Geobios 46 (2013) 243–252

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Original article

Ultrastructural study of *Arcellites humilis* Villar de Seoane and Archangelsky, 2008, from the Cretaceous of Patagonia, Argentina☆

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ARTICLE INFO

Article history:
Received 6 February 2012
Accepted 18 July 2012
Available online 17 April 2013

Keywords:
Ultrastructure
Megaspores
Marsileaceae
Cretaceous
Patagonia
Argentina

ABSTRACT

A scanning and transmission electron microscopy-based ultrastructural study of *Arcellites humilis* Villar de Seoane and Archangelsky, 2008, from the Kachaike Formation and Piedra Clavada Formation (Albian-Cenomanian) from several localities in Patagonia (Argentina), is presented in this paper. TEM-photographs of thin-sectioned specimens show a thick spore body wall composed of three layers: a thin and dense exine, an inner granular perine sublayer with irregularly disposed small channels and lacunae, and an outer alveolate perine sublayer with channels perpendicular to the external surface. Transverse sections of the *A. humilis* wall are compared with fossil and extant species of Marsileaceae. We show that the wall ultrastructure is similar in *A. humilis*, *A. santacrucensis*, *A. disciformis*, *A. stellatus* and *Regnellidium upatoiensis*. However, the body wall and acrolamella of *A. humilis* are more similar to those of *R. diphyllum* Lindman than to those of the *Marsilea* L. or *Pilularia* L. species. Water ferns such as members of the Marsileaceae played an important role in aquatic or semi-aquatic niches in Patagonian Cretaceous floras, suggesting that high humidity and temperature prevailed during the Albian-Cenomanian in this region of Argentina.

1. Introduction

The genus *Arcellites* was erected by Miner (1935) to refer to megaspores from the Upper Cretaceous in Greenland that have a small and circular body with few to several short tube-like appendages and a pitted external surface. The genus was later emended several times (Potter, 1963) until Ellis and Tschudy (1964), working with Cretaceous specimens from the western USA, included in the genus all megaspores with a proximal neck formed of leaf-like segments and adjusted the diagnosis of the type species *Arcellites disciformis*.

More recently, species of *Arcellites* have been described using scanning and transmission electron microscopy (Li and Batten, 1986; Baldoni, 1987; Baldoni and Taylor, 1988; Batten et al., 1996; Nowak and Lupia, 2004, among others). Currently, *Arcellites* Miner emend. Ellis and Tschudy contains approximately 20 species, of which morphological studies have been conducted and 10 species have undergone ultrastructural analysis. *Arcellites* belongs to the Marsileaceae, which includes water ferns exclusive to tropical America. These plants have highly specialized reproductive structures: the megagametophytes are enclosed within the spores (which are generally dispersed in water), and the embryo develops at the water/soil interface (Tryon, 1990). The family is represented by three extant genera: *Marsilea* L. with seventy species, *Pilularia* L. with five species, and the monotypic *Regnellidium* Lindman. *Marsilea* and *Pilularia* are cosmopolitan, whereas *Regnellidium* (with its single living species *R. diphyllum*) is native to Rio Grande do Sul and southern Santa Catarina, Brazil, and to the Corrientes province in Argentina, where it grows amidst aquatic vegetation along the shores of lakes and also in stagnant ponds (De la Sota and Mitchell, 1970; Tryon and Tryon, 1982). The family is clearly distinct and is not closely allied with other ferns (Tryon and Tryon, 1982).

Fossil specimens of *Arcellites* are restricted to the Cretaceous and mostly to the Early Cretaceous: late Valanginian – early Albian of England (Batten et al., 1996), P.R. China (Li and Batten, 1986), USA (Nowak and Lupia, 2004), and Argentina (Baldoni and Taylor, 1988; Batten et al., 1996). There are only a few known species from the Late Cretaceous (late Albian – Cenomanian) in the USA (Batten et al., 1996), Greenland (Batten et al., 1996), and Argentina (Villar de Seoane and Archangelsky, 2008). Four *Arcellites* species are known to occur in Southern Patagonia: *A. santacrucensis* Baldoni (Baldoni, 1987), *A. humilis* Villar de Seoane and Archangelsky, *A. pentagonalis* Villar de Seoane and Archangelsky, and *A.* sp. A (Villar de Seoane and Archangelsky, 2008).

In the present paper, the body, acrolamella and appendages wall ultrastructure of *Arcellites humilis* are studied using scanning and transmission electron microscopy (SEM and TEM), and

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http://dx.doi.org/10.1016/j.geobios.2012.07.002

compared with the wall structure of fossil species and the body and acrolamella structure of the extant genera *Marsilea*, *Pilularia*, and *Regnellidium*. The study is based on new specimens recovered from material collected at the type locality Cardiel Lake (Villar de Seoane and Archangelsky, 2008). The ultrastructural analyses of the body wall of *A. humilis* based on SEM and TEM observations adds new information that can be used to compare this species with other similar taxa. An emended diagnosis of *A. humilis* is therefore presented.

2. Material and methods

The described assemblages are from three localities of Western Santa Cruz Province: Bajo de La Comisión, Estancia La Federica and Cardiel Lake (Fig. 1). Bajo de La Comisión is located to the northeast of San Martín Lake, near a stream of the same name. Four samples (PBC6, PBC6′, PBC8 and PBC9) belonging to the middle part of the stratigraphic section located at 48°51′ S and 72°02′ W (Guler and Archangelsky, 2006) were studied. The Estancia La Federica section is located to the east of San Martín Lake. Two surface samples were studied, QEMK2 at 49°03′54″ S and 72°08′25″ W; and LLK3 at 49°00′09″ S and 72°08′25″ W. Cardiel Lake is surrounded by several hills that emerge from a basaltic plateau (Ramos, 1982). To the northwest of the lake two surface samples from Cerro Bayo, a low hill, were studied: PCB3 at 48°44′50″ S and 71°23′52″ W, and PCB5 at 48°44′32″ S and 71°24′18″ W.

From a stratigraphic point of view the material collected from Bajo de La Comisión and Estancia La Federica belongs to the Kachaike Formation, dated as Albian (Guler and Archangelsky, 2006), and the specimens collected from Cardiel Lake are referred to the Piedra Clavada Formation, also Albian in age, but which might extend into Cenomanian (Nullo et al., 1999).

