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REVIEW OF SOME *HYSTRICOSPORITES* REPRESENTATIVES FROM BOLIVIA

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ABSTRACT – A taxonomic study of megaspores with bifurcate-tipped processes from the Devonian-Carboniferous transition of Bolivia was performed. Megaspore specimens were isolated from palynological samples of the Pando X1 and Manuripi X1 boreholes. Their morphological characteristics and wall structures were described and illustrated with transmitted optical light, ultraviolet fluorescence, and scanning electron microscopy. This megaspore assemblage comprised six fossil species of *Hystricosporites* (*H. costatus*, *H. delectabilis*, *H. elongatus*, *H. expandus*, *H. furcatus* and *H. spiralis*). The morphologic features of their sporoderms confirm that these megaspores with bifurcate-tipped processes could have had a botanical affinity related to lycopsids. Additional future studies of wall ultrastructure using transmission electron microscopy will provide more information about their phylogenetic relationships with fossil and extant lycopsids.

Keywords: megaspores, lycopsids, Carboniferous, Devonian.

RESUMO – Foi realizado um estudo taxonômico de megásporos com processos de pontas bifurcadas da transição Devoniano–Carbonífero da Bolívia. Espécimes de megásporos foram isolados de amostras palinológicas dos furos Pando X1 e Manuripi X1 e suas características morfológicas e estruturas de parede foram descritas e ilustradas com luz óptica transmitida, fluorescência ultravioleta e microscopia eletrônica de varredura. Esta associação de megásporos era composta por seis espécies fósseis de *Hystricosporites* (*H. costatus*, *H. delectabilis*, *H. elongatus*, *H. expandus*, *H. furcatus* e *H. spiralis*). As características morfológicas de seus esporodermos confirmam que esses megásporos com processos com pontas bifurcadas poderiam ter tido uma afinidade botânica relacionada às licopsídeas. Futuros estudos de ultraestruturas de parede, utilizando microscopia eletrônica de transmissão permitirão obter mais informações sobre suas relações filogenéticas com licopsídeas fósseis e existentes.

Palavras-chave: megásporos, licopsídeas, Carbonífero, Devoniano.

INTRODUCTION

Megaspores with bifurcate-tipped processes are a characteristic element within Middle and Upper Devonian assemblages (Owens *et al*., 2022), they appeared towards the end of the Early Devonian and practically disappeared near the Devonian–Carboniferous boundary (Wellman, 2002).

The most common genera of megaspores with several species bearing bifurcate-tipped processes are *Hystricosporites* McGregor and *Ancyrospora* (Richardson) Richardson. Another one is *Nikitinsporites* Chaloner (*e.g*., Allen, 1965; Allen & Robson, 1981; Candilier *et al*., 1982; Higgs & Scott, 1982; Wellman, 2002), but it is less frequent. Although they have bifurcate-tipped processes as part of their ornamentation, there are important morphologic features to distinguish the three genera. *Hystricosporites* include azonate megaspores (McGregor, 1960), *Ancyrospora* presents an equatorial extension called "pseudo-flange" (Richardson, 1962), and *Nikitinsporites* differ by the structure of the processes since they lack the elongated cords at its base, frequently found in the other two genera (Urban, 1969; Taylor *et al*., 1980; Allen & Robson, 1981). Since these bifurcate-tipped processes have been recorded in several Devonian spore genera, it is likely that they are polyphyletic and have arisen through homoplasy, produced by different plant lineages that are potentially phylogenetically unrelated (Wellman, 2002). There are few *in situ* records of these megaspores, and consequently, their botanical affinities remain speculative.

Few works describe the ultrastructure of the wall of these megaspores with bifurcate-tipped processes. According to Scott & Hemsley (1992), who described the exospore of *Hystricosporites*, and Wellman (2002) who studied the exospore of *Ancyrospora*, these megaspores present a wall made up of two layers. The internal is dense, homogeneous, and laminated, and the external is thicker and spongier. In both cases, the ultrastructure is very similar to that of other lycophytes, which is why these authors consider them possible parental plants. This contribution presents a morphotaxonomic analysis of megaspores with bifurcate-tipped processes assigned to *Hystricosporites* obtained from Pando X1 and Manuripi X1 boreholes from northern Bolivia. Their botanical affinity and stratigraphic range are discussed in terms of previous palynologic information.

MATERIAL AND METHODS

Megaspores were isolated from palynological samples of Pando X1 (11º36'07''S, 67º56'45''W) and Manuripi X1 boreholes (11º36'01''S, 68º08'55''W) from Madre de Dios Basin, Bolivia (Figure 1). Descriptions of the two boreholes and palynologic results have been previously published (Isaacson & Díaz Martínez, 1995; Isaacson *et al*., 1995; Vavrdová & Isaacson, 1996; Vavrdová *et al*., 1996; di Pasquo, 2009; di Pasquo *et al*., 2015).

A total of 23 samples from Pando X1 (CICYTTP 729, 731, 733, 734, 2609, 2610, 2611, 2612, 2613, 2614, 2615) and Manuripi X1 boreholes (CICYTTP 564, 565, 566, 572, 573, 574, 575, 576, 577, 578, 579, 580) were collected. These correspond to the Toregua Formation (see Figure 2) of mid–late Tournaisian age (Suárez Soruco & Díaz Martínez, 1996; di Pasquo *et al*., 2015, 2019). Samples were processed at the Laboratorio de Palinoestratigrafía y Paleobotánica, Centro de Investigaciones Científicas y Transferencia de Tecnología a la Producción (**CICYTTP-CONICET**-Entre Ríos-UADER), and all the materials are housed under the acronyms of the Palynostratigraphic (**CICYTTP-Pl**) and megaspore collections (**CICYTTP-M**).

Samples were macerated by a softer laboratory technique to avoid destruction of megaspores (see Steemans *et al*., 2009). For each sample, 20–30 g of sediment were crushed in sizes greater than 5 mm and macerated using 25% HCl for 8 hours, followed by 45% HF for 48 hours. Samples were washed with distilled water to neutralize the acids. Then, residues were sieved using a 25 µm mesh. Megaspores with bifurcate-tipped processes were isolated manually from palynologic residues in a Petri dish under a stereomicroscope using needles and very fine pipettes. Those selected megaspores were mounted on glass slides (non-permanent slides) using water for microscopic observation, measurement, and illustration with white light (**LM**) under a Leica DM500. Some of those specimens, bearing different morphologies, also exhibited fractures of their walls determined under

Figure 1. Map of the study area: **1**, Pando X1 and **2**, Manuripi X1 boreholes, Madre de Dios Basin, Bolivia.

