

Reinterpretation of *Paleoazolla*: a heterosporous water fern from the Late Cretaceous of Patagonia, Argentina

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PREMISE: Undoubtedly, fossils are critical for understanding evolutionary transformations in deep time. Here, we reinvestigate the microspores and megaspores of *Paleoazolla patagonica*, a water fern found in Late Cretaceous sediments of the Chubut Province, Patagonia, Argentina, which provides novel evidence on the past history of the water fern clade. The study was based on recently collected specimens and additional observations of the original material.

METHODS: Most specimens analyzed herein were obtained from new palynological samples collected at the Cerro Bosta and Cañadón del Irupé localities, La Colonia Formation. Samples were mechanically disaggregated and treated with hydrofluoric and hydrochloric acid. Spores were studied using standard light microscopy and scanning electron microscopy. We also reexamined the original materials.

RESULTS: The newly described characters of *Paleoazolla* include the presence of heterosporangiate sori composed of one ellipsoidal megasporangium surrounded by three to four oval microsporangia, megasporangium containing one hairy massula that encloses two trilete megaspores (rarely one or three), and microsporangia containing numerous microspore massulae with non-septate multibarbed glochidia and one trilete microspore per massula.

CONCLUSIONS: The reinterpretation has revealed a novel set of characters for understanding the evolution of heterosporous water ferns. The presence of two megaspores in the megasporangium of *Paleoazolla* exposes serious gaps in the current knowledge on the evolution of monomegaspory in heterosporous water ferns, a fact that emphasizes the need of including fossils within phylogenies to elucidate patterns of character acquisition among water ferns.

KEY WORDS heterospory; Late Colonia Formation; Maastrichtian; megaspore; microspore massulae; Salviniaceae.

There is no doubt that fossils are critical for understanding evolutionary transformations in deep time. In the case of the heterosporous water ferns, complete fossil plants (macrofossils) and spores (microfossils) document their past morphologies showing the similarities between extant and fossil taxa (Bůžek et al., 1971; Collinson, 1991, 2001, 2002; Hoffman and Stockey 1994; Cúneo et al., 2013; Wang et al., 2014; Hermesen, 2019).

Heterosporous water ferns include two families, Salviniaceae Martinov and Marsileaceae Mirbel, both forming a monophyletic group within the order Salviniales Link (Smith et al., 2006; PPG I, 2016). Salviniaceae comprises two extant genera with worldwide distribution; *Azolla* Lamarck with approximately seven species, and

Salvinia Séguier with 10 species (Tryon and Tryon, 1982; Schneller, 1990a, b; Nagalingum et al., 2006, 2007, 2008; Reid et al., 2006; Metzgar et al., 2007; Pereira et al., 2011; PPG I, 2016).

Marsileaceae is represented by three extant genera: *Marsilea* L. comprising about 50 species with worldwide distribution; *Regnellidium* Lindman with one species restricted to a small region of southern Brazil and adjacent Paraguay and Argentina; and *Pilularia* L. represented by five species with nearly worldwide distribution (Tryon and Lugardon, 1991; Pryer, 1999; Nagalingum et al., 2007, 2008; Whitten et al., 2012; PPG I, 2016).

Salviniaaceous ferns are free floating and form extensive populations on the surface of low energy freshwater environments

such as swamps, ponds, and lakes because of their active vegetative multiplication (Tryon and Tryon, 1982), while marsileaceous ones are rhizomatous and grow in the shallow margins of ponds, lakes, marshes, rivers, and on floodplains, but a few grow in permanent water bodies (Tryon and Tryon, 1982).

In Salviniaceae, the megaspores are morphologically different in both genera. Megaspores of *Azolla* are characterized by having a series of vacuolated structures called “floats” that surround a central acrolamella above the trilete mark, whereas in *Salvinia* the megaspores have a thick vacuolated outer layer and a delicate acrolamella hidden by three large valves (Kempf, 1973; Lumpkin and Plucknett, 1980; Tryon and Lugardon, 1991). An interesting feature of this family is that the microspores form vacuolated massulae, which may have glochidia (hair-like to anchor-shaped elongated processes) that hook to the hairs of the megaspore (Lumpkin and Plucknett, 1980; Tryon and Lugardon, 1991). In Marsileaceae, the megaspores have a conspicuous acrolamella above the trilete mark, while the microspores are simple and occasionally have a small acrolamella (Tryon and Lugardon, 1991; Schneider and Pryer, 2002). Despite the differences in ecology and in many morphological characters between these families, they share several evolutionary innovations, such as monomegaspority, endospory, and spores with a highly modified perine (Schneider and Pryer, 2002).