The megaspores are very well preserved and therefore can be used for SEM and TEM analyses. In the SEM procedure, they were treated with HCl and HF to extract the organic residue from the sediments. Then, the residues were washed through a 250 μm mesh sieve, and the megaspores were isolated, mounted on circular stubs, and coated with gold-palladium. Observations were made using a Philips XL30 TMP microscope at the Electron Microscopy Service of the Bernardino Rivadavia Museum of Natural Sciences in Argentina. For TEM, the megaspores were treated with OsO4 and embedded in Spurr low viscosity resin (Spurr, 1969). Transverse sections (TSs) of the megaspores were cut with a diamond knife on a SORVAL manual ultramicrotome and mounted on single-hole grids coated with Formvar and stained with $KMnO_4$ (5–10 min) and uranyl acetate (30 s). Two specific areas were used to cut TSs, the first (A on Fig. 2(1)) located in the extremities of foliaceous leaf-like segments of the acrolamella, and the second (B on Fig. 2(1)) in the proximal face, where the acrolamella and appendages originate. Light micrographs of TSs were taken with a Leica DFC 280 and TEM observations were made on a Zeiss EM 109 microscope at the Electron Microscopy Service of the Cellular Biology Department at the Faculty of Medicine in Buenos Aires University. The specimens are stored in the Paleobotanical Collection of the museum under catalogue numbers with the prefixes BA Pb Pm, BA Pb MEB, and BA Pb MET.

The terminology of Schneider and Pryer (2002) was used in the ultrastructural analysis. The terminology used by Baldoni and Taylor (1988), Batten et al. (1996), Tryon and Lugardon (1991), and Schneider and Pryer (2002) relevant to the description and comparison of different species, is shown in Table 1. The dimensions include the specimens found at the five localities

Table 1
Comparative terminology used by several authors for the different spore wall layers. The terminology of Schneider and Pryer (2002) was used in the present study.

Schneider and Pryer (2002)	Tryon and Lugardon (1991)	Batten et al. (1996)	Baldoni and Taylor (1988)
Outer sublayer of gelatinous perine layer	——	——	——
Inner sublayer of gelatinous perine layer	——	——	——
Outer sublayer of solid perine layer	Epispore	Outer exoexine	Outer sexine
Inner sublayer of solid perine layer	Epispore	Inner exoexine	Inner sexine
Exine	Exospore	Intexine	Nexine
Intine	Pseudoendospore	——	——

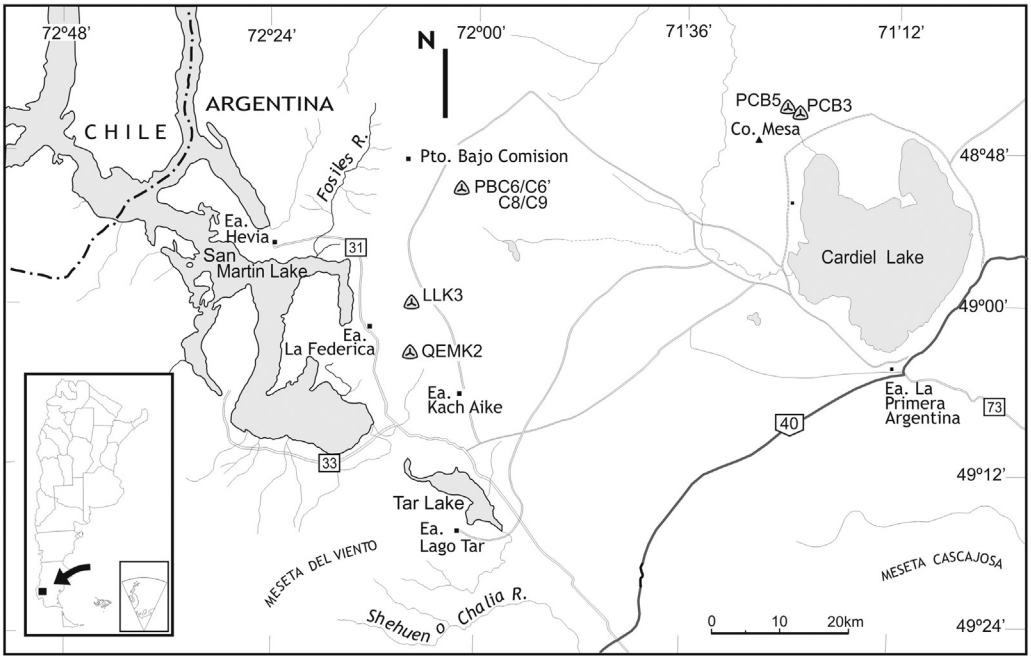

Fig. 1. Map of Argentina showing the localities studied in the western region of the Santa Cruz Province. Abbreviations: PCB: Cerro Bayo; LLK: La Lila; QEMK: El Quemado; PBC: Bajo de La Comisión.