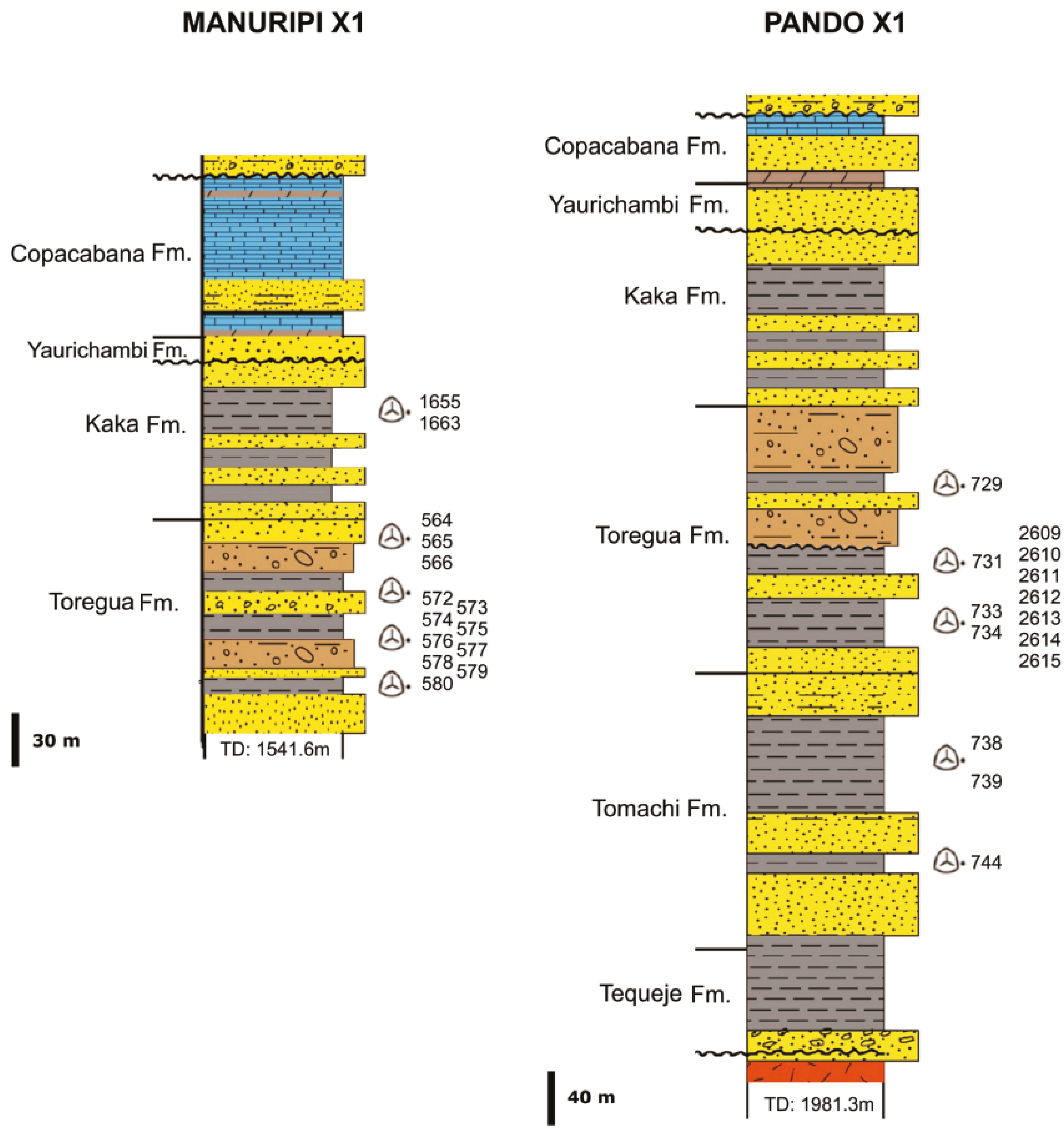

Figure 2. Stratigraphic columns of Pando X1 and Manuripi X1 boreholes with the location of the studied samples. Modified from Koltonik *et al*. (2019).

LM, they were transferred to stubs for a detailed study with scanning electron microscopy (**SEM**) JENCK PHENOM PRO at the CICYTTP (CONICET-Entre Ríos-UADER) and JEOL JSM 6360 LV at the Facultad de Ciencias Naturales y Museo (UNLP, La Plata, Buenos Aires province). Detailed measurements of processes and other features of the analysed megaspores were achieved using the program ImageJ version IJ 1.46r (Ferreira & Rasband, 2012). Maximum and minimum values were expressed in micrometers (µm).

SYSTEMATIC PALEONTOLOGY

In this contribution we followed Potonié (1893) for morphotaxonomic classification of megaspores, and Owens (1971), Chi & Hills (1976), and Punt *et al*. (2007) for morphologic terminology. This study describes six species of the genera *Hystricosporites* organized in alphabetical order within the morphotaxonomic scheme of Turmas.

Anteturma SPORITES Potonié, 1893
Turma TRILETES (Reinsch) Potonié & Kremp, 1954
Suprasubturma LAMINATITRILETES Smith & Butterworth, 1967
Subturma AZONOLAMINATITRILETES Smith & Butterworth, 1967
Infraturma DECORATI Neves & Owens, 1966

Hystricosporites McGregor, 1960

Type species. *Hystricosporites delectabilis* McGregor, 1960.

Hystricosporites costatus Vigran, 1964
(Figure 3A–D)

1964 *Hystricosporites costatus* – Vigran, p. 14, pl. 5, figs. 3–5.
1976 *Hystricosporites costatus* Vigran – Chi & Hills, p. 712, pl. 4, figs. 6–7.

Material. Specimens CICYTTP-M408, 491, 494 (Table 1, Appendix 1).
Description. Megaspore trilete, polarly compressed, is observed at the distal face; thus, laesurae with raised lips cannot be seen. Long, broad-based processes terminated with a triangular bifurcate fluke are variably distributed on the body surface. These are 45–60 µm long, 15–25 µm wide in its base, and 5–9 µm wide just below the apical fluke. The processes apical flukes are 7–15µm wide.
Dimensions. Three specimens measured. Megaspores are 117–200 µm wide along the equatorial axis.
Remarks. The studied specimens coincide with the original description of the morphospecies defined by Vigran (1964) and the description made by Chi & Hills (1976), where they describe its bifurcated processes as triangular or expanded triangular following Owens' classification (1971).
Chronostratigraphic distribution of the studied material. This species is considered part of the set of reworked Devonian palynomorphs incorporated into the Lower Carboniferous at Pando X1 borehole, Bolivia.
Stratigraphic and geographic range. Givetian–Frasnian of Norway (Vigran, 1964), Givetian–Famennian of Canada (Chi & Hills, 1976).

Hystricosporites delectabilis McGregor, 1960
(Figure 4A–F)

1960 *Hystricosporites delectabilis* – McGregor, p. 32, pl. 11, figs. 13–14; text-fig. 1.
1971 *Hystricosporites delectabilis* McGregor – Owens, p. 27, pl. 6, figs. 5–6; text-fig. 5.
1976 *Hystricosporites delectabilis* McGregor – Chi & Hills, p. 711, pl. 4, fig. 5.

Material. Specimens CICYTTP-M65, 66, 67, 71, 84, 128, 155, 274, 377, 434, 478, 485 (Table 1, Appendix 1).
Description. Megaspore trilete, polar, and laterally compressed. Proximal face with contact areas bearing a pattern of radiating ridges and a laesurae with raised lips, 30–110 µm high. Radiating ridges of 1–2 µm wide and with 3 µm the average distance between them. The surface of the contact areas lacks other type of ornamentation. The rest of the megaspore body has broad-based processes that are terminated with a laterally extended and reflexed bifurcate fluke. These processes are 15–45 µm long, 6–20 µm wide at the base, and 2–10 µm wide just below the apical fluke. The processes apical flukes are 5–25 µm wide. In the cross section, the wall of these processes, composed by the outer exospore, has a small-sized lumen. Through the observation of a fracture, it is possible to identify the outermost layer of the exospore, which is constituted by thick and short rodlets, 0.8–1.7 µm thick, anastomosed, delimiting heterogeneous spaces, and forming a closed network where the rodlets are arranged in a disorganized manner giving rise to a dense structure.
Dimensions. Twelve specimens measured. The overall length of the megaspore (including the elevated lips) is 110–250 µm, and the body's width is 90–260 µm.
Remarks. The studied specimens coincided with the original description of McGregor (1960). The size and general morphology of apical fluke processes in our specimens are also quite similar to those of *Hystricosporites bulbosus* Chi & Hills, 1976. They differ only in the presence of a more bulbous base in *H. bulbosus*. The analysed megaspores are also similar to *Hystricosporites gravis* Owens, 1971, given that they present processes with apical flukes of the same type, although these are longer than in *H. delectabilis*.
Chronostratigraphic distribution of the studied material. Mid–late Tournaisian, Toregua Formation, of Pando X1 and Manuripi X1 boreholes, Bolivia.
Stratigraphic and geographic range. Givetian–Famennian of Canada (McGregor, 1960; Owens, 1971; Chi & Hills, 1976), and Tournaisian of the United Kingdom (Allen & Robson, 1981).