The fossil record of the water ferns indicates a past worldwide distribution and goes back to the Late Jurassic or Early Cretaceous, although the group seems to have diversified during the Late Cretaceous, at the same time as the flowering plants. Fossils with putative affinities to Marsileaceae have been recorded from the Late Jurassic or Early Cretaceous (Yamada and Kato, 2002), although most of the macrofossils remains have been recovered from Late Cretaceous deposits (Sahni and Sitholey, 1943; Chitaley and Paradkar, 1972; Banerji, 1987; Skog and Dilcher, 1992; Lupia et al., 2000; Rich et al., 2001; Nagalingum, 2007; Hu et al., 2008; Herman and Kvaček, 2010; Cúneo et al., 2013; Hermsen et al., 2013; Puebla et al., 2014; Sender et al., 2014; Sun et al., 2014; Monje Dussán et al., 2016; Vallati et al., 2017; Estrada-Ruiz et al., 2018; Hermsen, 2019). The fossil record of Salviniaceae starts later, in the Late Cretaceous, and it is mostly based on dispersed megaspores and microspore massulae, while sporophytes are scarcely recorded (Hall, 1968; Bůžek et al., 1971, 1988; Sweet and Chandrasekharam, 1973; Lucas and Duckett, 1980; Melchior and Hall, 1983; Kovach and Batten, 1989; Batten and Kovach, 1990; McIver and Basinger, 1993; Hoffman and Stockey, 1994; Collinson et al., 2013; Wang et al., 2014; Vallati et al., 2017; De Benedetti et al., 2018; Hermsen et al. 2019).

There are several genera of dispersed fossil megaspores, practically restricted to the Cretaceous, such as *Ariadnaesporites* Potonié emend. Tschudy, *Arcellites* Miner emend. Ellis and Tschudy, *Azinia* Baluyeva, *Azollopsis* Hall emend. Sweet and Hills, *Balmeisporites* Cookson and Dettmann emend. Dettmann, *Capulisporites* Potonié, *Ghoshispora* Srivastava emend. Dettmann, *Glomerisporites* Potonié, *Hallisporites* Nowak and Lupia, *Molaspora* Schemel emend. Hall, *Monophyllosporites* Gunther and Hills, *Paleoazolla* Archangelsky, Phipps, Taylor et Taylor, and *Parazolla* Hall, which have been related to the water ferns stem lineage (Baluyeva, 1964; Ellis and Tschudy, 1964; Tschudy, 1966; Hall, 1967, 1968, 1969a, 1974, 1975; Gunther and Hills, 1972; Bergad, 1973; Sweet and Hills, 1974, 1976; Collinson, 1991, 2001; Batten et al., 1994, 1996, 1998, 2011a, b, 2016; Rothwell and Stockey, 1994; Dettmann, 1995; Archangelsky et al., 1999; Nowak and Lupia, 2005; Kutluk et al., 2011; Lupia, 2011; Kutluk and Hills, 2015; Santamarina et al., 2018). Some of these

with a worldwide distribution pattern, such as *Ariadnaesporites*, *Arcellites*, *Balmeisporites* and *Ghoshispora*, highlight the importance of the water ferns during the Cretaceous. Nevertheless, of particular interest, is the monotypic genus *Paleoazolla* that was erected by Archangelsky et al. (1999) to accommodate fossil spores of a heterosporous aquatic fern from the Late Cretaceous La Colonia Formation, northern Patagonia, Chubut Province, Argentina. In this study, we reinterpret the morphological and ultrastructural features of the genus *Paleoazolla* based on new observations of the original material and on new specimens. We also establish comparisons between this Patagonian genus and megaspores of the living heterosporous water ferns and other related fossil taxa. Our interpretation provides critical information for understanding the evolution of water ferns.

MATERIALS AND METHODS

Stratigraphy and fossil record

The La Colonia Formation outcrops at Chubut Province, Patagonia, Argentina (Fig. 1). The unit is considered to have been deposited during the Late Cretaceous (Maastrichtian), although its upper beds could be earliest Danian in age (Guler et al., 2014; Cúneo et al., 2014). It consists of thick, mostly clayey or silty beds (massive, laminated, or heterolithic) with sporadic fine-grained sand beds (Cúneo et al., 2013, 2014). These sediments were deposited in freshwater coastal environments and lagoons associated with coastal sand bars (for more details see Cúneo et al., 2014). It is underlain by the late Early to Late Cretaceous sediments of the Chubut Group, or the Puntudo Chico Formation at the Cerro Buitre/Mirasol Chico area, and is overlain by the Paleocene basalts of the El Buitre Formation, and by the Eocene to early Oligocene informally named “Baibian Beds” (Archangelsky and Zamaloa, 2014; Cúneo et al., 2014).

The La Colonia Formation contains a very rich fossil record composed of macro- and microvertebrates (including dinosaurs, plesiosaurs, turtles, mammals, birds, marine and freshwater fishes), macro- and microinvertebrates (including bivalves, ostracods, and foraminifera), and macro- and microplant remains representing wetland plant communities (including angiosperms, gymnosperms, pteridophytes, algae, dinoflagellates, and fungi) (Bonaparte, 1985; Gasparini and Spalleti, 1990; Archangelsky et al., 1999; Pascual et al., 2000; Gandolfo and Cúneo, 2005; Lawver et al., 2011; Malumián and Nández, 2011; Sterli and De La Fuente, 2011; Navarro et al., 2012; O’Gorman et al., 2013; Cúneo et al., 2014; Gallego et al., 2014; Gandolfo et al., 2014; Guler et al., 2014; Gasparini et al., 2015; Borel et al., 2016; De Benedetti et al., 2018). Fossil spores studied in this contribution were found among the macro and micro-plant remains and were recovered from four localities: Cerro Bosta, Cañadón del Irupé, Quebrada del Helecho, and Plesiosaurio (Fig. 1).

