflora* are widely used as animal food, especially in the dry season when there is no pasture, and some are also used as firewood, fencing stakes and popular medicines (Costa et al., 2002). Before the European colonisation, the bark and roots of *M. tenuiflora* were used by indigenous cultures to prepare a hallucinogenic drink called 'vinho de jurema' in northeast Brazil (Souza, 2008) or 'tepescohuite' in Mexico (Camargo-Ricalde, 2000).

Mohl (1835) was the first to study pollen of *Mimosa* with brief comments on *M. latispinosa* Lam. Afterwards, a considerable proportion of the species of *Mimosa*, around 204, has been subjected to detailed pollen-morphological studies (Erdtman 1945, 1952; Van Campo & Guinet, 1961; Barros, 1966; Barth & Yoneshigue, 1966; Guinet, 1969; Sorsa, 1969; Barth, 1973; Grether & Martínez-Bernal, 1996; Caccavari, 1985, 1986a, 1986b, 1987, 1988, 1989; Grether, 1997; Silvestre-Capelato & Melhem, 1997; Martínez-Bernal, 2006; Flores-Cruz et al., 2006; Lima et al., 2008; Buril et al., 2010). These works revealed that *Mimosa* pollen grains can be grouped into tetrads, bitetrads (eight grains) or polyads (12 or 16 grains). Furthermore, differences in the size, shape and exine ornamentation among the species were observed according to the grains arrangement.

Most of the *Mimosa* species studied so far have pollen dispersal units grouped in tetrads. Caccavari (1988, 1989) suggested that polyads with higher numbers of grains probably derived from tetrads, but according to the evolutionary progressions proposed by Barneby (1991), large compound polyads are plesiomorphic and tetrads are more derived.

In a recent study, Simon et al. (2011) investigated the evolution of a set of morphological characters based on a molecular phylogeny of *Mimosa*. They concluded that pollen type (the number of grains per polyad) is remarkably congruent with a plastid gene tree and it presents relatively low levels of homoplasy. Additionally, their results supported the hypothesis of pollen evolution proposed by Barneby (1991). According to Simon et al. (2011), other pollen characters such as shape and size of the polyads, presence of apertures, and structure of the exine, which were not investigated in that study, may also be of taxonomic significance and could be explored further.

The objective of the present work was to study the pollen of the species of *Mimosa* ser. *Leiocarpae*, searching for palynological characteristics that could be used in our revisionary and phylogenetic studies of the group, as well as to test current taxonomic and phylogenetic hypotheses.

Material and methods

The pollen data presented here are based on extensive sampling of all recognised species in *Leiocarpae* except for *Mimosa puberula* Benth., for which pollen material is not available because this species is known only from the types. One to three different specimens of each species were studied. A list of taxa with full voucher data is provided at the end of the paper under 'Specimens investigated'. Anthers from pre-anthesis buds from field collections made by the first author and from herbarium specimens were macerated and mounted on metallic stubs with adhesive tape. Pollen samples were subsequently sputter-coated with gold (Balzers SCD 050) and examined using a Jeol JSM-5800LV scanning electron microscope at the Laboratório de Microscopia Eletrônica of the Universidade Estadual de Campinas. The size of dispersal unit was based on the measurements of 15 to 20 units per sample. Details of pollen morphology were described using the terminology of Punt et al. (2007).

Results

General descriptions of *Mimosa* ser. *Leiocarpae* pollen grains

Pollen morphology of 29 species of *Leiocarpae* is described and summarised (Figures 1–3, Table I). For 18 species, palynological features are presented here for the first time, and for 11 species, previously published pollen morphology data are confirmed. Our results show that *Leiocarpae* pollen grains are grouped in tetrads or (less frequently) in bitetrads, which are isopolar (Figure 1B) or heteropolar, decussate, compressed in the equatorial plane (Figure 1B, C, G, H, I, K) or not (Figures 1A, E, J, 2E, J), ellipsoid (Figures 1E, J, 2E, J) or ellipsoidal (Figures 1B, C, 2G, K) in outline, and with outline in side view intersected (Figures 1A, E, J, 2E, J) or not (Figure 3G, H), rounded (Figure 1B, H) or in the form of the letter x (Figure 1A) or not (Figures 1D, 2I). The size of dispersal unit varied from 8.5 to 43.7 µm in the longer and 7.1 to 20 µm in the shorter dimension. Diads and triads were occasionally found in *Mimosa amnis-atricornis*, *M. coruscocaesia* (Figure 1F), *M. insignis*, *M. pteridifolia*, *M. schomburgkii*, *M. tenuiflora*, *M. trianae* and *M. verrucosa*. (Table I). In some species with ellipsoidal tetrad, we found more often irregular (more or less sigmoidal), tetrahedral and/or tetragonal tetrads (Figures 2L, 3B, 3D, Table I).