Hystricosporites elongatus Chi & Hills 1976
(Figure 5A–B)

1976 *Hystricosporites elongatus* – Chi & Hills, p. 720, pl. 5, figs. 10–12.

Material. Specimens CICYTTP-M190, 197 (Table 1, Appendix 1).
Description. Megaspore trilete, compressed in polar view. Proximal face having elevated laesurae lips (114 µm high) and contact areas with radiating ridges, although not well-distinguished, observed closer to the equatorial margin, 0.5–0.7 µm wide, and the average distance between them was 2–4 µm. The megaspore body bears broad-based processes with an expanded bifurcate fluke and, in some specimens, laterally extended, expanded, and reflexed. These processes are 35–60 µm long, 9–16 µm wide at the base, and 3–9 µm wide just below the apical fluke; apical flukes are 13–18 µm wide. Granules of 0.5–0.8 µm in diameter are observed

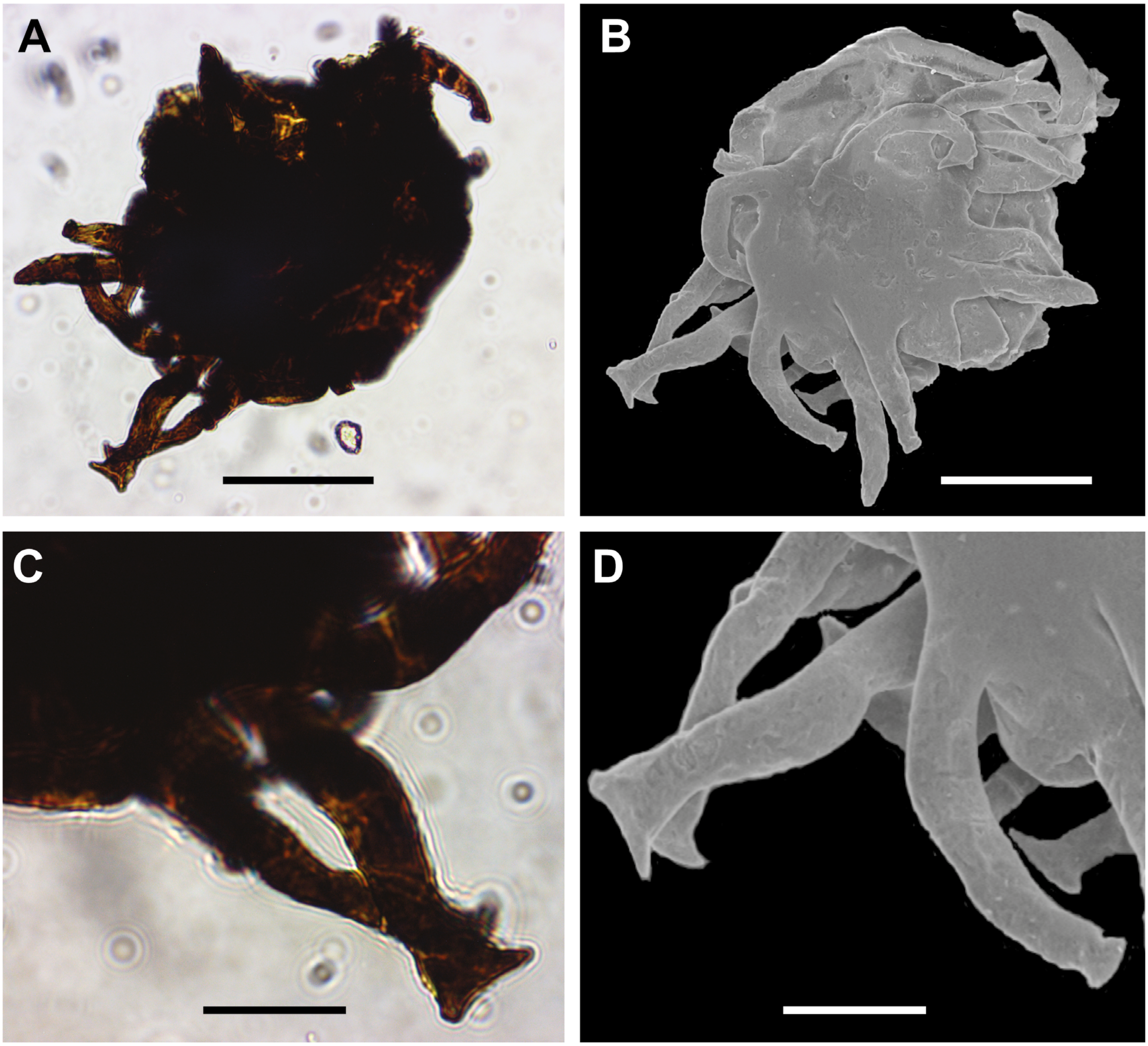

Figure 3. *Hystricosporites costatus* Vigran, 1964. Specimen CICYTTP-M408. **A**, **C**, Megaspore observed with LM. **B, D**, megaspore observed with SEM. **A**–**B**, megaspore general view. **A**, megaspore compressed in polar view. **B**, megaspore in distal view. **C**–**D**, detail of the ornamentation of the megaspore body. **C**, long, broad-based processes terminated in a triangular bifurcate fluke. **D**, processes with a triangular bifurcate fluke. Scale bars: A–B = 50 µm; C–D = 20 µm.

as secondary ornamentation between the bifurcate-tipped processes.

Dimensions. Two specimens measured. Megaspores are 135–250 µm wide along the equatorial axis.

Remarks. The studied megaspores coincided with the original description of the morphospecies made by Chi & Hills (1976). *Hystricosporites grandis* Owens, 1971 has a similar type of apical flukes. However, the processes can reach up to twice the length of *H. elongatus* and are wider both at the base and at the apical fluke. Moreover, in *H. grandis* the basal part of processes has elongate and thickened ribs which were not observed in *H. elongatus*. *Hystricosporites gravis* also has apical flukes of the same type, although little expanded. In addition, thickened radial ribs in the contact areas are absent in *H. elongatus*.

Chronostratigraphic distribution of the studied material. This species is considered part of the set of reworked Devonian palynomorphs incorporated into the Lower Carboniferous at Pando X1 borehole, Bolivia.

Stratigraphic and geographic range. Givetian–Famennian of Canada (Chi & Hills, 1976).

Hystricosporites expandus Chi & Hills, 1976
(Figure 5C–D)

1976 *Hystricosporites expandus* – Chi & Hills, p. 721, pl. 6, figs. 1–5.

