



## Morphology of the megaspore *Lagenosporites magnus* (Chi and Hills 1976) Candilier et al. (1982), from the Carboniferous (lower Mississippian: mid-upper Tournaisian) of Bolivia

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**Abstract:** The morphology and structure of megaspores assigned to *Lagenosporites magnus* from the Toregua Formation, Retama Group, mid-upper Tournaisian of Bolivia were studied. The analysis was performed with light, fluorescence and scanning electron microscopy. Megaspores were laterally compressed and presented a spherical body with a proximal gula, of the hologula type. Gula had verrucae ornamentation and the spore body presented complex processes consisting of a bulbous base and an internally partitioned projection with sharp apex. In addition to this main ornamentation, perforations were present throughout the spore surface. Megaspores showed well marked *curvaturae perfectae* due to the abrupt transition existing between the gula ornamentation and the spore body processes. These megaspores were assigned to heterosporous arborescent lycopsids of the Lepidocarpaceae family, as in section view, exospore structure presented a three-dimensional network of fused elements. Likewise, due to a similarity found between sporoderm and *Isoetes* L. structure, it is evident that megaspores structure has remained intact inside the heterosporous lycopsids. Therefore; the *L. magnus* structure not only would confirm its affinity with the Lycophyta fossils but also with the living ones.

**Key words:** Bolivia, Carboniferous, lycopsids, megaspores, Tournaisian.

### INTRODUCTION

Heterospory is one of the most important innovations in the history of plants since the Devonian (Traverse 2007). Heterospory earliest megafossil evidence is represented by *Chaleuria cirrosa* Andrews et al. from the Emsian (Andrews et al. 1974), whereas,

in the fossil record of dispersed spores, megaspores are known from the Pragian-Emsian (McGregor and Camfield 1976, Richardson and McGregor 1986). From the Emsian and throughout the Devonian, it is observed an increase in megaspores diversity (Scott and Hemsley 1996), but it is in the Carboniferous that a remarkable rise has been recorded (Braman and Hills 1980).

Compared to Pennsylvanian epoch, there is still limited information available about dispersed megaspores from the Mississippian due to a paucity

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of described assemblages (Scott and Hemsley 1996, Arioli et al. 2007, Wellman et al. 2009). There are few publications on Tournaisian megaspores from different regions of the world (e.g., Chaloner 1954, Winslow 1962, Alvin 1966, Mortimer et al. 1970, Allen and Robson 1981, Hills et al. 1984, Scott and Meyer-Berthaud 1985, Hemsley et al. 1994, Glasspool et al. 2000).

Taking into consideration Tournaisian assemblages, two groups of megaspores can be differentiated: (1) those linked to the Upper Devonian, dominated by small forms characterized by the presence of grapnel-tipped processes (e.g., Allen and Robson 1981, Candilier et al. 1982, Higgs and Scott 1982), and (2) those linked to the Pennsylvanian, dominated by larger forms (Arioli et al. 2007, Wellman et al. 2009). It is in this second group that the gulate megaspores, which are characterized by having a well-developed apical prominence or gula are found.

Megaspores presenting gula have been studied by certain authors (e.g., Dybová-Jachowicz et al. 1979, 1982, 1984, 1987, Candilier et al. 1982, Archangelsky et al. 1989, Cúneo et al. 1991, Ricardi-Branco et al. 2002, Arioli et al. 2007, Wellman et al. 2009, Steemans et al. 2011) and even principles for their classification have been proposed (Dybová-Jachowicz et al. 1979). There are several genus within the gulate megaspores, *Infraturma Gulati* (Bhardwaj 1957), such as *Lagenicula* and *Lagenosporites*, which have been well documented and studied at an ultrastructural level. However, only a few species from Upper Devonian-Mississippian have been fully studied (e.g., Glasspool et al. 2000, Arioli et al. 2007, Wellman et al. 2009), as most of them either have only been briefly described (e.g., Candilier et al. 1982), or have been focused on Pennsylvanian species (e.g., Thomas and Blackburn 1987, Scott and Hemsley 1993).