The individual pollen grain has a particular form. It can be pyramidal or have two distal surfaces, one pyramidal with a vertex located at the centre of the tetrad, and the other a trapezoid with the smaller side in the centre of the tetrad. The apertures were

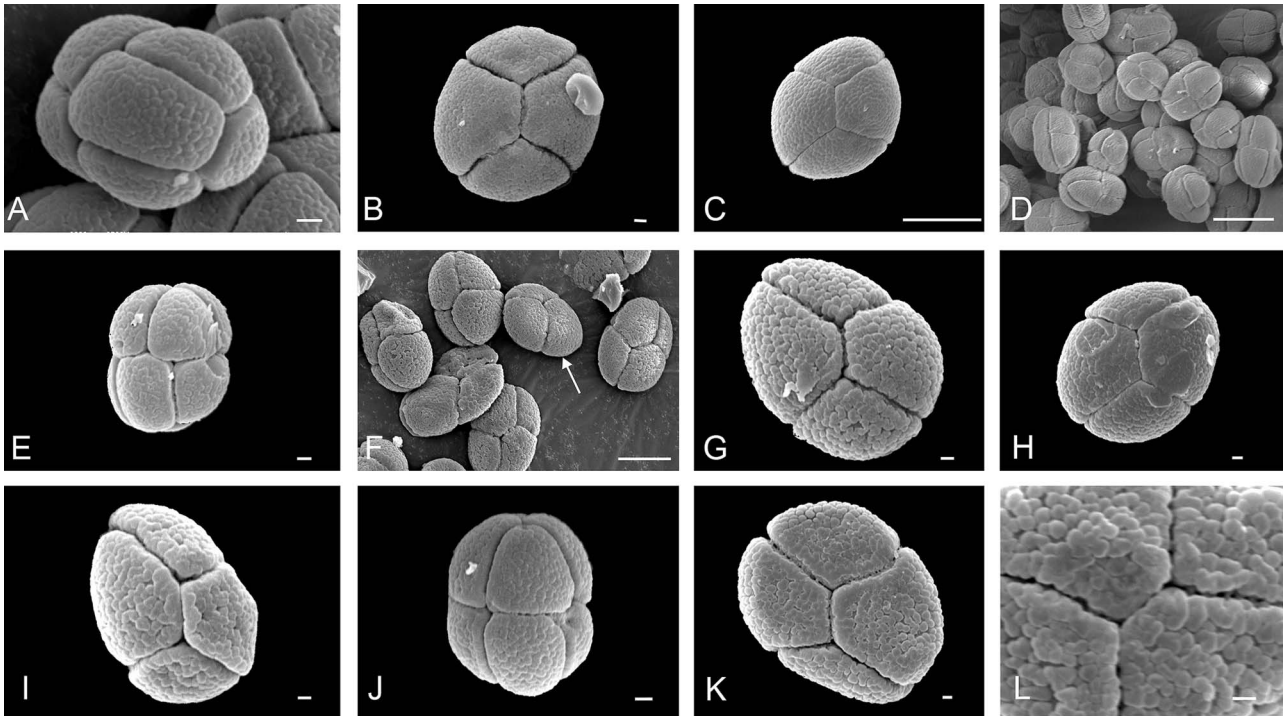


Figure 1. Scanning electron micrographs of ser. *Leiocarpae* pollen grains. **A.** *Mimosa acutistipula*, bitetrad at frontal view. **B.** *Mimosa amnis-atri*, tetrad at frontal view. **C.** *Mimosa apodocarpa*, tetrad at frontal view. **D, E.** *Mimosa arenosa*: **D.** Different grains, frontal and side view; **E.** Bitetrad, frontal view. **F, G.** *Mimosa coruscocaesia*: **F.** Triad and different tetrads, oblique frontal view; **G.** Tetrad, frontal view. **H.** *Mimosa craspedisetosa*, tetrad at frontal view. **I.** *Mimosa dalyi*, tetrad at frontal view. **J.** *Mimosa dichroa*, bitetrad at frontal view. **K, L.** *Mimosa fiebrigii*: **K.** Tetrad at frontal view; **L.** Magnified part of exine. Scale bars – 10 μm (C, D, F), 1 μm (A, B, E, G, H–L).

difficult to see; when seen, they were porate. The exine ornamentation is areolate-verrucate; individual verruca and areola are rounded with different dimensions (0.2–3.3 μm) and densely distributed all over the surface.

Description of the pollen types

Two pollen types are recognised based on polyad shape and grain number in the polyad:

Tetrad type (Figures 1B, C, F–I, K, L, 2A–D, F–I, L, 3A–I, Table I). — Pollen grains grouped predominantly isopolar or heteropolar, compressed in the equatorial plane, decussate, ellipsoidal tetrads; outline in polar view not intersected, rounded; longer diameter 19.0–26.3 μm ; shorter diameter 7.2–9.5 μm ; individual pollen grain having three flat proximal sides and one convex distal side, which determine two distal surfaces: One pyramidal with a vertex located at the centre of the tetrad, and the other a trapezoid with the smaller side in the centre of the tetrad; pores 3–4 in each pollen grain (when seen).

Taxa included. — *Mimosa adenophylla*, *M. amnis-atri*, *M. apodocarpa*, *M. artemisiana*, *M. coruscocaesia*,

M. craspedisetosa, *M. dalyi*, *M. ferricola*, *M. fiebrigii*, *M. gemmulata*, *M. glutinosa*, *M. hebecarpa*, *M. insignis*, *M. interrupta*, *M. nothopteris*, *M. pteridifolia*, *M. schomburgkii*, *M. sericantha*, *M. spixiana*, *M. tenuiflora*, *M. trianae*, *M. verrucosa*, *M. xavantinae*.

Bitetrad type (Figures 1A, D, E, J, 2E, J, K, Table I). — Polyads with eight pollen grains grouped in two decussate tetrads (bitetrads) that are either juxtaposed or rotated up to 45° off its meridian; bitetrads isopolar or heteropolar, ellipsoid, not compressed, outline in polar view intersected in the form of the letter x or not; longer diameter 19.7–24.2 μm ; shorter diameter 7.2–12.8 μm ; individual pollen grains probably with a pyramidal shape whose external side is triangular; pores could not be seen.