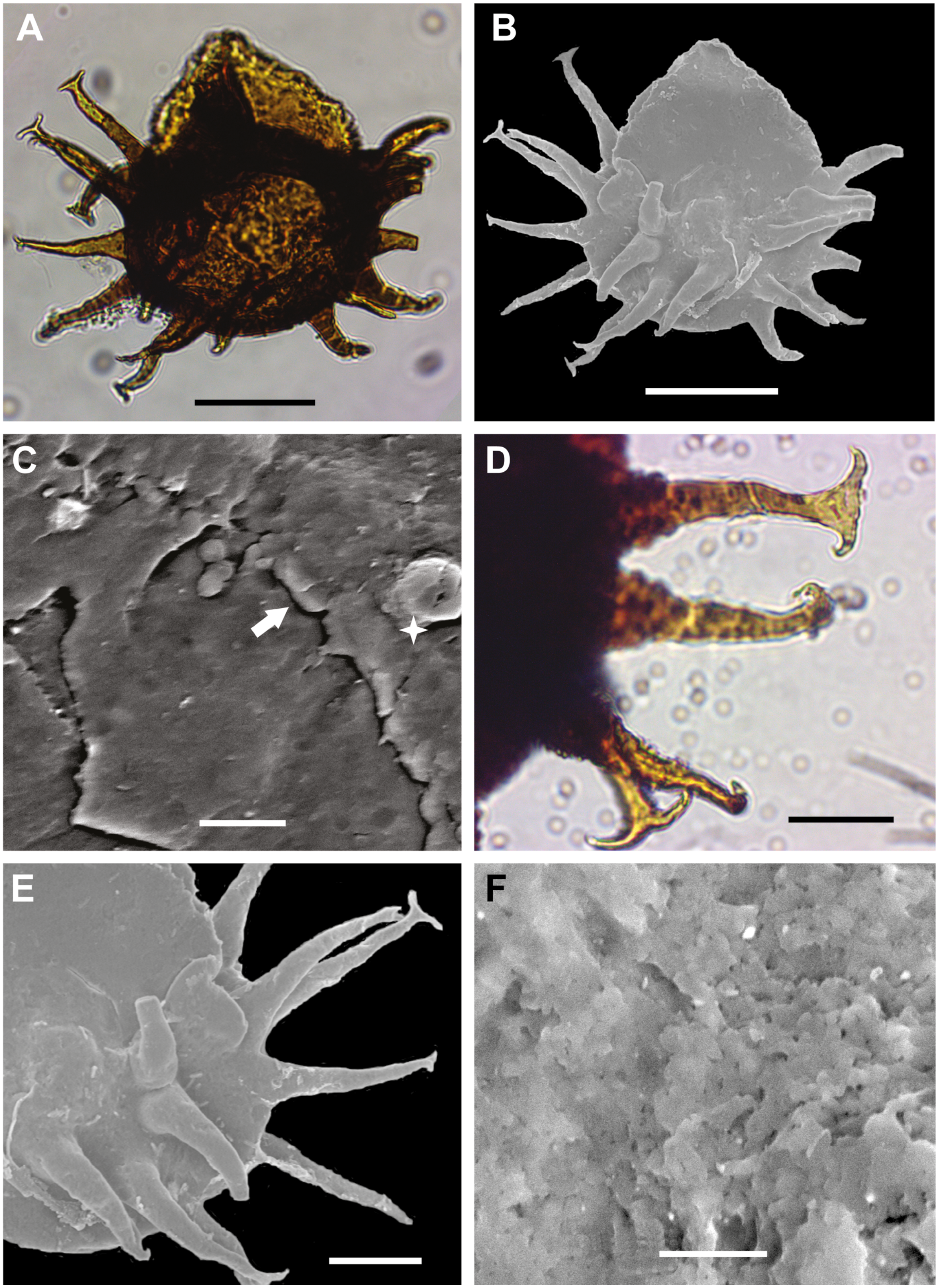

Figure 4. *Hystricosporites delectabilis* McGregor, 1960. **A, D**, megaspore observed with LM. **B–F**, megaspore observed with SEM. **A, B**, megaspore general view. Specimen CICYTTP-M377. **A**, megaspore laterally compressed where the elevation lips can be observed. **B**, laevigate contact areas and labiate laesura, and the processes of the body can be observed. **C–E**, detail of the megaspore surface. **C**, specimen CICYTTP-M128. Contact areas limited distally by radiating ridges (arrow). Cross section of a process formed by a wall that delimits a small-calibre lumen (star). **D**, specimen CICYTTP-M478. Broad-based processes terminated in a laterally extended and reflexed bifurcate fluke. **E**, specimen CICYTTP-M377. Processes with a laterally extended and reflexed bifurcate fluke. **F**, specimen CICYTTP-M155. Fracture of a megaspore wall where it is possible to identify the outermost layer of the exospore, composed of anastomosed rodlets delimiting heterogeneous spaces and forming a closed network where the rodlets are arranged in a disorganized manner giving rise to a dense structure. Scale bars: A–B = 50 µm; C–E = 20 µm; F = 10 µm.