Fossil specimen curation and preparation

The fossils are housed at the palynological collection of the Museo Paleontológico Egidio Feruglio (MEF; repository acronyms MPEF-PA for palynological collection), Trelew, Chubut Province, Patagonia, Argentina. Most specimens analyzed herein were obtained from newly collected samples at the Cerro Bosta and

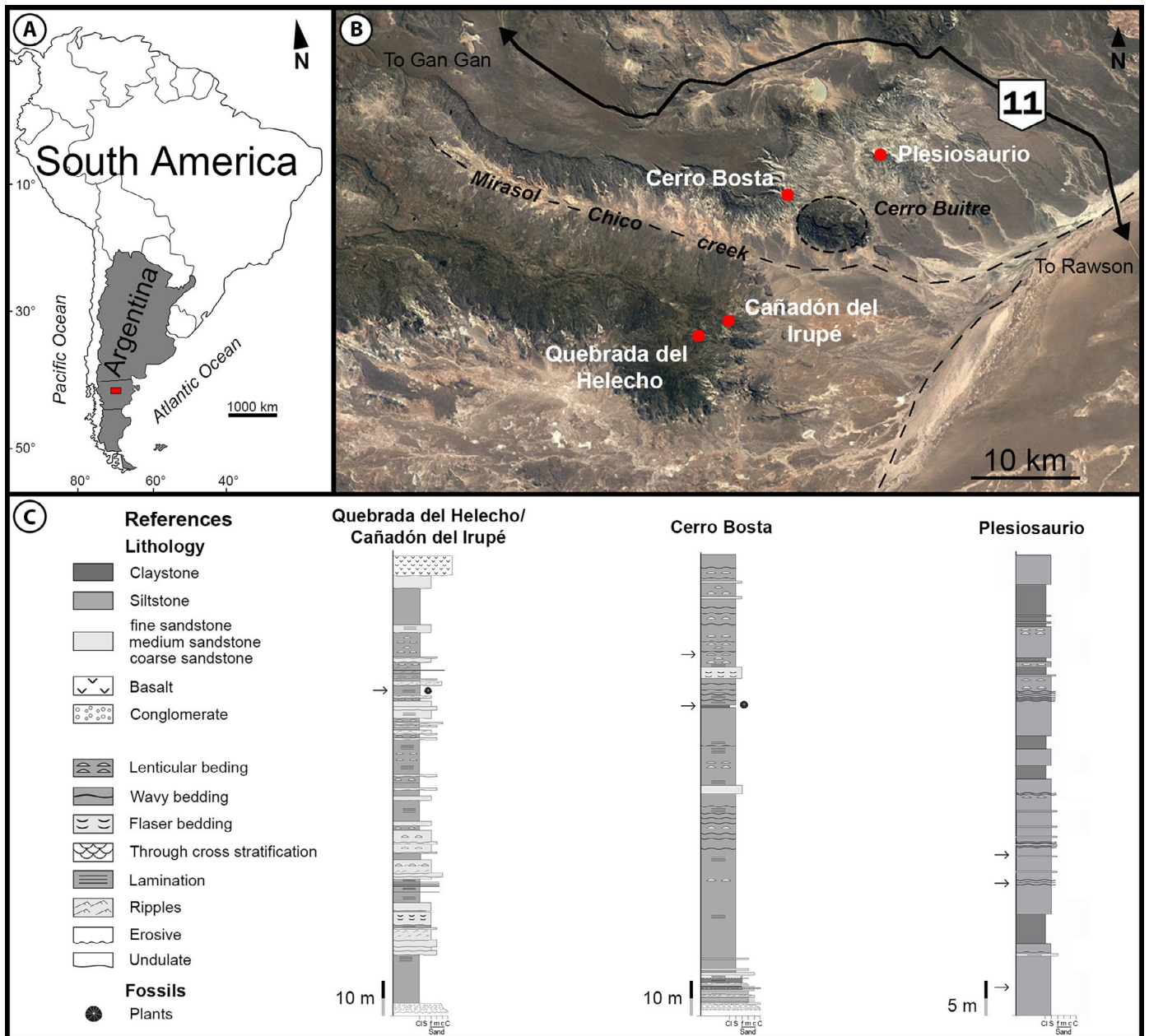


FIGURE 1. (A) Map showing the location of the outcrops of the La Colonia Formation, Chubut Province, Argentina. (B) Localities where fossils were collected (red dots). (C) Schematic profiles of the La Colonia Formation in the studied area, with indications of the samples where *Paleoazolla* spores were recovered (arrows).