Taxa included. — *Mimosa acutistipula*, *M. arenosa*, *M. dichroa*, *M. hapaloclada*, *M. mensicola*, *M. ophthamocentra*.

Discussion

Among the Mimosoideae, *Mimosa* is one of the best studied genera with regard to pollen morphology. The genus contains approximately 540 species (Simon et al., 2011), about 38% of which have been

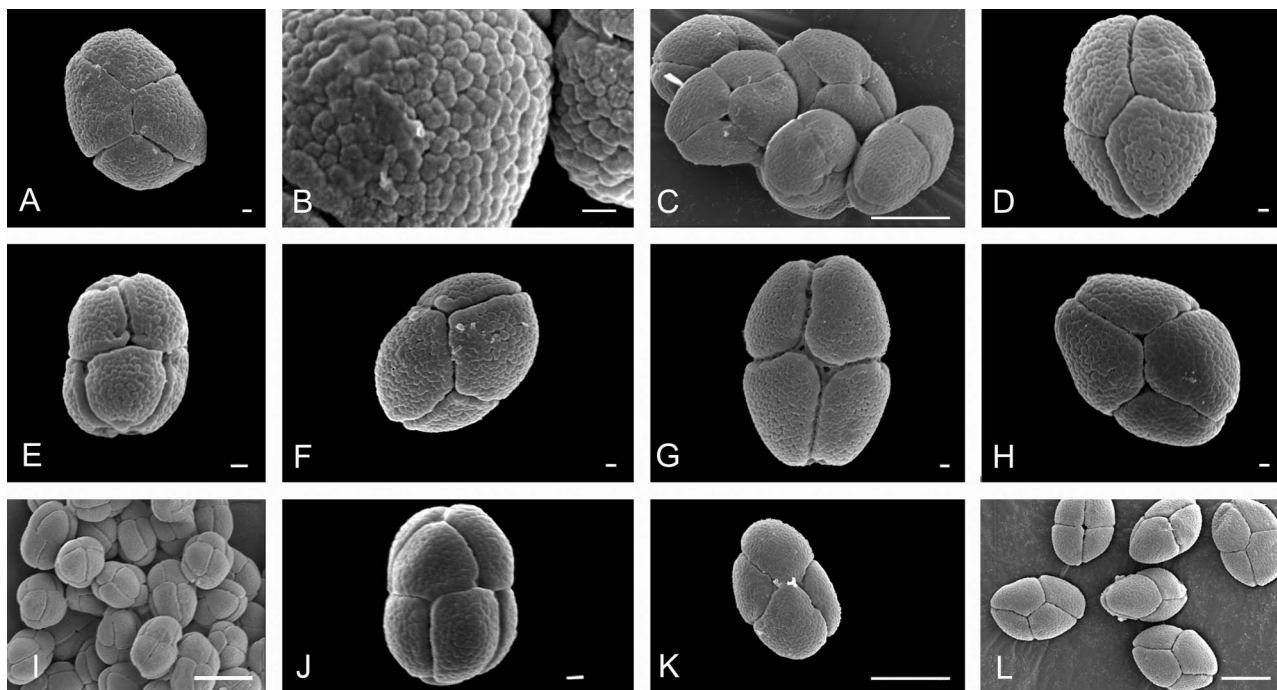


Figure 2. Scanning electron micrographs of ser. *Leiocarpace* pollen grains. **A, B.** *Mimosa gemmulata*: **A.** Tetrad, frontal view; **B.** Magnified part of exine. **C, D.** *Mimosa glutinosa*: **C.** Different tetrads, side and frontal view; **D.** Tetrad, frontal view. **E.** *Mimosa hapaloclada*, bitetrad at frontal view. **F.** *Mimosa hebecarpa*, tetrad at frontal view. **G.** *Mimosa insignis*, tetrad at frontal view. **H.** *Mimosa interrupta*, tetrad at frontal view. **I.** *Mimosa nothopteris*, tetrad at frontal view. **J, K.** *Mimosa ophthalmocentra*: **J.** Different tetrads at side and frontal view; **K.** Bitetrad at frontal view. **L.** *Mimosa schomburgkii*, different tetrads at side view. Scale bars – 10 µm (C, J), 1 µm (A, B, D–I).

analysed to date (Erdtman 1945, 1952; Van Campo & Guinet, 1961; Barros, 1966; Guinet, 1969; Sorsa, 1969; Barth, 1973; Silvestre-Capelato & Melhem, 1997; Caccavari, 1985, 1986a, 1986b, 1987, 1988, 1989; Grether & Martínez-Bernal, 1996; Grether, 1997; Martínez-Bernal, 2006; Flores-Cruz et al., 2006; Lima et al., 2008; Buriel et al., 2010). These studies revealed the group's diverse pollen morphology with respect to grain number per polyad (4, 8, 12 or 16), and polyad size and shape, characterising *Mimosa* as a eurypalynous genus.

Compound pollen units are not commonly observed in angiosperms, occurring only in 56 families (Knox & McConchie, 1986), but in Mimosoideae, mature pollen dispersed in polyads is very frequent. The presence of polyads was widely assumed to confer a selective advantage in reproduction, as it could provide an efficient way of pollen dispersal by insects (Krees, 1981; Kenrick & Knox, 1982). However, Wyatt et al. (2000) considered this hypothesis unlikely, because it would be difficult to understand why composite pollen grains should have originated as a solution to the problem of low pollination efficiency in only a few groups of flowering plants. An alternative hypothesis is that the structure of polyads may protect pollen grains from dehydrating in dry habitats.