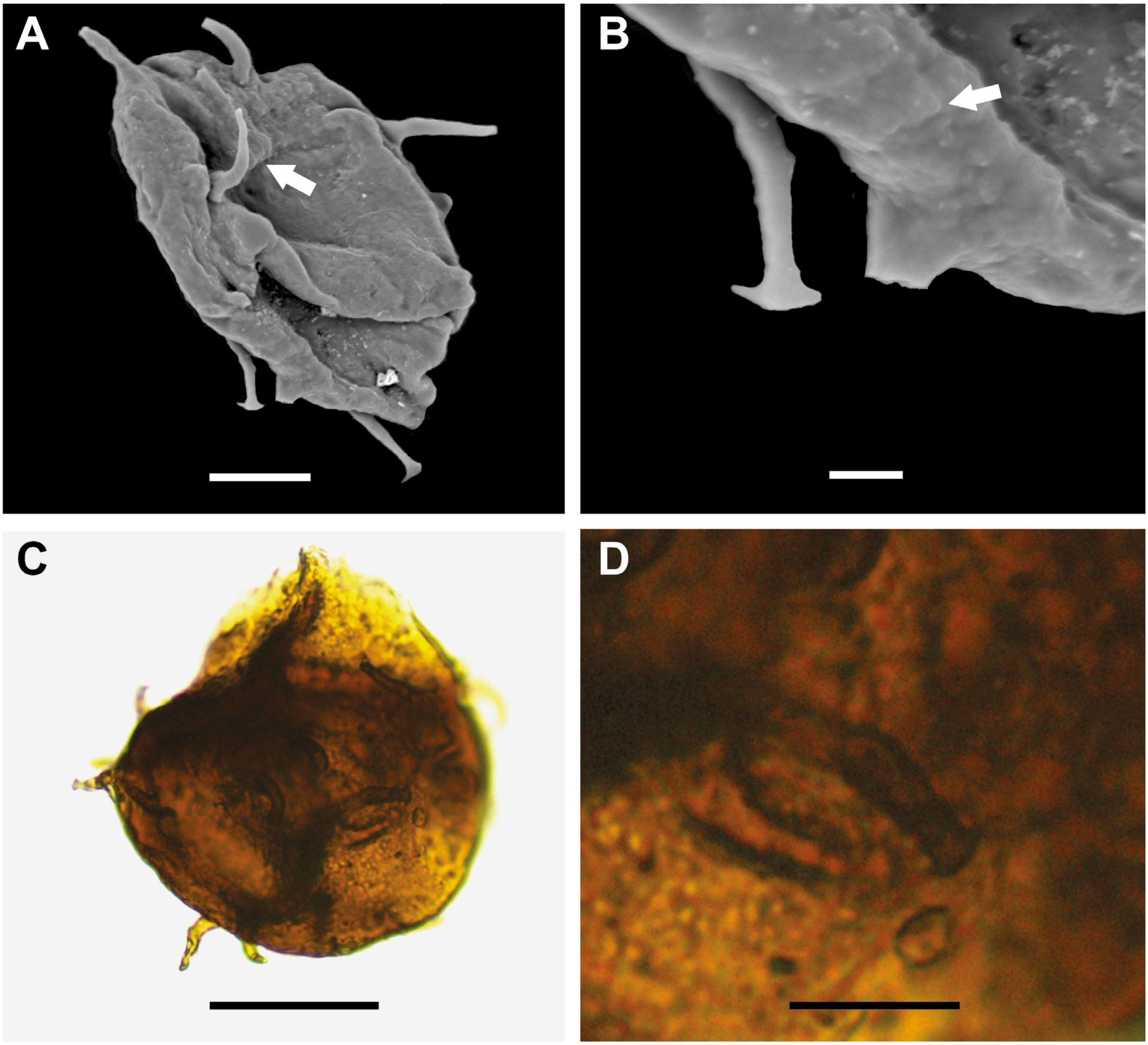

Figure 5. *Hystricosporites elongatus* Chi & Hills, 1976. Specimen CICYTTP-M190. **A–B**, Megaspore observed with SEM. **A**, megaspore compressed in polar view with elevated lips and laevigate surface of contact areas limited distally by radiating ridges (arrow). **B**, broad-based processes ended in laterally extended, expanded, and reflexed bifurcated flukes. Granules are observed between these processes (arrow). Scale bar = 10 µm. *Hystricosporites expandus* Chi & Hills, 1976. Specimen CICYTTP-M435. **C–D**, megaspore observed with LM. **C**, megaspore laterally compressed where the elevated lips of the laesurae are observed in proximal face. **D**, broad-based processes terminated in expanded bifurcated flukes. Scale bars: A, C = 50 µm; B, D = 10 µm.

Material. Specimens CICYTTP-M435, 488 (Table 1, Appendix 1).

Description. Megaspore trilete, polar, and laterally compressed. Proximal face with elevated laesurae lips (30 µm high) and smooth contact areas. The megaspore body has broad-based processes that end with an expanded bifurcate fluke. These are 9–20 µm long, 3–4 µm wide at the base, and 1.5–2 µm wide just below the apical fluke; apical flukes are 3–5 µm wide.

Dimensions. Two specimens measured. The overall length (including the raised lips that could only be observed in the laterally compressed specimen) is 100 µm, and the width of the body is 60–84 µm.

Remarks. The studied megaspores presented the same type of apical fluke as the one initially described by Chi & Hills (1976). *Hystricosporites grandis* and *H. elongatus* have also expanded bifurcate flukes, nonetheless they have laterally extended and reflexed bifurcate flukes.

Chronostratigraphic distribution of the studied material. This species is considered part of the set of reworked Devonian palynomorphs incorporated into the Lower Carboniferous at Pando X1 and Manuripi X1 boreholes, Bolivia.

Stratigraphic and geographic range. Givetian–Famennian of Canada (Chi & Hills, 1976).

Hystricosporites furcatus Owens, 1971
(Figure 6A–F)

1971 *Hystricosporites furcatus* – Owens, p. 28, pl. 6, figs. 7–9; text-fig. 6.
1976 *Hystricosporites furcatus* Owens – Chi & Hills, p. 714, pl. 4, figs. 8–9.

Material. Specimens CICYTTP-M64, 115, 273, 376, 422, 436, 437, 518, 519 (Table 1, Appendix 1).
Description. Megaspore trilete, polar, and laterally compressed. Proximal face with elevated laesurae lips (43–56 µm high), and smooth contact areas with well-marked curvaturae perfectae. The megaspore body has broad-based processes that end with a slightly extended bifurcate fluke. Processes are 12–47 µm long, 6–19 µm wide at the base, and 1–13 µm wide just below the apical fluke; apical flukes are 4–22 µm wide. In cross-section, these processes are observed as solid, and their diameter is 8–10 µm. Through the observation of a fracture in one specimen, it is possible to identify the innermost layer of the exospore, constituted by thick rodlets of 0.15–0.30 µm wide, anastomosed, delimiting heterogeneous spaces and forming a closed network where the rodlets are arranged in a disorganized manner giving rise to a dense structure.
Dimensions. Nine specimens measured. The overall length of the megaspore (including the elevated lips) is 93–121 µm, and the width of the body is 77–250 µm.
Remarks. The studied specimens present the same type of apical fluke and are within the size range of the species *Hystricosporites furcatus* originally described by Owens (1971). As previously remarked by Chi & Hills (1976), this species can be differentiated from all other species of *Hystricosporites* by its extended bifurcate flukes.
Chronostratigraphic distribution of the studied material. This species is considered part of the set of reworked Devonian palynomorphs incorporated into the Lower Carboniferous at Pando X1 and Manuripi X1 boreholes, Bolivia.
Stratigraphic and geographic range. Givetian–Famennian of Canada (Owens, 1971; Chi & Hills, 1976), Frasnian and late Famennian of Iran (Ghavidel-Syooki, 2003; Taherian *et al*., 2022).

Hystricosporites spiralis Chi & Hills, 1976
(Figure 7A–F)

1962 *Dicrospora* sp. – Winslow, p. 55, pl. 12, figs. 4-4a.
1976 *Hystricosporites spiralis* – Chi & Hills, p. 722, pl. 4, figs. 8–9.

Material. Specimens CICYTTP-M46, 76, 96, 143, 198, 400, 409, 417, 438, 439, 440, 441, 442, 490, 515 (Table 1, Appendix 1).
Description. Megaspore trilete, polar, and laterally compressed. Proximal face with smooth contact areas limited proximally by the laesurae having elevated lips and distally by radiating ridges. Laesurae lips are 48–100 µm high and have a 3–6 µm thickness. The rest of the megaspore body has broad-based processes, visibly branched and terminated with a laterally extended and reflexed or only reflexed bifurcate fluke. These processes are 20–79 µm long, 5–30 µm wide in its base and 4–16 µm wide just below the apical fluke; apical flukes are 7–33 µm wide. Granules of 1.3–1.6 µm in diameter are observed between the processes. In addition, the entire surface presents perforations as secondary ornamentation. In section, the exospore is formed by a thin and compact outer exospore of 0.5–0.8 µm thick, and immediately below, a visibly thicker inner exospore composed of thin rodlets that anastomose forming a closed and dense pattern of 1.5–4 µm thick. Processes are also formed by the outer exospore that delimits a small-calibre lumen.
Dimensions. Fifteen specimens measured. The overall length (including the elevated lips) is 85–278 µm, and the width of the body is 89–350 µm.
Remarks. The present species shares with *Hystricosporites varius* Hills, Hyslop, Braman & Lloyd, 1984 the width of the base of processes and the width of their apical flukes. However, in *H. varius*, the processes have extended, laterally extended, and reflexed bifurcate flukes, reaching a greater length (46–108 µm).
Chronostratigraphic distribution of the studied material. This species is interpreted to be part of the set of reworked Devonian palynomorphs incorporated into the Lower Carboniferous at Pando X1 and Manuripi X1 boreholes, Bolivia.
Stratigraphic and geographic range. Famennian of USA (Winslow, 1962), Givetian–Famennian of Canada (Chi & Hills, 1976).