The aim of this work is to study northern Bolivia *Lagenosporites magnus* mid- upper Tournaisian

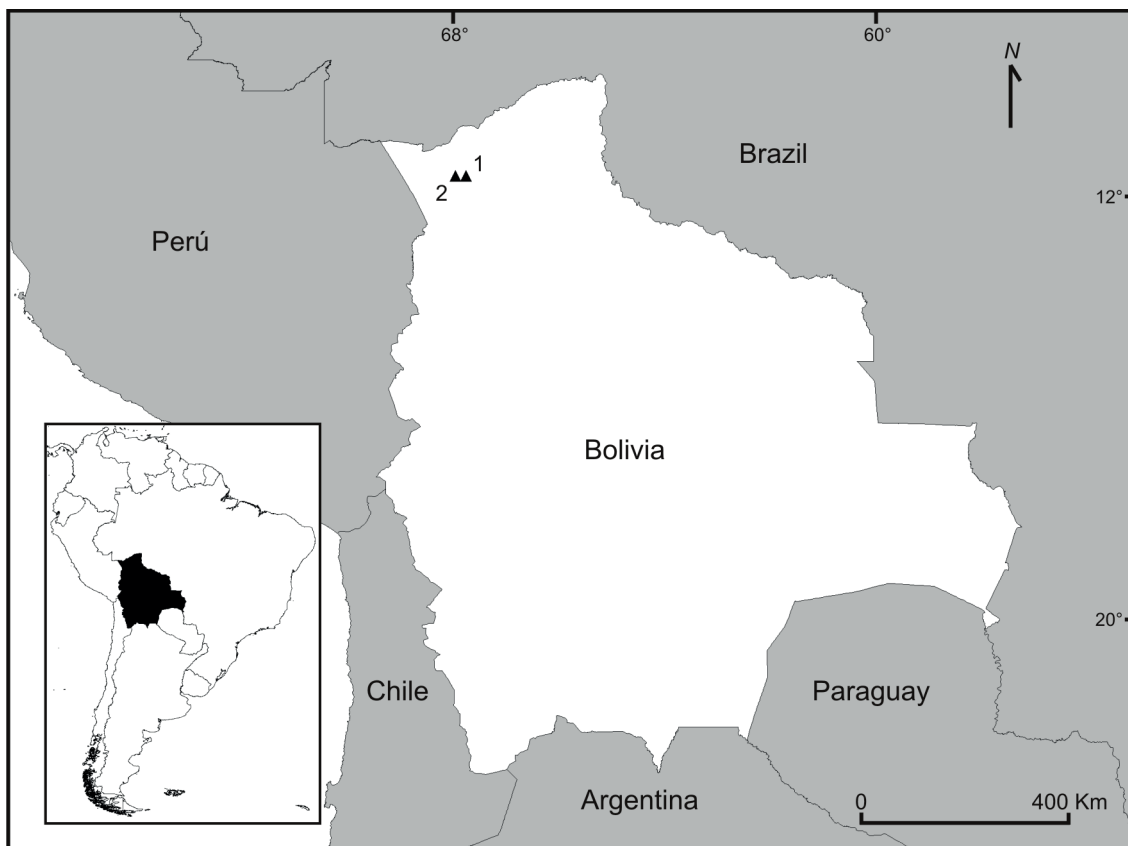
gulate megaspore morphology and; to achieve this, optical, fluorescence, and scanning electron microscopy were used. This study on Mississippian megaspore diversity was carried out to contribute with information that was not available, especially because no research on megaspores for this region and time period has ever been made.

## MATERIALS AND METHODS

### SAMPLES PROVENANCE

Megaspores were recovered in palynological samples from Pando X-1 (11° 36' 07" S, 67° 56' 45" W) and Manuripi X-1 wells (11° 36' 01" S, 68° 08' 55" W), Madre de Dios Basin, Bolivia (Fig. 1). Both boreholes were drilled in 1991 by Mobil Boliviana de Petroleos Inc. and Occidental Boliviana Inc., as continuous crowns from the Devonian/Silurian to the Pennsylvanian. Geological information-including descriptions of wells and palynological and chronological results- has been previously published (Isaacson et al. 1995, Vavrdová et al. 1996, Vavrdová and Isaacson 1996, di Pasquo 2009, 2015, di Pasquo et al. 2015, 2016, Quetglas et al. 2017).