In relation to the mechanism of polyad formation within *Mimosa*, only two studies have been performed so far (Caccavari & Galati, 1998; Seijo & Neffa, 2004). Light microscopic and ultrastructural aspects of pollen development and exine formation in *M. balansae* Mich. were described by Caccavari and Galati (1998). According to this study, the mature pollen grains of this species are released as permanent tetrads of very small size (diameter 6 µm). The exine ultrastructure shows a discontinuous granular tectum (areola), a granular infratectal layer and a homogeneous endexine layer, thicker than the ectexine.

Seijo and Neffa (2004) showed that in *Mimosa bimucronata* (DC.) Kuntze many polyads are formed in each pollen sac (*c.* 500) and anther (*c.* 2000). The apical cavity in the stigma has space just sufficient for one single bitetrad. Occasionally, more than one bitetrad have been observed attached to the pistils, but always outside the stigmatic cavity. In this species, the number of pollen grains per polyad (eight) corresponds to the mean number of ovules in the ovaries. Therefore, a single polyad can potentially fertilise all the ovules in an ovary. According to these authors, the number of pollen grains per polyad in *Mimosa* is determined by the number of cells that remain attached after the last mitotic division of

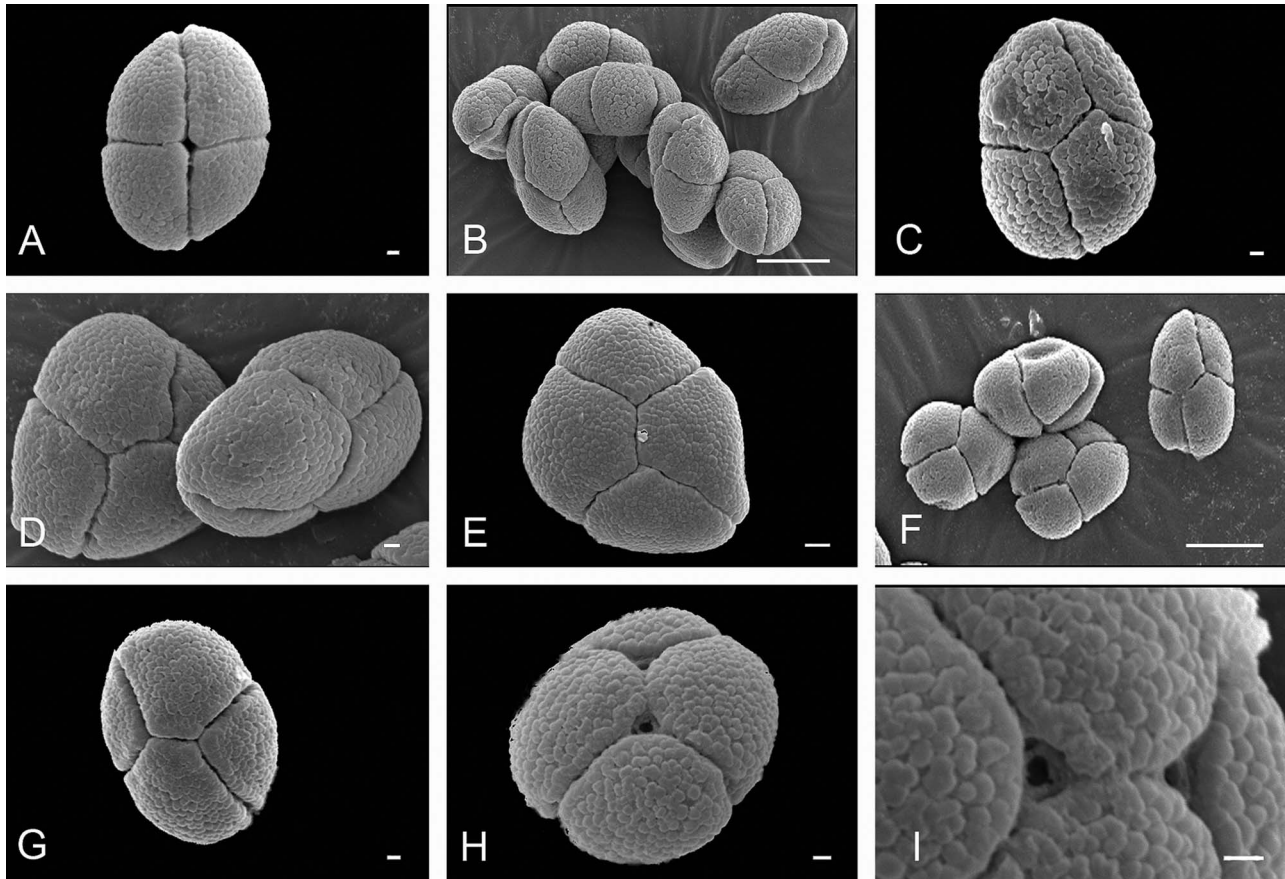


Figure 3. Scanning electron micrographs of ser. *Leiocarpae* pollen grains. **A.** *Mimosa schomburgkii*, tetrad frontal view. **B., C.** *Mimosa sericantha*: **B.** Different tetrads, side and frontal view; **C.** Tetrad, frontal view. **D., E.** *Mimosa spixiana*: **D.** Different tetrads, side and frontal view; **E.** Tetrad, frontal view. **F., G.** *Mimosa tenuiflora*: **F.** Triad and different tetrads, side and frontal view; **G.** Tetrad, frontal view. **H., I.** *Mimosa verrucosa*: **H.** Tetrad, frontal view; **I.** Detail of pores. Scale bars – 10 µm (B), 1 µm (A, C–I).

the sporogenous tissue, and not by the number of pollen mother cells per locule, as described in *Acacia* (Kenrick & Knox, 1982).