DISCUSSION

Botanical affinity

The megaspores with bifurcate-tipped processes found in this research correspond to the genera *Hystricosporites*. These, along with *Ancyrospora* and *Nikitinsporites* are the most common megaspores genera bearing bifurcate-tipped processes during the Devonian. According to Wellman (2002), the presence of these types of bifurcated flukes in various Devonian spore genera, would probably indicate that these megaspores are polyphyletic and have arisen through homoplasy produced by different plant lineages that are phylogenetically not related. Concerning *Nikitinsporites*, it was recovered from megasporangia of the fossil *Kryshtofovichia africani* (Nikitin, 1934), a Late Devonian lycophyte of Russia (Allen, 1980). Meanwhile *Ancyrospora*, in a detailed morphological study of the structure and ultrastructure (Wellman, 2002), was interpreted as having close similarities with lycophyte spores (*Borysthenostrobus mirandus* Ishchenko & Semenova, 1982 and *Krishtofovichia africani* Nikitin, 1934). This affinity was also suggested by Marshall (2000), who linked the relative abundance

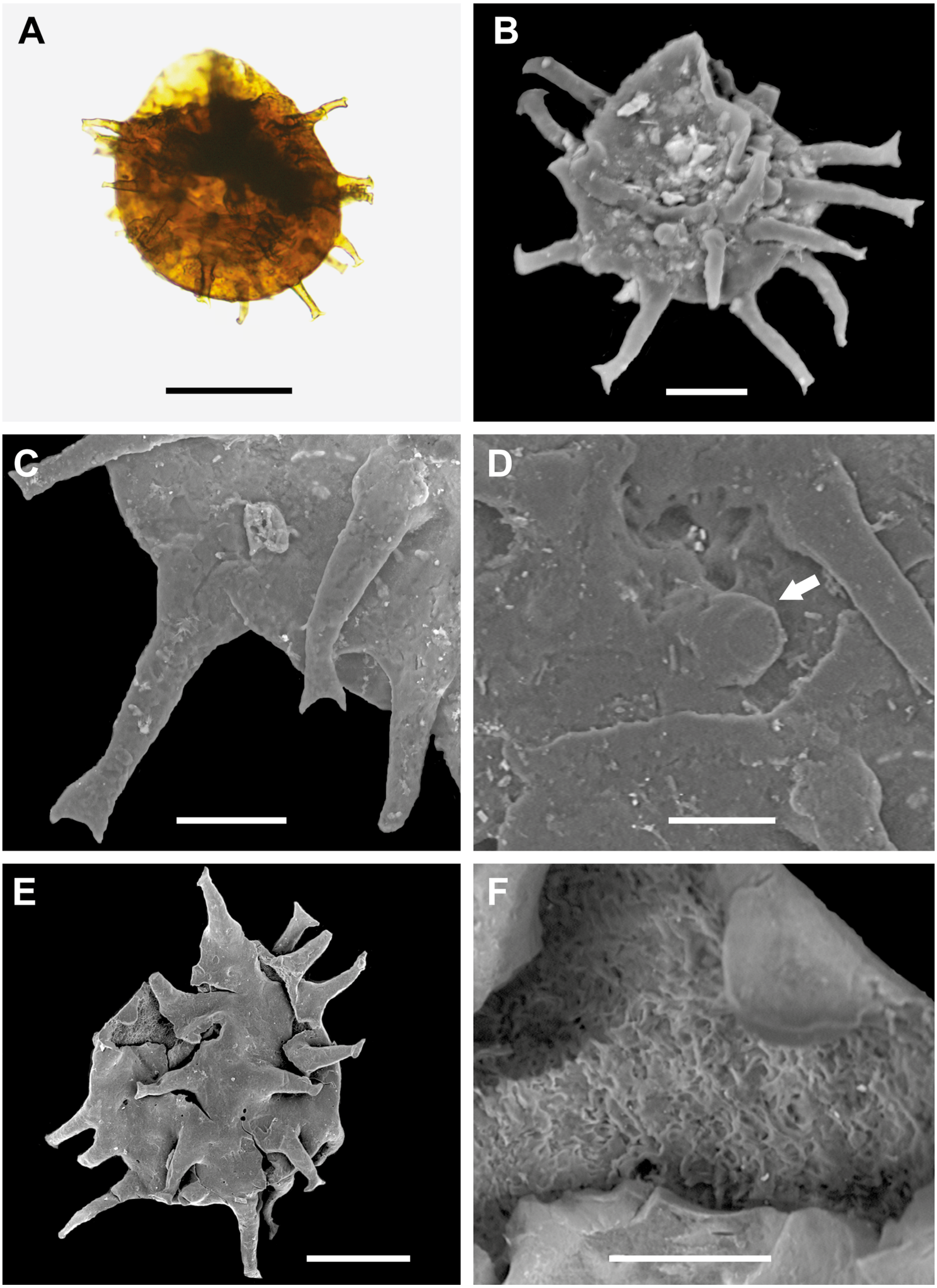

Figure 6. *Hystricosporites furcatus* Owens, 1971. **A**, megaspore observed with LM. **B**–**F**, megaspore observed with SEM. **A**–**B**, megaspore general view. **A**, specimen CICYTTP-M437. Megaspore laterally compressed where the elevated lips are observed. **B**, specimen CICYTTP-M436. Megaspore laterally compressed with smooth contact areas. **C**–**D**, detail of the megaspore body ornamentation. Specimen CICYTTP-M376. **C**, broad-based processes bearing slightly extended bifurcate flukes. **D**, cross section of the processes which are observed solid (arrow). **E**–**F**, detail of a fracture of a megaspore wall. Specimen CICYTTP-M422. **E**, megaspore fractured where it is possible to identify the innermost layer of the exospore. **F**, detail of picture E, where the exospore is observed to be constituted by thick rodlets, anastomosed, arranged in a disorganized manner, delimiting heterogeneous spaces, and forming a closed network giving rise to a dense structure. Scale bars: A, E = 50 μm; B–D = 20 μm; F = 10 μm.