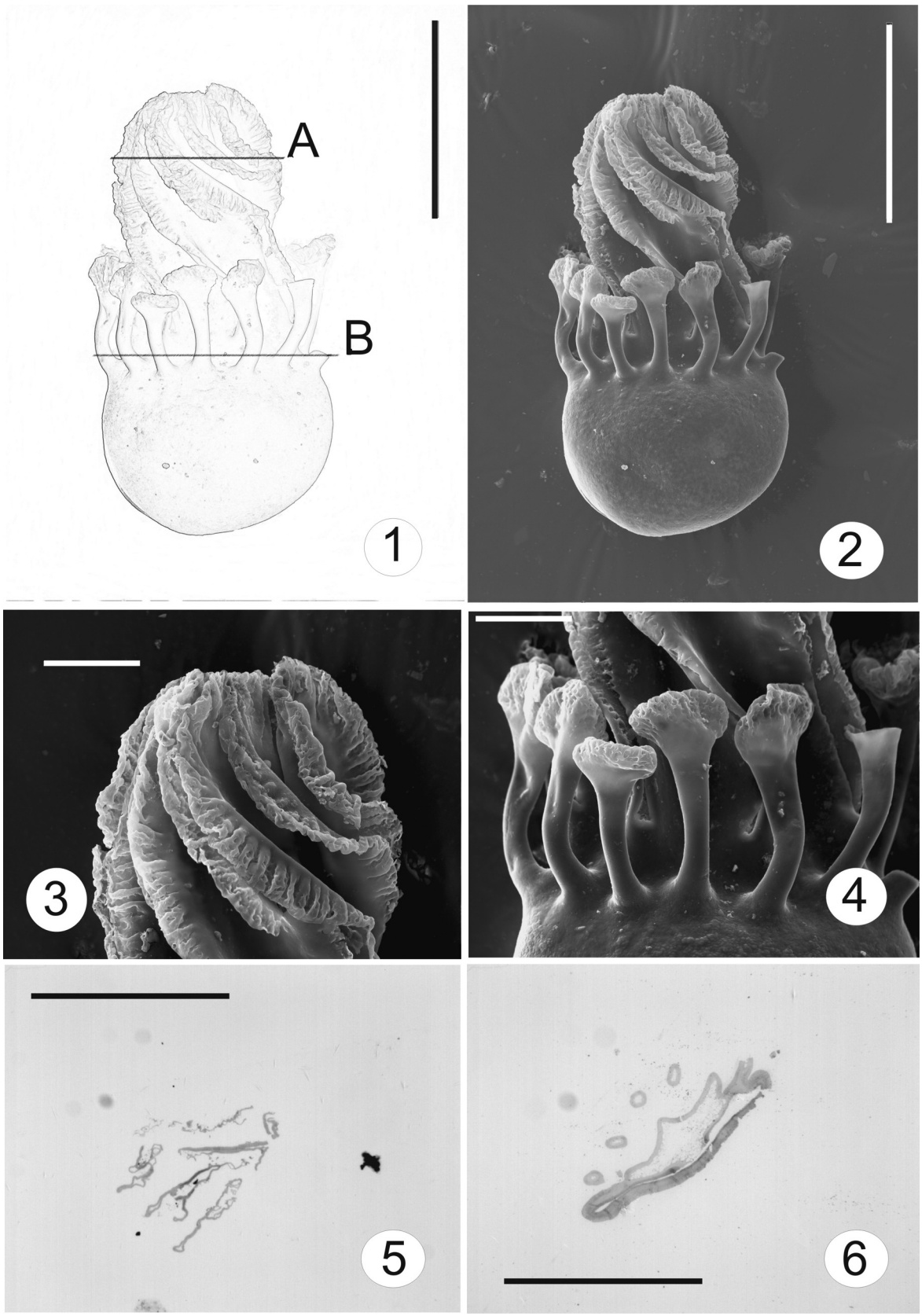

Fig. 2. *Arcellites humilis* Villar de Seoane and Archangelsky, 2008. **1**. Drawing showing lines A and B where the TEM transverse sections (TSs) were made. **2**. General view of specimen BA Pb MEB 241 under SEM. **3**. Details of the acrolamella of Image 2 under SEM. **4**. Details of the proximal face and appendages of Image 2 under SEM. **5**. LM image of TS on acrolamella extremities (A on Fig. 2(1)), BA Pb Pm 589. **6**. LM image of TS (oblique section) on the proximal face with the acrolamella origin and appendage bases (B on Fig. 2(1)), BA Pb Pm 588. Scale bars: 1, 2, 5, 6: 200 μm; 3, 4: 50 μm.

(Fig. 1; Villar de Seoane and Archangelsky, 2008); numbers in parentheses refer to the average of the 32 specimens.

3. Systematic paleontology

Family MARSILEACEAE Mirbel, 1802

Genus ***Arcellites*** Miner, 1935 emend. Ellis and Tschudy, 1964

Type species: *Arcellites disciformis* Miner, 1935 emend. Ellis and Tschudy, 1964.

Arcellites humilis Villar de Seoane and Archangelsky, 2008

Figs. 2–5

2008. *Arcellites humilis* nov. sp. – Villar de Seoane and Archangelsky, pp. 356–358, fig. 2C, D.

Material: BA Pb Pm 588, BA Pb Pm 589, BA Pb Pm 590 and BA Pb MET 214 (two specimens studied using TEM); BA Pb MEB 241 and BA Pb MEB 383 (eight specimens studied using SEM).

Measurements (based on 32 specimens): Spore body diameter, 211 (219) 225 μm; width of wall, 18.5 μm; length of acrolamella, 277 (290) 304 μm; width of acrolamella wall, 5.60 μm; width of trilete laesura wall, 9.50 μm; appendages: length, 100–125 μm; thickness, 11 (15) 22 μm; width at apex, 46 (55) 60 μm; width at base, 9–10 μm.

Emended diagnosis: Trilete megaspore with spherical body. Crown of 12–14 appendages around a twisted acrolamella at the proximal face. Body diameter of up to 225 μm. Short and hollow appendages with expanded apexes. Body exine slightly verrucate. Trilete laesura with short branches covered by the acrolamella. Outer surface of spore body and appendages slightly pitted. Three-layered spore wall with a dense exine layer and two perine sublayers. Inner granular perine sublayer with small channels and lacunae. Outer alveolate perine sublayer with channels perpendicular to the external surface.

Description: Megaspores have a spherical body with a pitted surface ornamented by low verrucae that are irregularly distributed (Fig. 3(4)). The proximal face has a trilete laesura that is covered by a strong acrolamella 160 μm-wide (Fig. 2(2)). The acrolamella is composed of seven leaf-like segments dextrally twisted. The undulating margin of the leaf-like segments has a foliaceous appearance (Fig. 2(3, 5)). At the apex of the megaspore body, there are 12–14 short appendages that form a crown surrounding the acrolamella (Fig. 2(2, 4)). The appendages have a constricted base and end with an expanded apex that presents a spongy and reticulated appearance and is 60 μm-wide. The external surface of the appendages is pitted (Fig. 3(6)).

The spore body wall is composed of three layers and has a similar width on each face. On the proximal face, it is 18.5 μm-thick, whereas on the distal face, it is 16.3 μm-thick (Fig. 2(6)). The exine layer is 2.75 μm-thick, dense and compact and sometimes has a granular appearance (Fig. 3(1, 5)). However, near the laesura, its width increases to 6.5 μm (Fig. 3(1, 2)). The inner perine sublayer is 6.5 μm-thick. It has dark, small channels and lacunae irregularly disposed (Figs. 3(1, 3), 4(1, 3), 5(2)). The outer perine sublayer is 7 μm-thick. It is alveolate and has channels that are perpendicular to the external surface (Figs. 3(4), 4(3)). Also, this sublayer has few perpendicular lacunae and short channels of 1.5 μm. Each channel ends at the outer surface with a hole 0.5 μm in diameter (Fig. 4(1, 3)).