The studied material consisted of 12 samples obtained between 1360 and 1240 m depth from the Pando X-1 well (CICYTTP 734, 731 and 729) and between 1535 and 1483 m depth from the Manuripi X-1 well (CICYTTP 580, 579, 578, 577, 576, 575, 574, 573 and 572), corresponding to the Toregua Formation (di Pasquo 2015). It is considered that these deposits have been influenced by marine-glacial and deltaic sedimentation (Isaacson and Díaz Martínez 1995). Palynological assemblages that contained the studied megaspores were similar in their composition and preservation in both boreholes (di Pasquo 2015). The presence of dispersed miospores such as *Vallatisporites ciliaris*, *Granulatisporites granulatus*, *Reticulatisporites waloweekii*, *Dibolisporites setigerus*, *Crassispora scrupulosa* and *Cristatisporites echinatus*,



**Figure 1** - Map of the study area and boreholes location: 1- Pando X-1 (11° 36' 07" S, 67° 56' 45" W) and 2- Manuripi X-1 (11° 36' 01" S, 68° 08' 55" W).

indicated a mid-upper Tournaisian age (di Pasquo 2015, di Pasquo et al. 2015).

#### PREPARATION AND TECHNIQUES

In order to prevent megaspores destruction, samples were exposed to a 'gentler' laboratory treatment (see Steemans et al. 2009). For each sample, 20-30 g of sediment were crushed in sizes greater than 5 mm and macerated using 30% HCl for 8 hours followed by 45% HF for 48 hours. To eliminate the acid from the samples (neutralization) several washes with distilled water were carried out. Then, residues were sieved using a 25  $\mu\text{m}$  mesh. Megaspores were picked directly from the residue under a stereoscopic microscope and mounted on glass slides for light microscope (LM) observation as well as with fluorescence. Those megaspores that presented,

either ornamentation that could not be determined with LM, or fractures in their wall were selected for a more detailed study with fluorescence and scanning electron microscopy (SEM). For SEM observation, megaspores were mounted with glue on stubs. Two types of microscopes were used, a JEOL JSM 6360 LV from Facultad de Ciencias Naturales y Museo (UNLP) - in this case samples were coated with gold - and a JENCK PHENOM PRO from Centro de Investigaciones Científicas y Transferencia de Tecnología (CONICET-Entre Ríos-UADER) - in this case samples did not require to be coated.

Megaspores size was obtained from the measurements made with LM and through the program ImageJ version IJ 1.46r (Ferreira and Rasband 2012). The maximum and minimum values were expressed in micrometers ( $\mu\text{m}$ ).

In this contribution, it was followed Paleozoic megaspores classification (Potonié 1893, Dybová-Jachowicz et al. 1979) and it was used Dybová-Jachowicz et al. (1979) and Punt et al. (2007) terminology.

Examined specimens are placed in the Laboratorio de Palinoestratigrafía y Paleobotánica (CICYTTP-CONICET-Entre Ríos-UADER) under the acronyms CICYTTP-M for the megaspores (di Pasquo and Silvestri 2014). The catalogue numbers are as follows: CICYTTP-M112, 119, 122, 129, 130, 136, 139, 140, 141, 144, 151, 152, 162, 163, 380 and 428.

## RESULTS

Anteturma SPORITES Potonié 1893

Turma TRILETES (Reinsch 1881) Potonié and Kremp 1954

Subturma LAGENOTRILETES Potonié and Kremp 1954 emend. Bhardwaj 1957

Infraturma GULATI Bhardwaj 1957

Genus LAGENOISPORITES (Potonié and Kremp 1954) Dybová-Jachowicz et al. 1979

*Lagenosporites magnus* (Chi and Hills 1976)

Candilier et al. 1982

Figures 2-4.

## DESCRIPTION

The 16 *Lagenosporites magnus* specimens examined were laterally compressed and presented a spherical body (distal surface) with a gula (proximal surface) of the hologula type (Fig. 2a-f). The contact areas were limited proximally by the laesurae and distally by the curvaturae perfectae, a curved line joining the laesurae ends (Fig. 2e-f). The overall length (including gula) ranged from 290-510  $\mu\text{m}$  and the width of the body ranged from 184-390  $\mu\text{m}$ , according to the equatorial axis.

The gula ranged from 80-280  $\mu\text{m}$  in height and from 85-332  $\mu\text{m}$  in width in its base. The gula had a verrucae ornamentation which was 2-7  $\mu\text{m}$  tall

(Fig. 3a-b) being either separated from each other or laterally fused. It was only present in contact areas and absent in laesurae lips (Fig. 3b).