Cañadón del Irupé localities. In addition, we reexamined original materials that are housed at the MEF (repository acronyms MPEF PALIN). Samples were mechanically disaggregated and treated with hydrofluoric acid (70%) and hydrochloric acid (30%). The resulting residues were decanted and washed several times and then filtered with 100 μm meshes. Spores were picked with needles under a binocular stereoscope. For light microscopy (LM) observations, the specimens were cleared with concentrated Schultz's reagent or with 25% sodium hypochlorite solution for 20–30 min and then mounted on slides with glycerin jelly (best results were obtained with this last technique). For scanning electron microscopy (SEM) observations, the specimens were mounted directly

on stubs using double-sided tape. Longitudinal and transversal sections were made using a razor blade. Light microscopy observations were made with a Nikon Eclipse 80i microscope coupled with a Nikon DS-L4 camera (Nikon Corp., Minato, Tokyo, Japan) at the MEF. Scanning electron microscope observations were made with a Philips XL30 TMP SEM (Philips, Amsterdam, North Holland, Netherlands) at the Natural Sciences Argentine Museum, Autonomous City of Buenos Aires, Buenos Aires Province, Argentina, and a JEOL JSM-6460 SEM (Jeol Ltd., Akishima, Tokyo, Japan) at Aluar S. A., Puerto Madryn, Chubut Province, Argentina. All images were edited using Adobe Photoshop CS6 (Adobe, San José, California, USA).

Terminology

The spores of extant and fossil heterosporous water ferns are the most complex reproductive structures among all pteridophytes. The terminology associated with the outer spore wall is particularly confusing among the different genera and at the intraspecific level (Hall, 1968, 1969; Kempf, 1969; Fowler, 1975; Sweet and Hills, 1976; Tryon and Lugardon, 1991; Batten et al., 1996, 1998, 2016; Nowak and Lupia, 2004; among others). In this study, we applied the term “exine” for the spore wall proper and “perine” for referring to the outermost exine as used by Collinson et al. (2013) and Schneider and Pryer (2002) for salviniaceae and marsileaceae spores, respectively.

The proximal extension of the perine, characteristic of numerous Mesozoic megaspore genera, has been called acrolamella, columella, gula, and trifolium (Tschudy, 1966; Hills, 1967; Kempf, 1969; Hall, 1975; Collinson, 1991; Batten et al., 2011a; Batten et al., 2016; among others). To facilitate comparisons, we used the term “acrolamella” for all the modern and fossil genera related to the heterosporous water ferns. In Fig. 2 we provide the terms applied to the spores of *Paleoazolla*.

SYSTEMATIC PALYNOLOGY

Order Salviniales Link 1833

Genus *Paleoazolla* Archangelsky, Phipps, Taylor et Taylor 1999 emend. De Benedetti et Zamalao

Original diagnosis: As for the type species (in Archangelsky et al., 1999; p.1201).

Emended diagnosis: Megaspore massula elliptic to ovate, containing two spherical trilete megaspores, rarely one or three; surface completely covered by a thick filusum. Microspore massula rounded to triangular, containing one spherical trilete microspore; surface covered by irregularly distributed multibarbed glochidia. Vegetative parts unknown.

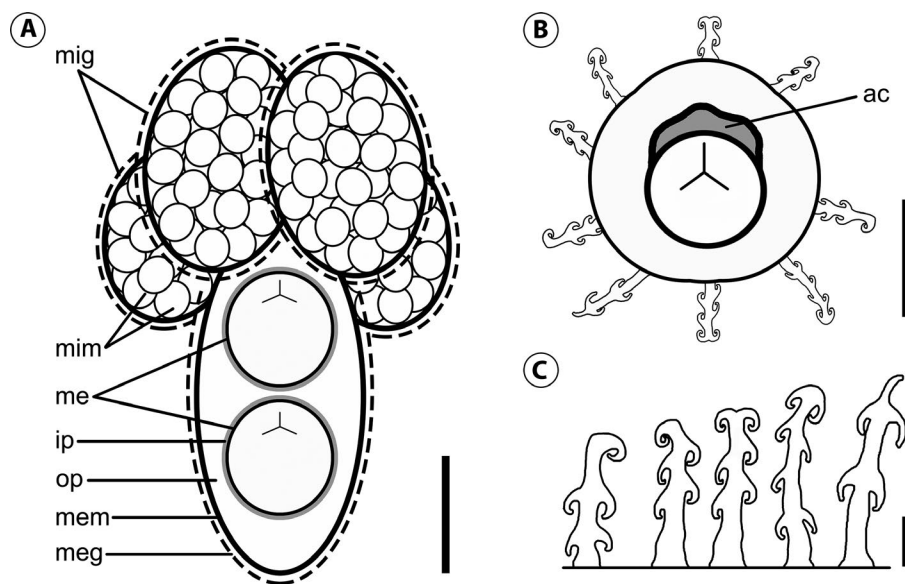


FIGURE 2. Schematic reconstruction of *Paleoazolla patagonica*. (A) Heterosporangiate sori: ip, inner perine; me, megaspore; meg, megasporangium; mem, megaspore massula; mim, microsporangium; mim, microspore massula; op, outer perine. (B) Microspore massula: ac, acrolamella. (C) Glochidia showing variation in tips and auxiliary hooks, from left to right: single-barbed, intermediate type, double-barbed, anchor-shape, anchor-shape with an apical projection. Scale bars: (A) 300 μm ; (B) 50 μm ; (C) 10 μm .