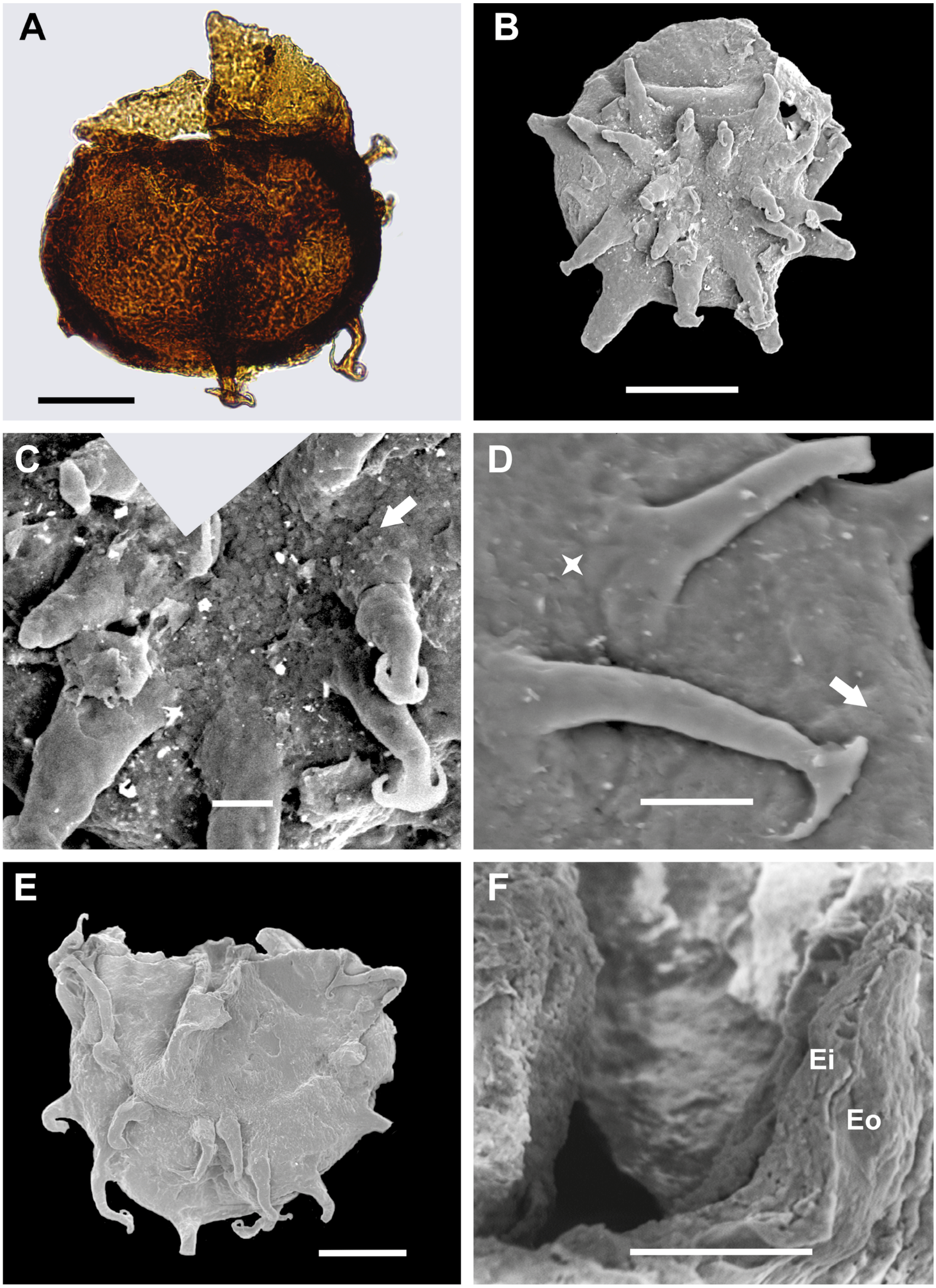

Figure 7. *Hystricosporites spiralis* (Winslow, 1962) Chi & Hills, 1976. **A**, megaspore observed with LM. **B–F**, megaspore observed with SEM. **A–B**, megaspore general view. **A**, specimen CICYTTP-M409. Megaspore laterally compressed where the elevated lips of the laesurae are observed. **B**, specimen CICYTTP-M143. Megaspore with contact areas devoid of ornamentation. **C–D**, detail of the megaspore surface. **C**, specimen CICYTTP-M143. Broad-based processes terminated with a laterally extended and reflexed, or only reflexed bifurcated flukes. Granules in the surface are observed between these processes (arrow). **D**, specimen CICYTTP-M198. Processes bearing branched basal portion (star) and between these elements the surface depicts perforations (arrow). **E–F**, detail of a fractured megaspore wall. Specimen CICYTTP-M417. **E**, megaspore fractured at the elevated lips (arrow) where it is possible to identify the exospore. **F**, detail of the fracture of picture E, where the exospore is observed to be constituted by a thin and massive outer exospore (**Eo**), and a thicker inner exospore (**Ei**) formed by thin rodlets that are anastomosed forming a closed and dense structure. Scale bars: A, E = 50 µm; B = 100 µm; C–D = 20 µm; F = 10 µm.

of *Ancyrospora* spores in strata containing remains of the *Thursophyton* lycophyte.

Regarding *Hystricosporites*, there are no *in situ* records to date and consequently, the botanical affinities of their parent plants remain speculative (Wellman, 2002). Scott & Hemsley (1992) analysed *Hystricosporites* with transmission electron microscopy, in research focused on controls upon the ultrastructural preservation of sporinite, and described two distinct regions: an inner one, dense, possibly laminated, and an outer one, spongy. According to these authors, *Hystricosporites* closely resembles *Nikitinisporites*. In the present work, some specimens of *Hystricosporites* (*H. delectabilis*, *H. furcatus* and *H. spiralis*) have been described at the level of their wall structure, which is formed mainly by a clearly spongy exospore (Figures 4F, 6F and 7F) comparable to the one described by Scott & Hemsley (1992). The ultrastructure we noted in *Hystricosporites* is also similar to the one observed in other fossil, such as *Lagenicula* (Bennie & Kidston, 1886) Potonié & Kremp, 1954, and extant lycophyte species, such as *Isoetes* Linnaeus, confirming a botanical affinity with Lycopsids. In addition, the practically unchanged wall features, such as the spongy exospore, that exhibit megaspores with grapnel-tipped processes with later Lycopsids would suggest stasis in the megaspore structure and therefore in its wall developmental processes. The latter being an implicative that monophyletic heterosporous lycopsids would have inherited a mode of megaspore wall formation that would have evolved through a simple modification of the basic developmental processes of its formation in the homosporous lycopsids, as proposed Arioli *et al*. (2007).

Biostratigraphy

Megaspores with bifurcated flukes, studied in this contribution and assigned to *Hystricosporites*, were found in the Toregua Formation of Carboniferous, mid–late Tournaisian age (Figure 8). All the megaspores species

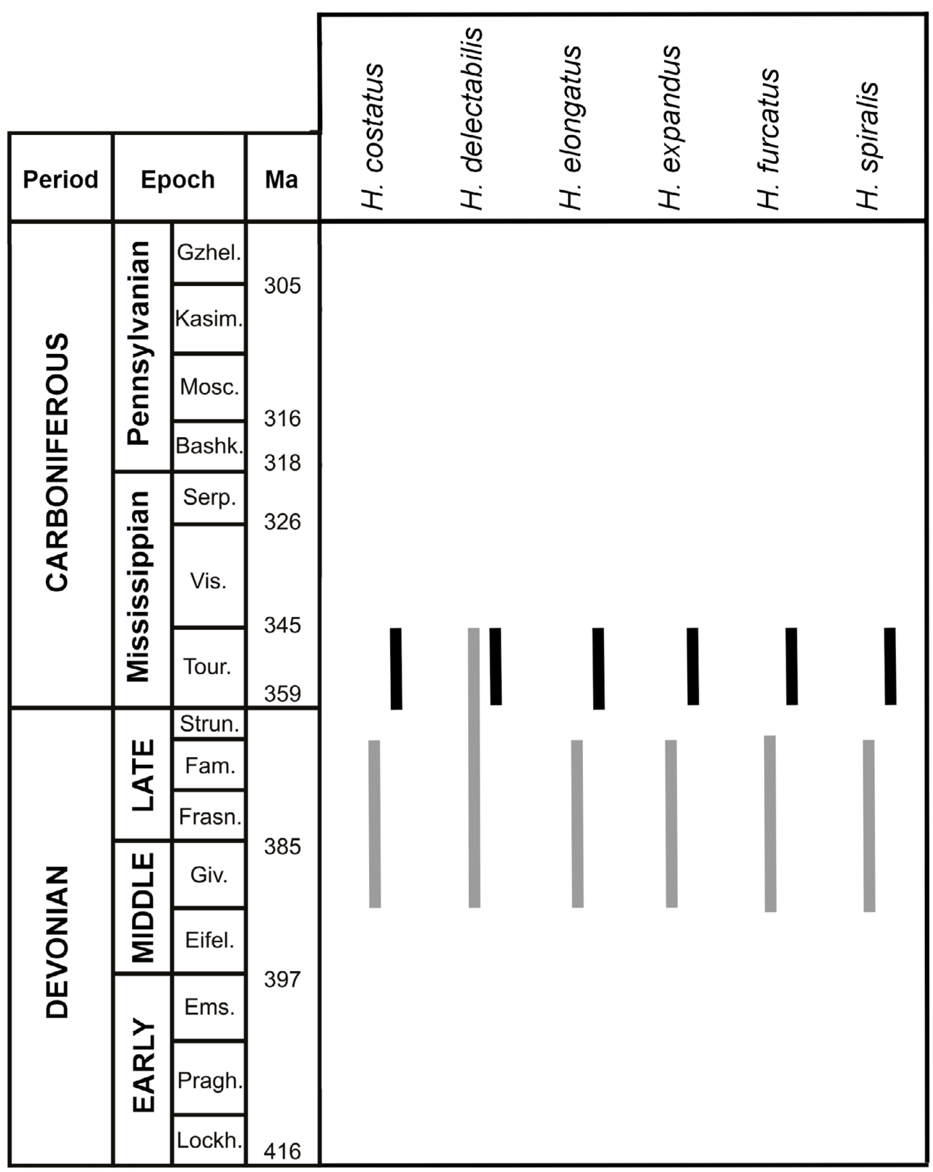

Figure 8. Range chart of the megaspores analysed in this study. Grey bars indicate the stratigraphic range of the morphospecies, and black bars the stratigraphic range of the studied material.