Pollen morphology was largely ignored in the various infrageneric classifications proposed for *Mimosa*. Caccavari (1988) was probably the first author to use pollen morphology as a taxonomic character in the genus. Subsequently, several studies investigated the relationship between pollen morphology and *Mimosa* classification (Caccavari, 1986a, 1987; Flores-Cruz et al., 2006; Lima et al., 2008). According to Simon et al. (2011), the non-monophyly of many of the infrageneric groups investigated in such studies have made it difficult to establish a correlation between palynological characteristics and infrageneric classification.

With a few exceptions, the pollen types recognised for the species of *Mimosa* corresponded to the infrageneric classification proposed by Barneby (1991). Furthermore, pollen evolution is remarkably congruent with the plastid gene tree and presents relatively low levels of homoplasy (Simon et al., 2011).

Polyads with 12 to 16 pollen grains are restricted to *Mimosa* sect. *Mimadenia* Barneby (Caccavari, 1987; Lima et al., 2008); they are morphologically very similar to the polyads observed in some species of *Piptadenia* Benth., making it difficult to distinguish these two groups based on pollen alone (Caccavari, 1987). The presence of this polyad type was interpreted by Barneby (1991) and by Simon et al. (2011) as the ancestral character state connecting *Mimosa* to other genera of the *Piptadenia* group, which also retain this character state. Tetrads and bitetrads can be found in section *Batocaulon*, whereas species of *M.* sect. *Calothamnos* Barneby, *M.* sect. *Habbasia* DC. and *M.* sect. *Mimosa* L. have the pollen grains arranged in tetrads.

With approximately 30 species, *Mimosa* ser. *Leiocarpae* sensu Barneby (1991) is the largest series in the section *Batocaulon*, and it is particularly abundant in the west-central and northeast regions of Brazil (about 24 spp.). It was defined by its high diverse trichome types (glandular, medusi-form, stellate, tector, verruciform), tetramerous and diplostemonous flowers in spicate inflorescences

and the fruit as a craspedium. However, this group is very heterogeneous: There is variation in the indumentum of the leaflets (eglandular or charged with either globose superficial or impressed glands which appear as minute glandular dots or scales), calyx shape (4-angulate, campanulate or crateriform), filament colour (white or pink) and fruit shape (plano-compressed or bluntly tetragonal craspedium). Barneby (1991) further divided *Leiocarpae* into five subsets of species based on morphological differences observed in vegetative and reproductive structures (Table II). According to this author, most of these subsets can be easily identified and might deserve some taxonomic status.

Palynological data obtained here, in addition to the molecular and morphological data, show that Barneby's (1991) division of the series is artificial (Table II). Of the five subsets, only one is supported by our results (group 1 in Table II). All species of group 1 share eglandulate leaflets and plano-compressed craspedia as well as polyads with eight

pollen grains, grouped into two decussate tetrads that are either juxtaposed or rotated up to 45° off its meridian.

A molecular phylogenetic analysis based on DNA sequences of the *trnD-trnT* non-coding plastid locus suggests that *Leiocarpae* is not monophyletic (Simon et al., 2011). In this work, the 16 sampled species were recovered as part of two clades (Figure 4). The first clade, nested in a larger clade that also contains Old World species, included three species of *Leiocarpae* (*Mimosa acutistipula*, *M. arenosa* and *M. ophthalmocentra*) that have aculei, white flowers and lack leaflet glands. Other species present in this clade are: sect. *Batocaulon* series *Caesalpinifoliae* Benth. (*M. caesalpinifolia* Benth. and *M. laticifera* Rizzini et A. Mattos), *Ceratoniae* Barneby (*M. ceratonia* L.) and *Bimucronatae* Barneby (*M. bimucronata* and *M. pseudosepiaria* Harms) and *Farinosae* Barneby (*M. farinosa* Griseb.). The second clade contained most sampled species of *Leiocarpae* and is mainly characterised by unarmed species with pink flowers and glands on leaflets.

Table I. Pollen morphological features of the taxa studied of *Mimosa* ser. *Leiocarpae*.