Type species: *Paleoazolla patagonica* Archangelsky, Phipps, Taylor et Taylor 1999 emend. De Benedetti et Zamalao

Original diagnosis: (in Archangelsky et al., 1999; p.1201). “Megaspore apparatus up to 950 μm long, 700 μm wide, composed of an ellipsoidal, commonly flattened megaspore and a complex of three or four floats; megaspore up to 650 μm long, 350 μm wide, and 115 μm thick; floats nearly spherical, 200–350 μm in diameter and attached directly to one end of the megaspore; float divided into a smooth inner zone up to 6 μm thick and an irregularly alveolate outer zone up to 25 μm thick covered by multibarbed hairs (glochidia); megaspore wall up to 52 μm thick, divided into a 7–10 μm thick exine and a 28–42 μm thick perine; perine organized into a dense spongy endoperine 4–10 μm thick and a loosely organized, irregularly packed 18–20 μm thick exoperine covered by a 6–12 μm thick infrafilusum. Massulae irregular, 350–490 μm diameter, with a pseudocellular alveolar structure from which the glochidia arise as external

FIGURE 3. *Paleoazolla patagonica* Archangelsky, Phipps, Taylor et Taylor 1999; scanning electron micrographs.

(A–C) “Heterosporangiate sori” showing the elliptical megaspore massula (= megasporangium contents) attached to four (A), three (B) and two (C) spherical to ovoid clusters of microspore massulae (= microsporangia contents). (A) Neotype MPEF PALIN 6; (B, C) MPEF-PA 104.

(D) Damaged specimen, showing the individual microspore massulae becoming detached from each other. MPEF PALIN 4.

(E) Transversal section of the complex showing microspores (white arrows) inside the “microsporangia” and a megaspore inside the “megasporangia”. MPEF-PA 201.

(F) Longitudinal section of the complex showing microspores (white arrows) and two megaspores. MPEF-PA 204.

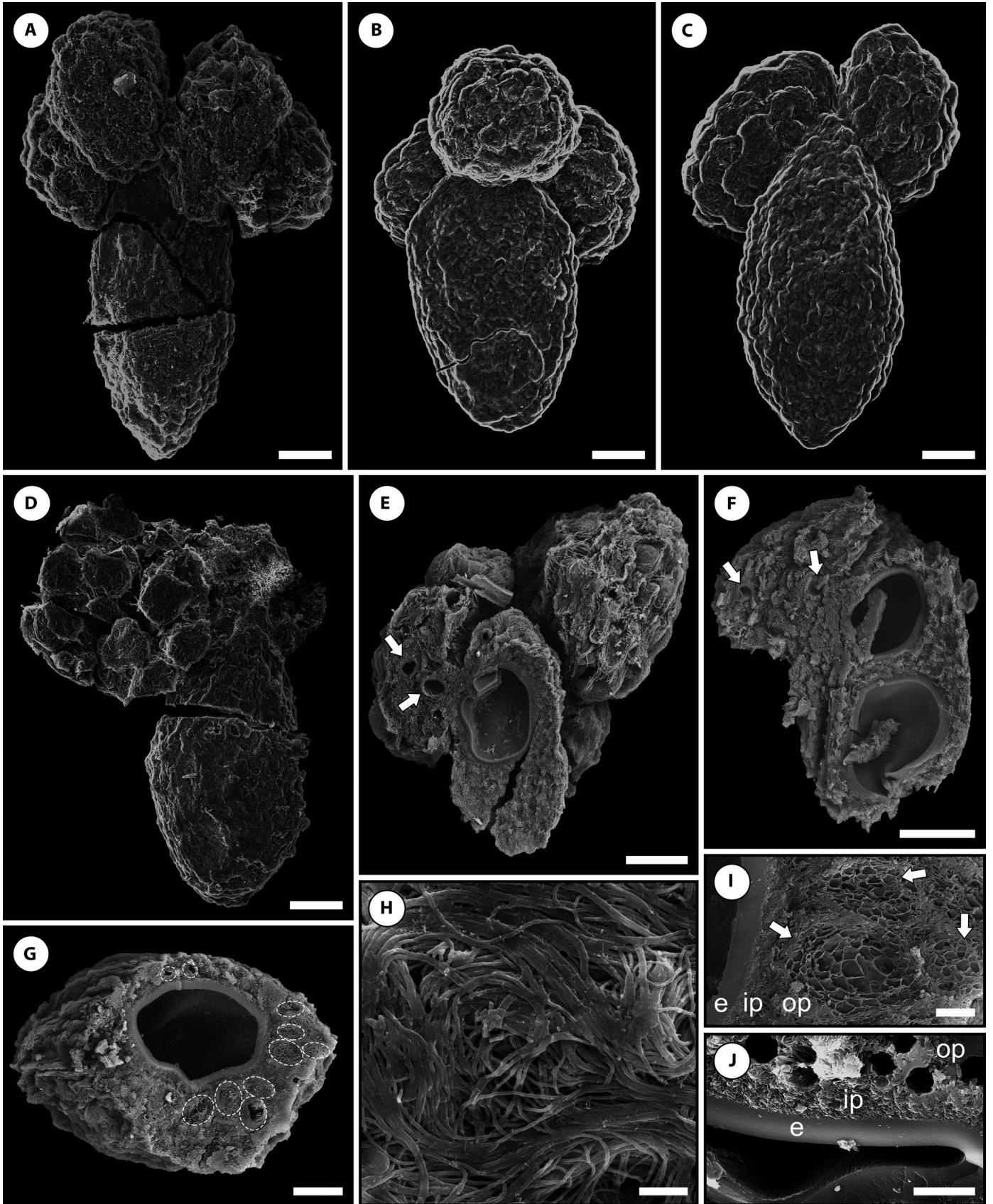
(G) Transversal section of a megaspore massula. Note the floats embedded in the outer perine (dotted white circles). MPEF-PA 201.