described for this formation have been previously recorded from Givetian to Famennian rocks in other parts of the world (McGregor, 1960; Winslow, 1962; Vigran, 1964; Owens, 1971; Chi & Hills, 1976; Ghavidel-Syooki, 2003; Taherian *et al*., 2022), except for *Hystricosporites delectabilis* that has also been documented in the Tournaisian (Allen & Robson, 1981). Although *Hystricosporites* is known to occur in Lower Mississippian rocks (*e.g*., Cormier & Kelly, 1964; Allen & Robson, 1981; Candilier *et al*., 1982), it is more common in Middle to Late Devonian assemblages, and its presence in Carboniferous deposits might be due to the erosion of underlying Devonian rocks (see di Pasquo *et al.*, 2015, 2019a, 2022) as in the present work.

CONCLUSIONS

This research contributes with morphological information about megaspores of the genus *Hystricosporites*, which are characterized by having bifurcated processes. The excellent preservation of specimens recovered plus the combination of optical and scanning electron microscopy, allowed us a thorough and detailed study, not only of the ornamentation, but also, in some cases, of the sporoderm structure, more specifically of the exospore which is clearly spongy as in other lycopsids.

Future additional studies will analyse these megaspores with transmission electron microscopy (TEM) in order to describe further the ultrastructure of the wall and therefore obtain more information about its phylogenetic relationship with fossil and extant Lycopsids.

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APPENDIX 1

Table 1. Stratigraphic information of the examined megaspores specimens.

Specimen	Horizon	Taxonomic assignment	Borehole	Formation	Age
CICYTTP-M 46	579	*Hystricosporites spiralis*	Manuripi X1	Toregua	mid-late Tournaisian
CICYTTP-M 64	579	*Hystricosporites furcatus*	Manuripi X1	Toregua	mid-late Tournaisian
CICYTTP-M 65	579	*Hystricosporites delectabilis*	Manuripi X1	Toregua	mid-late Tournaisian
CICYTTP-M 66	579	*Hystricosporites delectabilis*	Manuripi X1	Toregua	mid-late Tournaisian
CICYTTP-M 67	579	*Hystricosporites delectabilis*	Manuripi X1	Toregua	mid-late Tournaisian
CICYTTP-M 71	579	*Hystricosporites delectabilis*	Manuripi X1	Toregua	mid-late Tournaisian
CICYTTP-M 76	579	*Hystricosporites spiralis*	Manuripi X1	Toregua	mid-late Tournaisian
CICYTTP-M 84	578	*Hystricosporites delectabilis*	Manuripi X1	Toregua	mid-late Tournaisian
CICYTTP-M 96	578	*Hystricosporites spiralis*	Manuripi X1	Toregua	mid-late Tournaisian
CICYTTP-M 115	577	*Hystricosporites furcatus*	Manuripi X1	Toregua	mid-late Tournaisian
CICYTTP-M 128	577	*Hystricosporites delectabilis*	Manuripi X1	Toregua	mid-late Tournaisian
CICYTTP-M 143	576	*Hystricosporites spiralis*	Manuripi X1	Toregua	mid-late Tournaisian
CICYTTP-M 155	576	*Hystricosporites delectabilis*	Manuripi X1	Toregua	mid-late Tournaisian
CICYTTP-M 190	729	*Hystricosporites elongatus*	Pando X1	Toregua	mid-late Tournaisian
CICYTTP-M 197	729	*Hystricosporites elongatus*	Pando X1	Toregua	mid-late Tournaisian
CICYTTP-M 198	729	*Hystricosporites spiralis*	Pando X1	Toregua	mid-late Tournaisian
CICYTTP-M 273	1389	*Hystricosporites furcatus*	Pando X1	Toregua	mid-late Tournaisian
CICYTTP-M 274	1389	*Hystricosporites delectabilis*	Pando X1	Toregua	mid-late Tournaisian
CICYTTP-M 376	1327	*Hystricosporites furcatus*	Pando X1	Toregua	mid-late Tournaisian
CICYTTP-M 377	1327	*Hystricosporites delectabilis*	Pando X1	Toregua	mid-late Tournaisian
CICYTTP-M 400	1380	*Hystricosporites spiralis*	Pando X1	Toregua	mid-late Tournaisian
CICYTTP-M 408	1338	*Hystricosporites costatus*	Pando X1	Toregua	mid-late Tournaisian
CICYTTP-M 409	1338	*Hystricosporites spiralis*	Pando X1	Toregua	mid-late Tournaisian
CICYTTP-M 417	1334	*Hystricosporites spiralis*	Pando X1	Toregua	mid-late Tournaisian
CICYTTP-M 422	1334	*Hystricosporites furcatus*	Pando X1	Toregua	mid-late Tournaisian
CICYTTP-M 434	729	*Hystricosporites delectabilis*	Pando X1	Toregua	mid-late Tournaisian
CICYTTP-M 435	729	*Hystricosporites expandus*	Pando X1	Toregua	mid-late Tournaisian
CICYTTP-M 436	578	*Hystricosporites furcatus*	Manuripi X1	Toregua	mid-late Tournaisian
CICYTTP-M 437	729	*Hystricosporites furcatus*	Pando X1	Toregua	mid-late Tournaisian
CICYTTP-M 438	573	*Hystricosporites spiralis*	Manuripi X1	Toregua	mid-late Tournaisian
CICYTTP-M 439	729	*Hystricosporites spiralis*	Pando X1	Toregua	mid-late Tournaisian
CICYTTP-M 440	731	*Hystricosporites spiralis*	Pando X1	Toregua	mid-late Tournaisian
CICYTTP-M 441	731	*Hystricosporites spiralis*	Pando X1	Toregua	mid-late Tournaisian
CICYTTP-M 442	734	*Hystricosporites spiralis*	Pando X1	Toregua	mid-late Tournaisian
CICYTTP-M 478	564	*Hystricosporites delectabilis*	Manuripi X1	Toregua	mid-late Tournaisian
CICYTTP-M 485	565	*Hystricosporites delectabilis*	Manuripi X1	Toregua	mid-late Tournaisian
CICYTTP-M 488	565	*Hystricosporites expandus*	Manuripi X1	Toregua	mid-late Tournaisian
CICYTTP-M 490	566	*Hystricosporites spiralis*	Manuripi X1	Toregua	mid-late Tournaisian
CICYTTP-M 491	566	*Hystricosporites costatus*	Manuripi X1	Toregua	mid-late Tournaisian
CICYTTP-M 494	566	*Hystricosporites costatus*	Manuripi X1	Toregua	mid-late Tournaisian
CICYTTP-M 515	733	*Hystricosporites spiralis*	Pando X1	Toregua	mid-late Tournaisian
CICYTTP-M 518	733	*Hystricosporites furcatus*	Pando X1	Toregua	mid-late Tournaisian
CICYTTP-M 519	733	*Hystricosporites furcatus*	Pando X1	Toregua	mid-late Tournaisian