Although an external gelatinous perine layer is not preserved in fossil specimens, *A. humilis* has an irregular dark layer, with a thickness of 1.5 μm, on its external surface. This layer may be a remnant of the gelatinous perine (Figs. 4(3), 5(2, 3)).

At the end of the laesura branches, the inner perine sublayer is divided into two layers (Figs. 2(6), 3(1)), of which the innermost and the exine extend up to the laesura, whereas the outermost and outer perine sublayer form the acrolamella wall (Figs. 2(6), 3(1*)).

The acrolamella wall is composed of two layers (Fig. 3(3)). The outermost layer of the inner perine sublayer is 2.0 μm-thick and has a spongy appearance with anastomosed channels formed by the joining of long and circular elements, 0.05–0.10 μm in diameter (Fig. 3(1)). These elements form a net with large lacunae inside the cavity body (Fig. 3(3)). The outer perine sublayer is 4.50 μm-thick and has an alveolate appearance, with small channels and lacunae combined with long and circular elements (Figs. 3(3), 4(3)). This sublayer has large perpendicular lacunae that in some cases form long channels (1.0–2.0 μm) that end at the external surface with a hole 0.5 μm in diameter (Figs. 3(3), 4(3)). The extremity wall of the foliaceous leaf-like segments is thinner (1–2 μm) than the acrolamella wall and is composed of a compact exine layer (Figs. 2(5), 5(3)).

The trilete laesura wall is 9.50 μm-thick and has two layers (Fig. 2(6)). The exine layer is 2.75 μm-thick, dense and compact, and increases in width near the laesura (Fig. 2(6)). The innermost layer of the inner perine sublayer is 6.75 μm-thick. It has anastomosed elements that form an open net with large lacunae in the acrolamella cavity, especially in the trilete zone (Figs. 2(6), 3(1)).

The wall of the appendages is composed of two layers (Figs. 2(6), 3(6)); it is 9.35 μm-thick at the base and increases distally where the appendages are more flattened. The inner perine sublayer is 2.50 μm-thick and has an alveolate appearance with closed channels in which long and circular elements are combined. It has an open net aspect with elements 0.15–0.25 μm in diameter (Figs. 4(2), 5(1)). The outer perine sublayer is 4.50 μm-thick. It is compact but, on the outer surface, shows small pits with short channels 0.75 μm-long (Figs. 4(2), 5(1)). The appendages have expanded apexes with a foliaceous appearance (Fig. 2(4)). The wall (4.0 μm-thick) is composed of a compact exine layer.

Remarks: *A. humilis* is compared with *Arcellites santacrucensis* and other fossil species with a known spore wall ultrastructure. Morphological comparisons with other *Arcellites* species have been already published (Villar de Seoane and Archangelsky, 2008).

A. santacrucensis from the Kachaike Formation, dating back to the late Aptian-early Albian in the Santa Cruz Province (Argentina), has been studied twice with TEM. We use only the material analysed by Batten et al. (1996) because the specimens described by Baldoni and Taylor (1988) are significantly deteriorated: the ultrastructure of the layers is not very clear, and the exoexine has a loose texture. *A. santacrucensis* has a thicker spore wall than *A. humilis*, with a few different ultrastructural elements in the body and appendages. Baldoni and Taylor (1988) described the spore wall as having four layers and being up to 20 μm-wide but indicated that, in the neck base, it is only 5 μm-wide because some layers have disappeared due to degradation. In general, the nexine appears more compact and thinner (4 μm). The sexine is solid and dark with large holes, but often the outer sexine is missing, so the wall anatomy is very irregular. Batten et al. (1996) studied new specimens of *A. santacrucensis* and divided the spore wall into three layers. The wall is 7.8–12.0 μm-thick and composed of a granular outer exoexine (3.1–5.3 μm-thick), a finely granular inner exoexine (2.6–3.8 μm-thick), and a dense intexine perforated by small channels (1.7–2.4 μm-thick). Batten et al. (1996) suggested that the differences between the first and second descriptions are due to the large amount of degradation affecting the specimens studied by Baldoni and Taylor (1988). The surface of well-preserved specimens is pitted and lacks verrucae. Extending from the apex of the megaspore body are 10–12 slender appendages with a length of 294–349 μm, a width of 7–11 μm at the base, and expanded apexes. These appendages form a crown that surrounds the acrolamella, similar to appendages of *A. humilis*. In *A. humilis*, the neck is clearly an extension of the perine sublayers and particularly the outer perine sublayer (Figs. 2(6), 3(1, 3)), which