(H) Surface of megaspore massula showing the filusum. MPEF PALIN 4.

(I) Detail from (G) Transversal section of a megaspore massula. Note the homogeneous exine (e), the inner perine (ip), and the vacuolated floats (arrows) immersed in the outer perine (op). MPEF-PA 201.

(J) Transversal section of a megaspore massula. Note the homogeneous exine (e), the inner perine (ip), and the strongly vacuolated structure of the outer perine (op). MPEF-PA 104.

Scale bars: (A–F): 100 μm ; (G): 50 μm ; (H): 5 μm ; (I–J): 10 μm .



projections; glochidia multibarbed, up to 60 μm long, and 5 μm wide, nonseptate, commonly with irregularly distributed anchor-shaped tips adpressed to the massula surface; numerous rounded, inaperturate microspores per massula, microspores 42–63 μm in diameter.”

Emended diagnosis. Megaspore massula up to 890 μm long and 480 μm wide, elliptic to ovate, containing two spherical trilete megaspores, rarely one or three; each megaspore surrounded by a dense inner perine, and all spores surrounded by a spongy outer perine; surface completely covered by a thick filiosum. Microspore massula up to 140 μm diameter, rounded to triangular, containing one spherical trilete microspore; surface covered by irregularly distributed multibarbed aseptate glochidia. Vegetative parts unknown.

Dimensions. Length of sori 740(857)1125 μm , width of sori 477(574)700 μm (10 specimens measured). Length of megaspore massulae 440(624)890 μm , width of megaspore massulae 270(336)480 μm (30 specimens measured). Thick of perinal hairs 0.35(0.45)0.65 μm (30 specimens measured). Diameter of megaspore 240(290)340 μm (14 specimens measured). Maximum diameter of microsporangia 213(355)480 μm (40 specimens measured). Maximum length of microspore massulae 68(89)140 μm (50 specimens measured). Diameter of microspore 42(55)75 μm (20 specimens measured).

Holotype: In Archangelsky et al. (1999; MPEF PALIN 1, fig. 2), the specimen was on a SEM stub that is lost.

Lectotype designated here: MPEF PALIN 6; Fig. 3, A.

Remarks: A lectotype was selected from one of the specimens collected by Archangelsky et al. (1999; not illustrated), because the holotype and all other specimens illustrated by Archangelsky et al. are lost.

Specimens examined: MPEF PALIN 2–6; MPEF-PA 86; MPEF-PA 100–106; MPEF-PA 201–217; MPEF-PA 301–319; MPEF-PA 580, 586, 587, 609.

Repository: Palynological Collection at the Museo Paleontológico Egidio Feruglio (MPEF-PA and MPEF PALIN), Trelew, Chubut Province, Argentina.

Type locality: Cerro Bosta (= Cerro Buitre in Archangelsky et al., 1999), La Colonia Formation, Chubut Province, Argentina.

Other localities: Cañadón del Irupé, Quebrada del Helecho, and Plesiosaurio (La Colonia Formation, Chubut Province, Argentina).

Stratigraphic range and distribution: Maastrichtian of La Colonia Formation, Chubut Province, Argentina.

REINTERPRETATION OF *PALEOAZOLLA PATAGONICA*

Archangelsky et al. (1999, Figs. 1–4), described the megaspore apparatus as an elliptical megaspore with 3–4 oval floats grouped at