The megaspore body surface was covered by complex processes formed by a 5-9  $\mu\text{m}$  diameter bulbous base with a spheroid outline and an internally partitioned projection with an acute apex, which was generally curved like a hook (Fig. 3c-e). These processes were 5-19  $\mu\text{m}$  tall and 4-7  $\mu\text{m}$  width. Ornamentation distribution varied from one specimen to another. Specimens that presented dense ornamentation throughout their megaspore body surface usually had their process basal parts fused together. Other specimens presented a denser ornamentation near the curvaturae perfectae area, which was well marked by the abrupt transition existing between the gula ornamentation verrucae and the main body complex processes. Likewise, in this area, ornamentation constituted a well-differentiated 'necklace' formed by complex processes fused together in their base (Fig. 3d-e).

Megaspore gula and the body surface showed perforations of different sizes (Fig. 3f). These perforations did not affect the ornamentation bases and were not present in the laesurae lips.

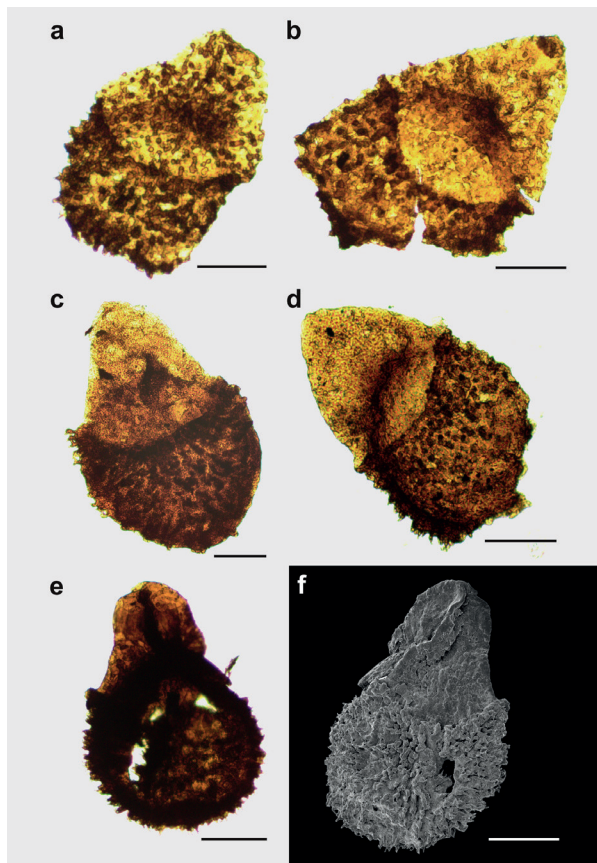
It was after observing a fracture in one gula specimen (CICYTTP-M 136; Fig. 4a-b) that the exospore outermost layer could be identified. This layer presented a spongy structure formed by a three-dimensional network of fused rodlets, which were circular in section delimiting heterogeneous spaces (Fig. 4c-d). These anastomosed rodlets were arranged in different levels having diverse orientations. Rodlets ranged from 0.2-0.4  $\mu\text{m}$  width.

## DISCUSSION

### COMPARISONS AND LYCOPSID BOTANICAL AFFINITY

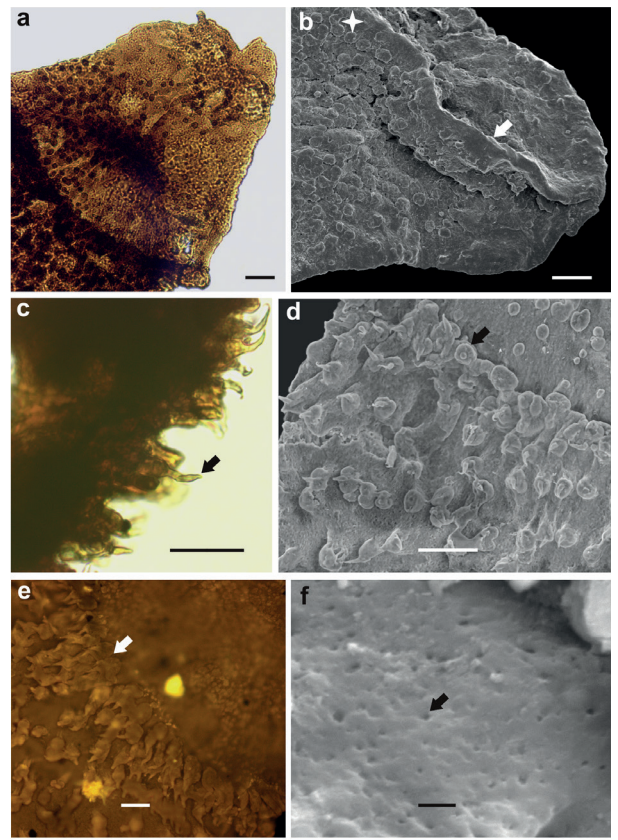
*Lagenosporites magnus* specimens recovered from Toregua Formation, Bolivia, had a similar morphology and wall structure to those described