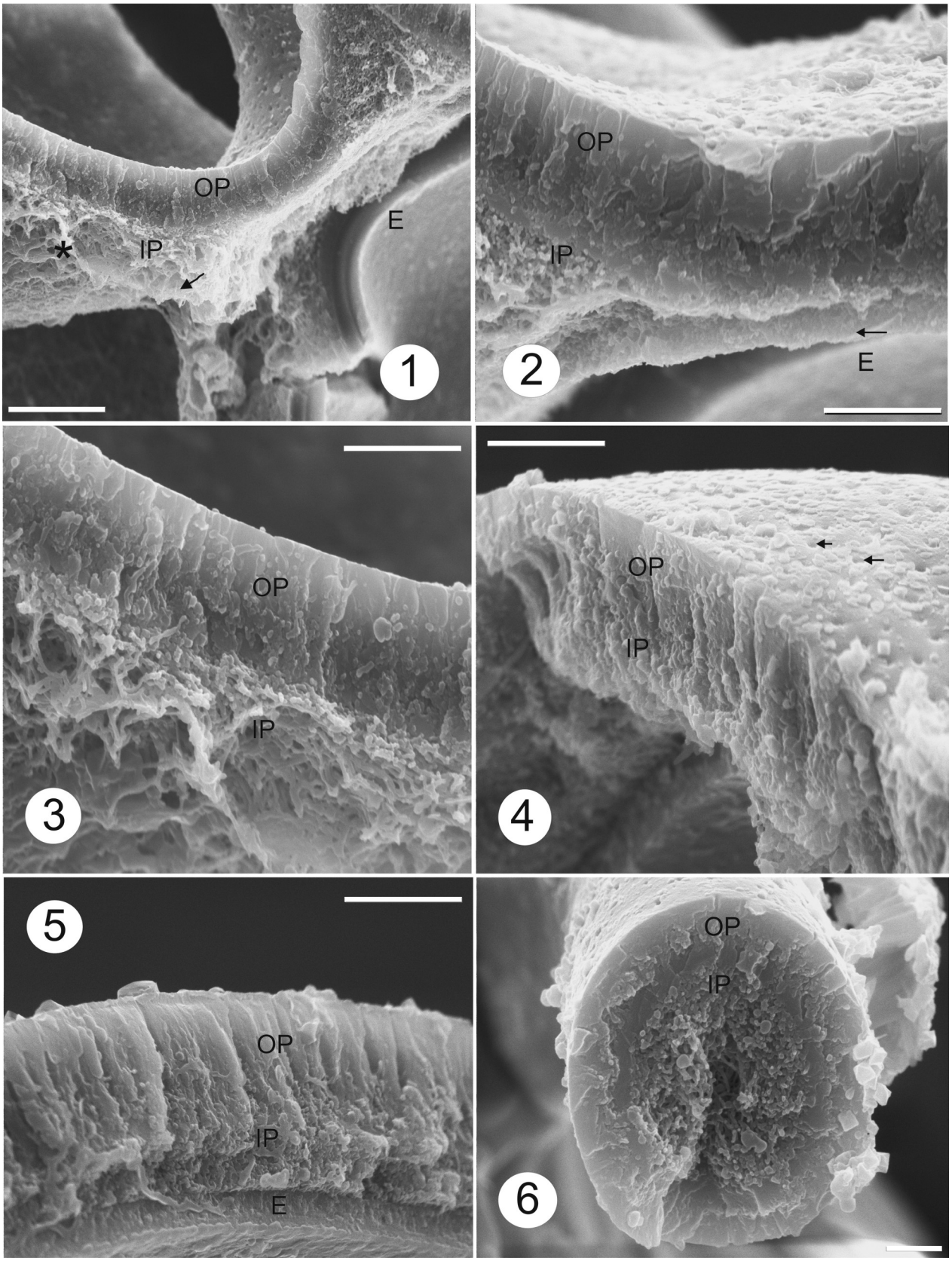

Fig. 3. *Arcellites humilis* Villar de Seoane and Archangelsky, 2008. SEM views of specimen BA Pb MEB 383. **1**. Transverse section (TS) of the proximal face where the inner perine sublayer is divided into two layers (arrow). **2**. Details of the proximal face where the perine layer is separated from the exine (arrow). **3**. Details of the inner and outer perine sublayers. **4**. TS of the body wall showing the pits on the external surface (arrows) connected with the channels (short arrows) of the perine sublayers. **5**. TS of the body wall showing the exine and perine sublayers. **6**. TS of one appendage showing the perine sublayers. E: exine; IP: inner perine sublayer; OP: outer perine sublayer. Scale bars: 1: 10 μm; 2–5: 5 μm; 6: 2 μm.

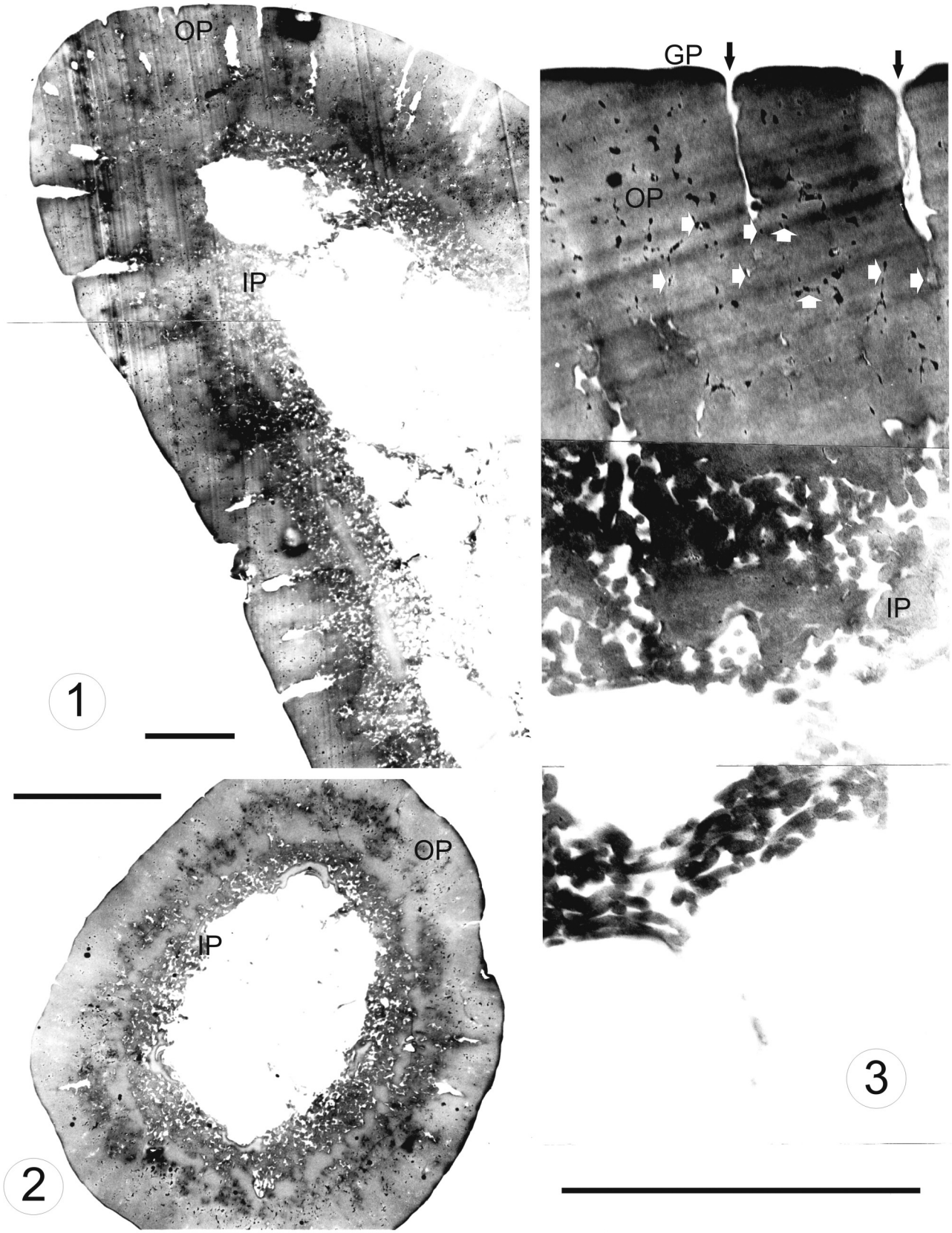

Fig. 4. *Arcellites humilis* Villar de Seoane and Archangelsky, 2008. TEM views of specimen BA Pb MET 214. **1**. Transverse section (TS) of the body wall on the proximal face. **2**. TS of the appendage wall (section at line B of Fig. 2(1)). **3**. Details of the exine, solid perine sublayers and gelatinous perine layer of the body wall. IP: inner perine sublayer; OP: outer perine sublayer; GP: gelatinous perine layer. Scale bars: 10 μm.