Species	N	LD	SD	SH	P	F
<i>M. acutistipula</i>	8	16(17.9)20	11(12.1)15	ellipsoid	H	1A
<i>M. adenophylla</i>	4	19(22)25	14(15.5)17	ellipsoidal, compressed	I	
<i>M. amnis-atric</i>	(3) 4	11.3(12.7)17.5	10(13.1)16.5	ellipsoidal, compressed	(H) I	1B
<i>M. apodocarpa</i>	4	12.1(14.2)18.7	10.7(14.5)17.1	ellipsoidal, compressed	I	1C
<i>M. arenosa</i>	8	13(14.5)16.3	8(9.1)11	ellipsoid	H, I	1D, E
<i>M. artemisiana</i>	4	14(25)39	11(15.5)20	ellipsoidal, compressed	I	
<i>M. coruscocoesia</i>	(3) 4	17.8(19.7)22.5	12.5(13.8)15.6	ellipsoidal, compressed	(H) I	1F, G
<i>M. craspedisetosa</i>	4	17.1(18.5)23.3	12.5(15.3)18.6	ellipsoidal, compressed	I	1H
<i>M. dalyi</i>	4	14.8(15.6)17	11.8(13)14	ellipsoidal, compressed	I	1I
<i>M. dichroa</i>	8	10(10.5)11	8.5(8.9)9	ellipsoid	H, I	1J
<i>M. ferricola</i>	4	10.5(12)13	8(9.1)9.5	ellipsoidal, compressed	I	
<i>M. fiebrigii</i>	4	15.6(17.1)20.5	10.6(14.5)16.5	ellipsoidal, compressed	I	1K, L
<i>M. gemmulata</i>	4	19(22.1)25	15(17)20	ellipsoidal, compressed	I	2A, B
<i>M. glutinosa</i>	4	16.8(18.5)20	10(12.8)14.5	ellipsoidal, compressed	I	2C, D
<i>M. hapaloclada</i>	8	19.7(22.1)24.2	10(15.5)17.7	ellipsoid	H	2E
<i>M. hebecarpa</i>	4	17.2(18.1)22	13(14)15.2	ellipsoidal, compressed	I	2F
<i>M. insignis</i>	(3) 4	13.5(16.6)20	11.2(12.5)13.1	ellipsoidal, compressed	(H) I	2G
<i>M. interrupta</i>	4	15(17.5)19	9(10.5)13.5	ellipsoidal, compressed	I	2H
<i>M. mensicola</i>	8	13(14.6)17	11(11.5)12	ellipsoid	I	
<i>M. nothopteris</i>	4	10.5(16.5)18.4	10(13)15	ellipsoidal, compressed	I	2I
<i>M. ophthalmocentra</i>	8	11.1(15.3)19.6	7.1(10.5)12.8	ellipsoid	H	2J, K
<i>M. pteridifolia</i>	(2,3) 4	19(21.2)26.3	14(16.5)18.1	ellipsoidal, compressed	(H) I	
<i>M. schomburgkii</i>	(2,3) 4	17.5(30)43.7	12.5(16.1)20	(tetragonal, tetrahedral) ellipsoidal, compressed	(H) I	2L, 3A
<i>M. sericantha</i>	4	17.2(23)31	10(11.9)13	(tetrahedral) ellipsoidal, compressed	(H) I	3B, C
<i>M. spixiana</i>	4	17.5(18.2)22.5	13.5(16)18	(tetrahedral) ellipsoidal, compressed	(H) I	3D, E
<i>M. tenuiflora</i>	(2,3) 4	11(15.5)17	7.8(9)10	ellipsoidal, compressed	(H) I	3F, G
<i>M. trianae</i>	(2,3) 4	13.2(15.5)20	12.9(14)15	ellipsoidal, compressed	(H) I	
<i>M. verrucosa</i>	4	15.6(17.9)21	8(11.9)17.4	ellipsoidal, compressed	I	3H, I
<i>M. xavantinae</i>	(3) 4	10(14.5)16.7	9(13)15	ellipsoidal, compressed	I	

Note: All measurements in micrometres (µm); N, number of pollen grains per polyads; LD, longer diameter; SD, shorter diameter; SH, shape of polyads; P, polarity; H, heteropolar; I, isopolar; F, figure number. The values indicated in parentheses correspond to the average.