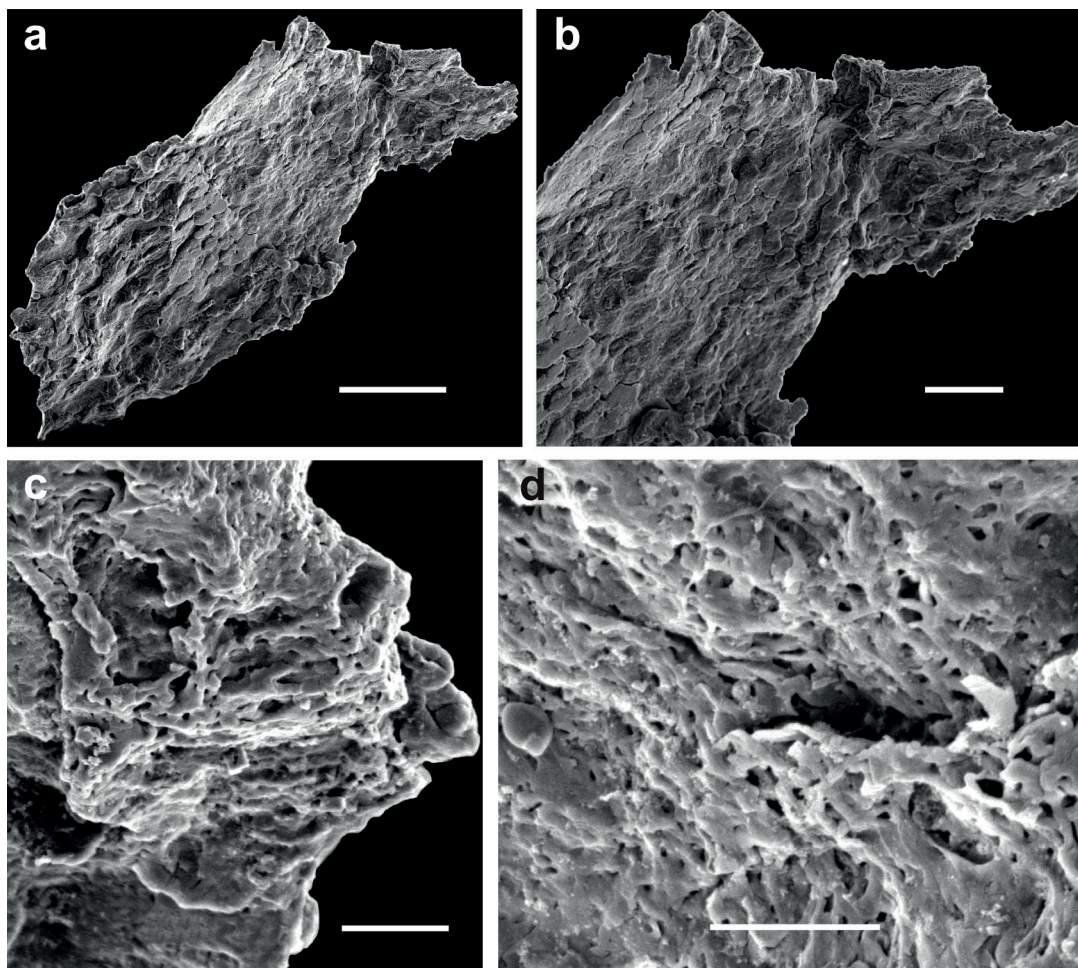
**Figure 2** - *Lagenosporites magnus* megaspores compressed laterally where the main body, the gula and the curvaturae perfectae can be distinguished. **a-e**. Megaspores observed with light microscope (LM). **a**: Specimen CICYTTP-M 139. **b**: Specimen CICYTTP-M 140. **c**: Specimen CICYTTP-M 144. **d**: Specimen CICYTTP-M 151. **e**: Specimen CICYTTP-M 162. **f**. Megaspores observed with scanning electron microscope (SEM). Specimen CICYTTP-M 162. Scale bar: 100  $\mu$ m.