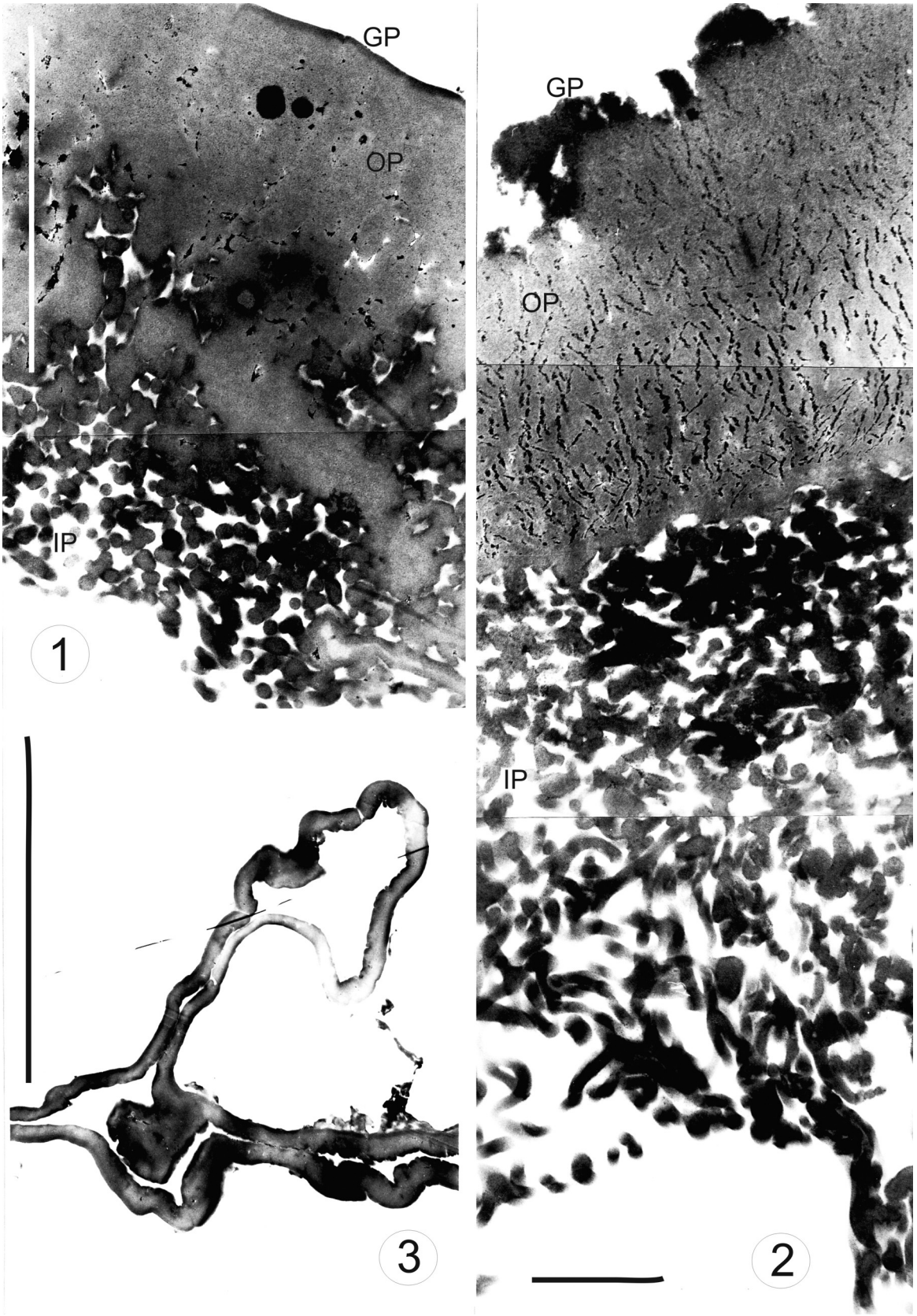

Fig. 5. *Arcellites humilis* Villar de Seoane and Archangelsky, 2008. TEM views of specimen BA Pb MET 214. **1**. Details of the gelatinous perine layer, the outer perine sublayer with scarce channels, and the inner perine sublayer with circular elements on the appendage wall. **2**. Details of the irregular gelatinous perine layer, the outer perine sublayer with vertical and branched channels, and the inner perine sublayer with elongated elements on the distal face. **3**. Detail of the transverse section of acrolamella foliaceous extremities showing the compact exine layer (section at line A of Fig. 2(1)). IP: inner perine sublayer; OP: outer perine sublayer; GP: gelatinous perine layer. Scale bars: 5 μm.

Table 2
Ultrastructural comparison of *Arcellites humilis* with fossil and extant species from the Marsileaceae and their stratigraphic occurrences.