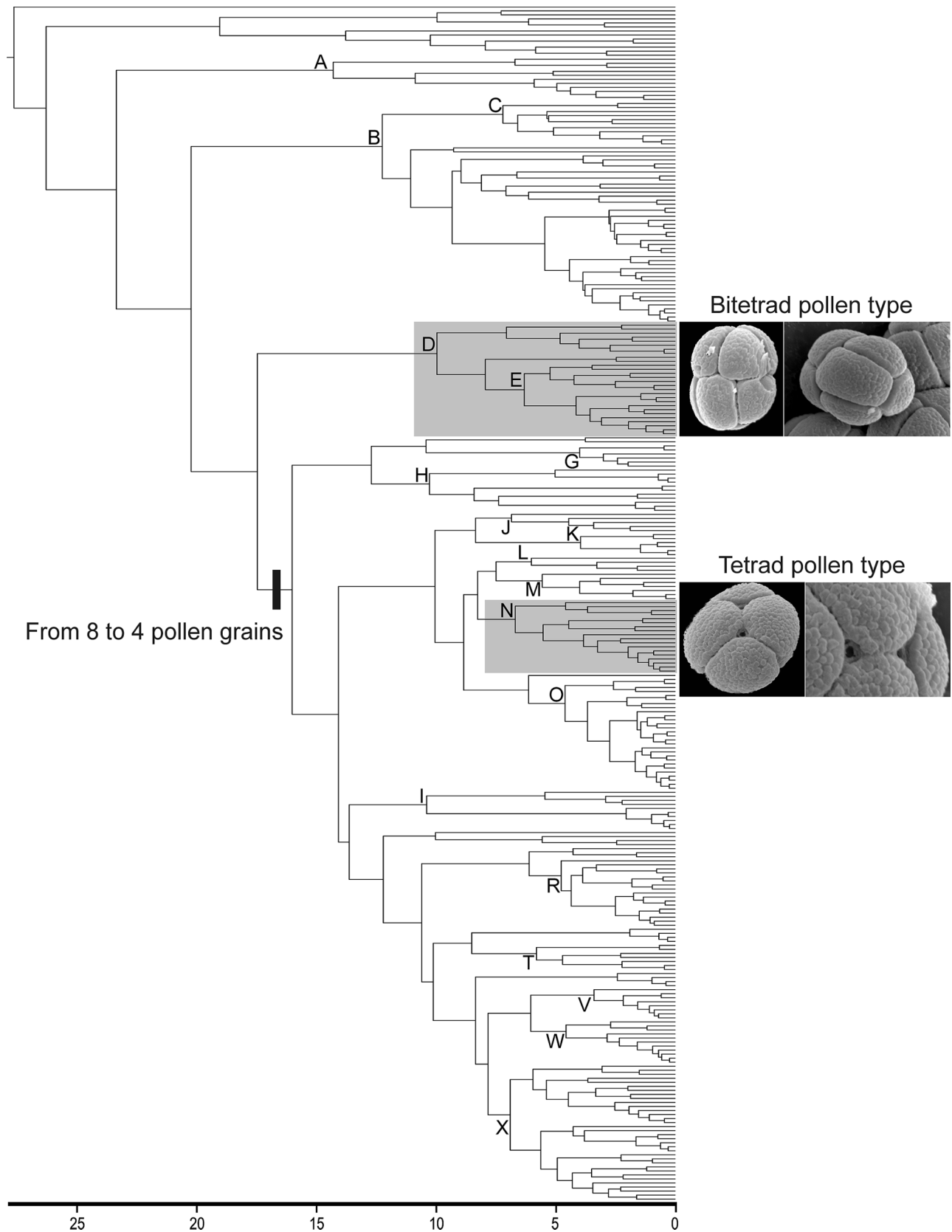


Figure 4. Molecular phylogeny of the genus *Mimosa* (chronogram), showing that the placement of species of ser. *Leiocarpace* in two distinct groups (clades D and N) is supported by pollen morphology. Ellipsoid bitetrads occur in clade D, whereas compressed tetrads predominate in clade N. The vertical bar shows the shift in polyad morphology from bitetrads to tetrads, hypothesised to have occurred only once in *Mimosa*. Modified from Simon et al. (2011). Scale bar in million years.

The pollen data obtained in our study as well as by other authors (Caccavari, 1988; Grether, 1997; Lima et al., 2008; Buril et al., 2010) do not support the monophyly of *Leiocarpae*, providing additional evidence for the grouping found by Simon et al. (2011) (Figure 4). This series is highly heterogeneous with respect to number of grains per polyad and polyad size and shape, and two obvious groups were observed (Figure 4). The first group has pollen grains arranged in bitetrads (*Mimosa acutistipula*, *M. arenosa*, *M. dichroa*, *M. hapaloclada*, *M. mensicola* and *M. ophthalmocentra*). These species are more closely related to each other and to other taxa present in clade D than to other members of *Leiocarpae*. In addition to pollen type, they share the absence of glands on the abaxial leaflet surface and the usually white filaments. The other group comprises 22 species with pollen in tetrads, which is a derived character state in *Mimosa*. All members of this group have glands on the abaxial leaflet surface that appear as minute glandular dots or scales, and predominantly pink filaments. Most of the species are part of clade N, which also includes *M. lewisii* Barneby (ser. *Bimucronatae*), which has the pollen grains also grouped in tetrads (Lima et al., 2008) but lacks glands on the leaflets and its flowers are in squatly ovoid or hemispherical capitula (versus flowers on spicate inflorescences in *Leiocarpae*).

Areolate-verrucate ornamentation of the exine was constant within *Leiocarpae*. The individual verrucae and areolae are rounded, of different sizes and densely distributed all over the surface. This pattern is also common in many other species of the genus *Mimosa* (Guinet, 1969; Sorsa, 1969; Barth, 1973; Caccavari, 1985, 1986a, 1986b, 1988; Silvestre-Capelato & Melhem, 1997).

Lima et al. (2008) analysed 39 species of *Mimosa* from northeast Brazil and observed some exceptions to the general ornamentation pattern: small granules on verrucae on the exine of *M. filipes* Mart., *M. gemmulata* and *M. invisita* Mart. ex Colla, and orbicular granules of sporopollenin on areolae of *M. ursina* Mart. These structures were not found in the sampled species of *Leiocarpae*.

The number of pollen grains per polyad is an important feature of *Leiocarpae* pollen. A small number of species in this series showed polyads with eight pollen grains. Bitetrads have been reported in some species of sect. *Mimadenia* (*Mimosa* ser. *Glanduliferae* Benth., *M. ser. Revolutae* Barneby), and are relatively common in sect. *Batocaulon* (36% of 70 spp. sampled). Ancestral state reconstruction of pollen type suggests that bitetrads – the most common state in the outgroup – are the ancestral state in *Mimosa* (Simon et al., 2011). This study also indicates that a single reversal from tetrads to

Table II. Comparative morphology of the five subsets of species of *Mimosa* ser. *Leiocarpae* sensu Barneby (1991).