by Chi and Hills (1976) and Candilier et al. (1982). We observed that *L. magnus* presented an ornamentation formed by verrucae in the gula and by complex processes in the rest of the megaspore body. However, some differences in the ornamentation distribution and in the sculptural elements dimensions were observed (see below). According to Chi and Hills (1976) verrucae are present throughout the megaspore surface, however, they do not mention the presence of spines on the bulbous bases of the body. Candilier et al. (1982) found differences between the gula and the megaspore body ornamentation, whereas



**Figure 3** - *Lagenosporites magnus*. Surface details. **a-f**. **a**: Specimen CICYTTP-M 380. Verrucae of the gula (LM). Scale bar: 40  $\mu$ m. **b**: Specimen CICYTTP-M 162. Ornamentation of verrucae and laesurae lips (arrow) of the gula (SEM). Note that some verrucae are fused together (star). Scale bar: 20  $\mu$ m. **c**: Specimen CICYTTP-M 162. Detail of the complex processes of the spore body, formed by a bulbous base and an internally partitioned projection (arrow) with sharp apex (LM). Scale bar: 20  $\mu$ m. **d**: Specimen CICYTTP-M 428. Complex processes of the spore body and detail of the curvaturae perfectae surface (arrow; SEM). Scale bar: 20  $\mu$ m. **e**: Specimen CICYTTP-M 144. Complex processes of the spore body and detail of the curvaturae perfectae surface (arrow) observed with fluorescence LM. Scale bar: 20  $\mu$ m. **f**: Specimen CICYTTP-M 162. Detail of the spore body surface that shows perforations (arrow; SEM). Scale bar: 2  $\mu$ m.

the gula presents verrucae, the spore body presents bulbous elements with an acute apex. Although the latter were of a relatively smaller size than those described in this work; they did coincide with the sizes described by Chi and Hills (1976). Size differences and ornamentation distribution in *L. magnus* could be considered an intraspecific variation- this circumstance had already been



**Figure 4** - *Lagenoisporites magnus*. Detail of the megaspore fracture observed with SEM. **a-d**. Specimen CICYTTP-M 136. **a**: Megaspores compressed laterally with a fracture in the gula. Scale bar: 50  $\mu\text{m}$ . **b**: Detail of the megaspore fracture. Scale bar: 20  $\mu\text{m}$ . **c**: Detail of the gula exospore. **d**: Detail of the exospore which consists of fused rodlets that form a three-dimensional network with heterogeneous spaces of diverse diameters and are arranged at different levels with dissimilar orientations. Scale bar: 5  $\mu\text{m}$ .

described for *Lagenicula* species (Wellman et al. 2009).

Arioli et al. (2007) described similar sculptural elements for *Lagenicula variabilis* (Winslow 1962) Arioli et al. (2004), from the Lower Carboniferous of Ohio, USA. According to these authors, *L. variabilis* presents an ornamentation consisting of large spines with wide bulbous bases. Although *L. magnus* had a similar ornamentation, the difference in size was important as *L. variabilis* measured 45-180  $\mu\text{m}$  high (Arioli et al. 2007) while *L. magnus* did not exceed 20  $\mu\text{m}$  high. These small size

processes constitute a diagnostic character of the *Lagenoisporites* genus (Dybová-Jachowicz et al. 1979). In addition, *L. variabilis* spine processes may bifurcate or even trifurcate (Arioli et al. 2007), whereas *L. magnus* spines did not divide, being always discrete. As for the gula ornamentation, *Lagenicula variabilis* bears verrucae, cones or spines that in several specimens may bifurcate or trifurcate (Arioli et al. 2007), whereas in *L. magnus* only verrucae were observed. Verrucae presence in the gula was frequently found within the gulate megaspores. However, the body ornamentation



described for *L. magnus*, has not been found in other known gulate megaspores.

Megaspores surface perforations could be determined by the three-dimensional structure of the exospore, as it was previously explained by Wellman et al. (2009). The same characteristic was observed in *Lagenicula acuminata* Dijkstra and Piérart 1957 (Glasspool et al. 2000), *Lagenicula variabilis* (Arioli et al. 2007) and *Lagenicula mixta* Winslow 1962 (Wellman et al. 2009).