Species	Wall width (μm)	Surface sculpture	Perine layers	Exine	Acrolamella	Appendages	Age
Arcellites humilis	18.5	Verrucate and pitted	Outer: alveolate Inner: granular	Compact to granular	Dextrally twisted	12–14 short	Albian-Cenomanian
Regnellidium diphyllum (Schneider and Pryer, 2002)	30.0	Psilate to slightly rugulate	Outer: alveolate Inner: smooth to granular	Compact	Twisted	—————	Extant
Regnellidium upatoiensis (Lupia et al., 2000)	18.5	Baculate and pitted	Outer: alveolate Inner: granular	Psilate	Twisted	—————	Santonian
Arcellites santacrucensis (Batten et al., 1996)	7.8–12	Pitted and smooth	Outer: granular Inner: granular	Perforated	Dextrally twisted	10–12 long	Late Aptian-early Albian
Arcellites stellatus (Nowak and Lupia, 2004)	31	Small dimpled elements	Outer: granular Inner: fibrous	Perforated	Straight	15–34 short	Aptian
Arcellites disciformis (Batten et al., 1996)	6.5	Pitted and smooth	Outer: granular Inner: finely granular	Perforated	Dextrally twisted	Numerous and short	Barremian-Cenomanian
Arcellites plicatus (Li and Batten, 1986)	14–24	Reticulate	Outer: granular Inner: granular	Compact	Straight	—————	Barremian-Aptian
Arcellites yiminensis (Li and Batten, 1986)	7.5–10	Pitted	Outer: spongy Inner: spongy	Compact	Straight	Numerous and long	Barremian-Aptian
Arcellites hexapartitus (Batten et al., 1996)	8.5–12	Perforated	Outer: granular Inner: granular	Perforated	Twisted	Numerous and short	Late Barremian
Arcellites vectis (Batten et al., 1996)	16–17.5	Scabrate-rugulate	Outer: granular Inner: granular-spongy	Perforated	Straight	Numerous and long	Barremian
Arcellites medusus (Batten et al., 1996)	8–18	Rugulate	Outer: granular Inner: granular	Granular	Straight	Numerous and long	Late Valanginian

coincides with the outer layer of the exoexine in the neck of *A. santacrucensis* (Batten et al., 1996).

Other species with known body wall ultrastructure show similarities and differences with *A. humilis* (Table 2). The spore body wall of *A. humilis* is thinner than that of *Arcellites stellatus* Nowak and Lupia, 2004 from the Early Cretaceous Potomac Group, USA, and *Arcellites plicatus* Li and Batten, 1986 from northeast inner Mongolia, P.R. of China. *A. stellatus* has a similar wall (31 μm-thick) composed of three layers, an inner exine layer (4 μm) and two perine sublayers. The inner perine sublayer is fibrous and the outer perine sublayer is granular, with an irregularly crumpled surface with small and dimpled sculptural elements. The megaspore body is covered with 15–34 slender and hollow appendages of 20–200 μm in length that terminate bluntly or are very slightly swollen, with an irregularly granulate and pitted surface (Nowak and Lupia, 2004). *A. plicatus* has a dense and compact intexine (4 μm) with smooth inner and granular outer layers. The exoexine (10–20 μm) is loosely structured internally but is denser and finely perforated towards the outer surface of the spore body, forming the muri and lumina of the reticulum and the acrolamella (Li and Batten, 1986).

The spore body wall of *A. humilis* is thicker than that of: *A. disciformis* (Miner) Ellis and Tschudy, 1964 from the Barremian-Aptian Patuxent Formation, Potomac Group, USA (Hueber, 1982) and the Late Albian-Cenomanian Atane Formation in West Greenland (Batten et al., 1996); *Arcellites hexapartitus* (Dijkstra) Potter, 1963 from the late Barremian Wealden in the Netherlands and England (Hughes, 1955; Potter, 1963; Batten et al., 1996); *Arcellites medusus* (Dijkstra) Potter, 1963 from the late Valanginian Upper Tunbridge Wells Formation in southeast England (Batten et al., 1996); *Arcellites vectis* (Hughes) Potter, 1963 from the Barremian Wessex Formation on the Isle of Wight (Batten et al., 1996); and *Arcellites yiminensis* Li and Batten, 1986 from the Early Cretaceous in northeast Inner Mongolia.

The similar wall of *A. disciformis* (6.5 μm-thick) has three layers. The outer exoexine has a fairly open texture and a coarsely granular appearance, with numerous pits perpendicular to the external surface that give it a palisade-like appearance. The inner exoexine is more finely granular than the outer exoexine, and the intexine is irregularly channelled (Batten et al., 1996). Hueber (1982) studied the ultrastructure of this species using SEM and described an outer-pitted exoexine, an inner reticulate exoexine, and a compact and almost amorphous intexine. The megaspore body is covered with slender, hollow short appendages that form extensions of the outer exoexine and do not have pits except at the apex of the appendages, where the layer is thinner and more granular. The inner exoexine extends very slightly into the base of each appendage. Hueber (1982) also mentioned that the appendages are formed of an extension of the same layers.

A. hexapartitus has an exine (8.5–12.0 μm-thick) composed of three distinct layers. The outer exoexine (3.5–5.5 μm-thick) has a coarsely granular aspect, and moving outwards, these sporopollenin elements form a smooth, finely pitted surface. The inner exoexine (3.0–4.0 μm-thick) is also finely granular. The intexine (2.5 μm–thick) is darker and irregularly channeled, becoming more granular towards the base. The surface of the spore body has hollow and short appendages that form extensions of the outer exoexine; they are finely perforated and end with swollen, crumpled, foveo-reticulate tips (Batten et al., 1996).

A. medusus and *A. vectis* show a different wall structure. The former has an exine of 8.0–18.0 μm-thick with three layers. The outer exoexine (0.7–1.1 μm) forms an undulated to ribbed exterior surface, whereas the inner exoexine (7.0–14.0 μm) and the intexine (1.6–3.1 μm-thick) have a granular appearance. The body has long, ribbed appendages with a rugate surface that is parallel to their sides (Batten et al., 1996). *A. vectis* has an exine (16.0–17.5 μm) composed of an exoexine and an intexine. The outer exoexine (1.5 μm-thick) has granular elements, and the inner exoexine (11.5–13.0 μm-thick) has a granular-spongiose appearance. The intexine (3.0–3.6 μm-thick) is perforated by narrow channels (Batten et al., 1996).

A. yiminensis has a dense intexine (2–3 μm) that is smooth on the inner surfaces and granular on the outer surfaces and that adheres closely to the exoexine. The exoexine (4.5–7 μm-thick) is spongy, with small holes in the inner layer and a more open outer layer towards the pitted surface (Li and Batten, 1986).