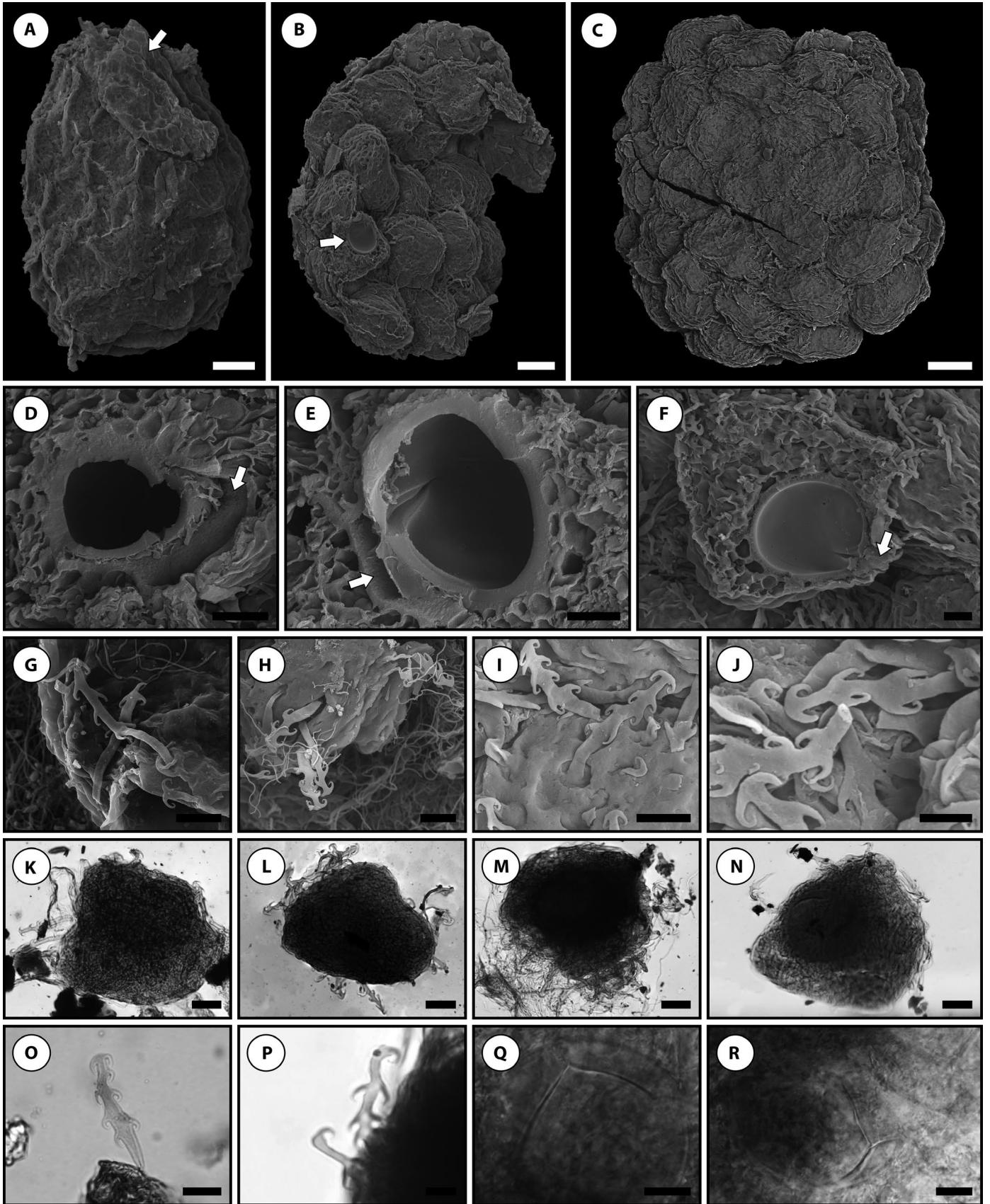
one end, each “float” showing an alveolate surface and multibarbed glochidia practically equal to those of the microspore massulae. In actuality, each “float” definitely corresponds to the complete content of one microsporangium. Indeed, each of the so-called “floats” is composed of smaller structures (elliptical, circular, or triangular in outline), which are individual microspore massulae (Fig. 3A–D). In addition to characteristics such as multibarbed glochidia and an alveolate surface, the size of these subunits is identical to that of isolated massulae (68–140 μm diameter) found among other palynomorphs in the same samples (Fig. 4K–N). Furthermore, in transversal sections of the “floats,” microspores surrounded by the alveolar tissue of the massulae are observed (Fig. 3E, F).

The microsporangia are 213–480 μm in maximum diameter and they are commonly found as isolated units (Fig. 4A–C). The number of massulae per microsporangium is probably 32 or 64 (Fig. 4B, C), and each massula contains a single microspore (Fig. 4B, F, K–N). The microspores are trilete and circular to elliptical in outline (\approx 42–75 μm diameter), with each laesura extending up to one third the diameter of the spore (Fig. 4Q, R). Under SEM, cross sections of the microspores reveal an acrolamella above the trilete laesurae (Fig. 4D–F). The microspore exine is 1.5–2 μm thick and appear homogeneous (Fig. 4D–F). The multibarbed glochidia are nonseptate (Fig. 4O, P), up to 60 μm long and 1.5–6 μm wide, with alternate or opposite barbs (5–7 μm long) along the stem and ends with variable forms ranging from anchor-shaped (more commonly), anchor-shaped with an apical projection, double-barbed or single-barbed (Fig. 4G–J, O, P; Fig. 2C).

The large size attributed to the microspore massulae (\approx 350–490 μm diameter) by Archangelsky et al. (1999, p. 1201), which is even larger than the supposed “floats” (200–350 μm diameter), indicates that the measurements were taken from clusters of massulae, probably from the complete contents of microsporangia. This confusion was probably caused by the strongly adpressed disposition of the massulae in the clusters, making it difficult to determine the limits among them. Also, Archangelsky et al. (1999, Fig. 6), mention “numerous inaperturate microspores per massula,” but each massula contains a unique trilete microspore (Fig. 4, K–N, Q and R). It is possible that the interpretation by Archangelsky et al. was due to the treatment with Schultz’s reagent that degraded the clusters of massulae and the exine of the spores, making the laesurae not discernible. Moreover, in their Fig. 7, three massulae are shown and each one appears to contain one trilete microspore.