According to Glasspool et al. (2000), as far as gulate megaspores ultrastructure is concerned, the exospore is divided into an outer, an intermediate, an inner layer and a basal lamina, whereas for Arioli et al. (2007) and Wellman et al. (2009), the exospore presents an outer and an inner layer. In *L. magnus*, exospore outermost layer was only observed, which was composed of a three-dimensional network of fused rodlets giving it a spongy structure appearance. Thus, this wall structure demonstrated the close phylogenetic relationship that exists with the rest of the Carboniferous gulate megaspores, which allowed us to assign them to heterosporous arborescent lycopsids of Lepidocarpaceae family (Arioli et al. 2007).

#### BRIEF APPROACHES ON THE EVOLUTION OF GULATE MEGASPORES

Heterospory has evolved once inside the lycopsids (Bateman and Di Michele 1994). The monophyletic heterosporous lycopsids group could have inherited the mode of megaspore wall formation, which evolved from a simple modification of the basic development process in the homosporous lycopsids (Arioli et al. 2007). This megaspore wall formation mode and; therefore, its ultrastructure, persisted relatively without changes in the heterosporous lycopsids and this condition can be determined by the lack of diversity in megaspores ultrastructure, from gulate megaspores up to the living ones (Wellman et al. 2009). Extensive researches made on extant and fossils lycopsids megaspores

strongly suggest stasis in wall structure (Arioli et al. 2007, Tryon 1986). According to Arioli et al. (2007), stasis is only present in the Selaginellaceae Willk. However, stasis has been indicated in the Isoetaceae Reichenb in relation to the inner portion of the spore wall, which presents stable structural components in contrast to the outermost portion which would present a variable structure with the ability to differentiate in response to external factors, representing adaptations to special environmental conditions (Tryon 1986).

Numerous fossil megaspores assigned to lycopsids present similarities in their ultrastructure with the Isoetales (Wellman 2002). This is the case of *L. magnus* in which a spongy exospore coincident with the general scheme described by Lugardon et al. (2000) for extant lycopsids was observed. In addition, it was recognized a structural setup of sporoderm elements similar to those described in extant species of *Isoetes* L, which present a three-dimensional network of fused rodlets forming heterogeneous spaces (Macluf et al. 2003). Tryon (1986) proposal on stasis in megaspore wall structure is hereby clearly evidenced. Finally, the exospore of *L. magnus* and the exospore of extant Isoetales were very similar, evidencing that megaspore structure has remained stable over the time.

#### CONCLUSIONS

This study on *Lagenosporites magnus* was carried out to contribute with information about lower Mississippian (Tournaisian) megaspores from Bolivia, especially because no research on megaspores for this region and time period has ever been made. In addition, *L. magnus* have only been briefly described (Chi and Hills 1976, Candilier et al. 1982), where no descriptions at wall structural level have been done, in contrast with a few species from Upper Devonian- lower Mississippian that have been fully studied (e.g.,

Glasspool et al. 2000, Arioli et al. 2007, Wellman et al. 2009). Nevertheless, the excellent preservation and the amount of specimens recovered of this species, plus the combination of optical, fluorescence and scanning electron microscopy, allowed us a thorough and detailed study not only of the ornamentation but also of that considered as background, distributed among the main sculptural elements and represented by perforations. This also allowed us to know the sporoderm internal structure, more specifically of the exospore, which was formed by a three-dimensional network of fused rodlets, similar to the ultrastructure described in extant species of *Isoetes* (Lycophyta).

The morphology and structure of the megaspore studied has allowed to assign it to the Lepidocarpaceae and to determine its botanical affinity with extant lycopsids. Likewise, it has been possible to corroborate the conservative nature of the megaspore wall structure by comparing fossil and extant lycopsids ultrastructure.

Future additional studies using transmission electron microscopy will allow us to deepen the wall ultrastructure to obtain more information about its phylogenetic relationship with the extant lycopsids

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#### AUTHOR CONTRIBUTIONS

MQ obtained megaspores from residues, prepared them for LM, fluorescence and SEM microscope observation, interpreted the results and wrote the manuscript. CM contributed to preparation and description of megaspores. MD provided the sample residues and geologic-palynologic context. CM and MD critically reviewed the manuscript and supervised the research.

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