	Group 1	Group 2	Group 3	Group 4	Group 5
Species	<i>M. acutistipula</i> <i>M. arenosa</i> <i>M. dichroa</i> <i>M. hapaloclada</i> <i>M. mensicola</i> <i>M. ophthalmocentra</i>	<i>M. dalyi</i> <i>M. insignis</i>	<i>M. tenuiflora</i>	<i>M. adenophylla</i> <i>M. amnis-atric</i> <i>M. apodocarpa</i> <i>M. coruscocoesia</i> <i>M. fiebrigii</i> <i>M. gemmulata</i> <i>M. glutinosa</i> <i>M. hebecarpa</i> <i>M. interrupta</i> <i>M. nothopteris</i> <i>M. pteridifolia</i> <i>M. sericantha</i> <i>M. spixiana</i> <i>M. xavantinae</i>	<i>M. artemisiana</i> <i>M. schomburgkii</i> <i>M. trianae</i> <i>M. verrucosa</i>
Leaflet	eglandular	eglandular	charged with globose superficial of impressed lenslike glands	charged with globose superficial of impressed lenslike glands	charged with globose superficial of impressed lenslike glands
Calyx	campanulate or crateriform	campanulate or crateriform	4-angulate	campanulate or crateriform	tubulate
Corolla	not stellate	not stellate	not stellate	not stellate	usually stellate
Pollen type	bitetrad	Tetrad	tetrad	tetrad	tetrad
Pod	plano-compressed	bluntly tetragonal with dilated replum	valves of craspedium bullately dilated	plano-compressed or not, valves of craspedium not bullate	plano-compressed or not, valves of craspedium not bullate

bitetrads is hypothesised to have occurred in *Mimosa* about 0.5 Mya (*M. sinaloensis* Britton et Rose, clade G; Figure 4; Simon et al., 2011). Dispersal units grouped in tetrads occur in over 70% of the studied species of *Leiocarpaceae*. This pollen type is predominant in *Mimosa* as verified in a large group of species (135 of the 204 studied species; Erdtman, 1945, 1952; Van Campo & Guinet, 1961; Barros, 1966; Barth & Yoneshigue, 1966; Guinet, 1969; Sorsa, 1969; Barth, 1973; Silvestre-Capelato & Melhem, 1997; Caccavari, 1985, 1986a, 1986b, 1987, 1988, 1989; Flores-Cruz et al., 2006; Lima et al., 2008; Buril et al., 2010).

The size of the polyads of *Leiocarpaceae* varies among the different species as well as within species. The smallest polyads were observed in *Mimosa dichroa*, with the longer dimension measuring 10–11 µm and the shorter measuring 8.5–9.0 µm (Figure 1J). In contrast, a specimen from *M. schomburgkii* has the largest pollen grains, with the longer dimension measuring 17.5–43.75 µm and the shorter measuring 12.5–20.0 µm (Figures 2L, 3A). Our measurements agree with the results of previous studies (Lima et al., 2008).

The dyads and/or triads verified among the predominantly tetrads of *Mimosa amnis-atrui*, *M. coruscocaesia* (Figure 1F), *M. insignis*, *M. pteridifolia*, *M. schomburgkii*, *M. tenuiflora*, *M. trianae* and *M. verrucosa* have probably been caused by genetic abnormalities during pollen development process, or they can be developmental deformities. The presence of dyads in *Mimosa* has been reported previously by Lima et al. (2008) in *M. adenophylla* and *M. ursina*, but we think that tetrads are the predominant pollen type in these species.

Within species of the tetrad type, intraspecific variation in the shape and arrangement of grains were observed. In some species with ellipsoidal tetrad, we found more often irregular tetrads, which are more or less sigmoidal, but sometimes, we also observed tetrahedral and/or tetragonal tetrads (Figures 2L, 3B, D). This irregular arrangement of tetrads has been observed by Caccavari (1985) in species of *Mimosa* sect. *Habbasia* (e.g. *M. pigra* L.), who considered that this variation can represent different evolutionary line.

Conclusion

Mimosa ser. *Leiocarpaceae* is remarkably diverse in its pollen morphology. Our results did not support the monophyly of this group, but instead provided additional evidence for the phylogenetic hypotheses obtained in molecular phylogenetic studies. We could not identify any pollen morphological synapomorphies for the group as a whole, casting doubt on

the validity of this infrageneric group. This study also confirmed that the number of pollen grains per polyad and polyad shape are valuable characters in the characterisation of *Mimosa* species.

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Specimens investigated

- Mimosa acutistipula* (Benth.) Mart. Brazil: Bahia, Barreiras. 13 May 1997; F. França 2262 (HUEFS); Piauí, Oeiras. July 1987; A. J. Castro s.n. (UEC 46098).
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- Mimosa amnis-atrui* Barneby. Brazil: Goiás, Mineiros. 9 April 2011, J. S. Silva 976 (UEC); Chapadão do Céu. 7 July 1999; M. Batalha 3589 (UB).
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- Mimosa artemisiana* Heringer et Paula. Brazil: Distrito Federal, Brasília. 15 May 1991; E. P. Heringer 6858 (UEC); Minas Gerais, Itaobim. 22 May 1978; H. F. Leitão Filho 7922 (UEC).
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- Mimosa craspedisetosa* Fortunato et Palese. Bolivia: Santa Cruz. 7 February 1998; A. Fuentes and G. Navarro 2195 (NY).
- Mimosa dalyi* Barneby. Bolivia: Chiquitos. 23 November 1989; M. Nee 37871 (NY).
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- Mimosa fiebrigii* Hassl. Brazil: Mato Grosso do Sul, Porto Murtinho. 10 March 2011; J. S. Silva and G. H. Shimizu 911 (UEC); idem, 24 March 1998; S. A. Faria and H. C. Lima s.n. (SMF 1432).
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