4. Discussion

According to Schneider and Pryer (2002) *Marsilea* megaspores have a prolate outline and an acrolamella in the shape of a disk, where the perine layer is extremely reduced. This layer is thin and undifferentiated, with a surface that is more or less psilate. The surface body is reticulate. Also, *Pilularia* has megaspores with a prolate outline. The acrolamella has six to seven straight leaf-like folds; the perine layer has a uniform structure, and its surface is psilate. The ornamentation of the body wall is reticulate. Finally, *Regnellidium* megaspores have a spheroidal outline and a prominent acrolamella with six or seven twisted leaf-like folds. The perine layer is alveolate and thin and has a psilate surface. The body surface exhibits rugulate or baculate ornamentation.

A. humilis has a body wall and acrolamella structure that are partly similar to those of the extant genus *Regnellidium*. However, the body wall is thicker in *Regnellidium*, whereas its acrolamella is shorter than that of *A. humilis*. Nevertheless, in both the extant and the fossil specimens, the acrolamella is formed of six to seven twisted prominent leaf-like segments (Schneider and Pryer, 2002: fig. 8E; Fig. 2(2, 3)). In *A. humilis* the neck is principally an extension of the outer sublayer of the solid perine that forms a small chamber as it does in the extant species. The inner sublayer and the exine extend up to the laesura (Schneider and Pryer, 2002: fig. 8F; Fig. 3(1, 2)).

The comparison between *A. humilis* and *Regnellidium diphyllum* has shown that they have a partially comparable ultrastructure and that the two taxa have a similar geographic-paleogeographic distribution. A comparison of their TS walls conducted using SEM reveals an alveolate palisade in *Regnellidium* (Schneider and Pryer, 2002: fig. 8F), while that of *A. humilis* is slightly more compact, possibly as a result of the fossilisation process (Fig. 3(3, 4, 5)). Near the acrolamella, the exine is separated from the solid perine layer in a similar way (Schneider and Pryer, 2002: fig. 8F; Fig. 3(1)).

R. diphyllum has a psilate and rugulate-to-baculate surface, whereas the surface of *A. humilis* is slightly verrucate and pitted. The TSs of the body walls in both species are formed of three layers. A thin exine and a solid perine layer are composed of two sublayers. The inner sublayer can vary from smooth to granulate, and the outer sublayer has an alveolate ultrastructure and is thicker than the inner one (Schneider and Pryer, 2002: fig. 8F; Figs. 3(3, 5), 4(1, 3)).

In *Regnellidium upatoiensis* from the Eutaw Formation (Santonian, Late Cretaceous) along Upatoi Creek, Georgia, USA, Lupia et al. (2000) included three sporocarps with megaspores and microspores. The authors suggested that these sporocarps represent the first instance of *Regnellidium* in the fossil record. *R. upatoiensis* megaspores have an 18.5 μm-thick wall composed of an inner exine and outer perine. The exine is psilate and 2.5 μm-thick, whereas the perine (16 μm-thick) is composed of two sublayers, a granular inner sublayer and an alveolate outer sublayer grading into hollow baculae.

The extinct morphogenus *Molaspora* Schemel, 1950 emend. Hall, 1963 is very similar to *Regnellidium* (Batten, 1988; Collinson, 1992; Batten et al., 2011). This observation indicates that the *Regnellidium* lineage may extend from the Albian to the Paleocene.

As Marsileaceae are amphibious ferns, their sporocarps are units for long-distance dispersal and dormancy. When the megaspores are released into water, they germinate quickly, so they have some elements to protect them and the future gametophyte. The gelatinous layer represents a special adaptation to reproduction; it provides to the megaspores with a mechanism to float at the water/air interface for an extended period. On the other hand, the acrolamella has a harmomegathic mechanism by which the water is transferred and a small chamber is formed between the exine and the perine layers. The sperm cells enter this chamber and release the male gametophytes that swim as far as the archegonium and fertilize it (Schneider and Pryer, 2002). The neck, appendages and ultrastructure of *A. humilis* suggest an adaptation to environmental conditions similar to those associated with extant marsileaceous species, including floating, fertilization and the sinking of the young embryo into the water-soil interface. This type of environment was frequent in the Albian formations that are found in the Austral Basin of Southern Patagonia, in which megaspores are abundantly represented.

The new material of *A. humilis* from the type locality allows a deeper ultrastructural analysis of the body wall based on further SEM and TEM observations and a new, emended diagnosis of the species is presented. The comparison with fossil species with a known body wall ultrastructure suggests that *A. humilis* is similar to *A. santacrucensis*, *A. disciformis* and *A. stellatus*. However, differences exist in the surface sculpture, wall width and exine type and with regard to geologic age in some cases: *A. santacrucensis* and *A. disciformis* show a body sculpture pitted and smooth, while *A. stellatus* has a sculpture with small dimpled elements and its acrolamella is straight. *A. disciformis* and *A. stellatus* have numerous short appendages. On the other hand, *A. humilis*, *R. upatoiensis* and the extant *R. diphyllum* have a granulate inner perine sublayer and an alveolate outer perine sublayer, whereas *A. santacrucensis*, *A. disciformis* and *A. stellatus* have a very similar wall morphology but feature granular outer perine sublayers. *A. plicatus*, *A. yiminensis*, *A. hexapartitus*, *A. vectis* and *A. medusus* are different morphological and ultrastructurally: *A. plicatus* has a reticulate surface and straight acrolamella, *A. yiminensis* has spongy perine layers, straight acrolamella and numerous and long appendages, morphologically similar to *A. vectis* and *A. medusus*, while *A. hexapartitus* has numerous but short appendages (Table 2).

Fossil and extant species of Marsileaceae show a similar ultrastructure in terms of the body and acrolamella walls. These findings suggest that this family may extend its paleogeographical and chronological distribution to the Albian in Southwestern Gondwana. Water ferns evolved during the Mesozoic and diversified during the late Cretaceous simultaneously with flowering plants (Pryer, 1999, Lupia et al., 2000).

Acknowledgments

Thanks are due to Orlando Cárdenas for his technical assistance in processing the samples, Amalia González for drawing Fig. 1, Fabián Tricárico of the Electron Microscopy Service at the Bernardino Rivadavia Museum of Natural Sciences for his help with the SEM studies, and Isabel Farías for technical assistance in preparing the megaspores for TEM. The authors are grateful to the Editor in Chief Dr. Gilles Escarguel, Associate-Editor Dr. Marc Philippe, and reviewers Dr. Gaëtan Guignard and Dr. Edith L. Taylor for all the valuable suggestions that have improved the present manuscript. The funding for this study was provided by the National Agency of Scientific and Technological Promotion, Grant PICT 433 - 2007.

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