Archangelsky et al. (1999, Figs. 8–9, 12–15) also described a single elliptical megaspore with a wall divided into exine and perine, the last one divided into a dense spongy inner perine and a loosely organized outer perine from which a thick “infraciliosum”

FIGURE 4. *Paleoazolla patagonica* Archangelsky, Phipps, Taylor et Taylor 1999; scanning electron (A–J) and transmitted light (K–R) micrographs. (A) Entire content of one microsporangium. Note remains of the netlike tissue of the microsporangium (or perhaps indusial) wall (arrow). MPEF-PA 207. (B) Partial content of a microsporangium. Note part of a microspore (arrow). MPEF-PA 207. (C) Cluster with more than 30 microspore massulae that probably represent the entire content of one microsporangium. MPEF-PA 104. (D–F) Cross sections of microspore massula. Note the acrolamella (arrows) and the vacuolated tissue of the massula. (D, E) MPEF-PA 201; (F) MPEF-PA 207. (G, H) Microspore massula attached to the perinal hairs of the megaspore massula. Note the different types of glochidia. (G) MPEF-PA 86; (H) MPEF-PA 203. (I, J) Detail of glochidia. Note the recurved tips of all barbs. (I, J) MPEF-PA 104. (K–N) Microspore massula. Note the only microspore inside the vacuolated tissue and the multibarbed glochidia. (K) MPEF-PA 580; (L) MPEF-PA 312; (M) MPEF-PA 587; (N) MPEF-PA 586. (O, P) Detail of nonseptate multibarbed glochidia. (O) MPEF-PA 609; (P) MPEF-PA 308. (Q, R) Detail of microspores showing the trilete laesurae. (Q, R) MPEF-PA 318. Scale bars: (A–C): 50 μm ; (D–I), (O–R): 10 μm ; (J): 5 μm ; (K–N): 20 μm .



is originated. However, in cleared and sectioned specimens, we observed that the “elliptical megaspore” is in fact a “megaspore massula” that contains two megaspores of equal size, rarely one or three (Figs. 3E, 5A–F). The megaspores are trilete and circular in outline (up to 340 μm diameter), with each laesura extending one third or up to half the radius of the spore (Fig. 5B–E). The megaspores are arranged one at each end (in case there are two of them) (Figs. 3E, 5B, D) or slightly overlapped if there are three (Fig. 5C). Interestingly, in some specimens there are one or up to three partially developed megaspores above the only well-developed megaspore (Fig. 5E, F). Because there are up to three aborted megaspores, we infer that all the spores enclosed in the megaspore massula of *Paleoazolla* correspond to the product of only one tetrad after meiosis.

In sectioned megaspores observed with SEM (Fig. 3E–G, J), the exine is 4–7 μm thick (up to 10 μm in Archangelsky et al. 1999) and appear homogeneous. However, Archangelsky et al. (1999, p. 1201, Figs. 12–14) described two exine zones in TEM sections; the inner composed by two rows of perforations irregular in shape and size, and the outer with few large perforations. We observed that the exine of each megaspore is covered by a dense spongy inner perine 4–10 μm thick of fibrous-to-granular appearance, built from a fibrous meshwork of fine sporopollenin fibrils (Fig. 3J). A thick, spongy outer perine of very vacuolated and loosely organized construction surrounds all the spores (Figs. 3E–G). The outer perine is composed of thicker sporopollenin threads than those of the endoperine (Fig. 3J; see also TEM sections of Archangelsky et al. 1999, Figs. 12, 13, 15). In some specimens, there are large alveoli of up to 8 μm in diameter (Fig. 3J), and in one case it was possible to observe some ovoid areas with a more vacuolated tissue, which could correspond to imbedded floats (Fig. 3G, 3I). Therefore, this suggests that probably the outer perine is the result of a loosely organized tissue in which numerous vacuolated and sometimes poorly developed floats are immersed and strongly appressed. The entire surface of the megaspore massula is covered by a dense layer of perinal hairs that constitute the filsum (Fig. 3H). In this particular case, it is incorrect to speak about the thickness of the outer perine, because it acts as the vacuolated pseudocellular structure of the microspore massulae, and the thickness varies according to the arrangement and number of megaspores inside it. It is also incorrect to determine the presence of supra or infrafilsum, because this distinction is based on the presence of an acrolamella, a collar, or floats on the proximal pole of *Azolla* megaspores, structures that are not present at all in *Paleoazolla*.

The sectioned “float” described by Archangelsky et al. (1999, Figs. 5 and 11), in fact, correspond to a megaspore massula with two inner spores, with the “smooth inner zone” corresponding to the exine and the “irregularly alveolate outer zone” to the perine. Archangelsky et al. (1999) indicated that multibarbed glochidia cover the surface of the floats (their Fig. 10), but the image they show corresponds to a different specimen and not to the section shown at their Fig. 5. None of the sectioned “floats” in the new specimens shows the clearly demarcated separation between these two zones, as illustrated by Archangelsky et al. (1999; Figs. 5 and 11).

We have found several hundred specimens with three to four clusters of microspore massulae attached to one end of the megaspore massula suggesting that this is a natural condition. Evidence of sporangial (or perhaps indusial) walls occur as netlike tissues or thin films covering the surface of some specimens (Fig. 4A) strongly supporting the presence of both types of sporangia in the same sorus.

Based on this new evidence, we conclude that *Paleoazolla* is characterized by producing heterosporangiate sori with one elliptical megasporangium (enclosing one megaspore massula) surrounded at one end by three or four spherical-to-ovoid microsporangia (enclosing multiple microspore massulae); the megaspore massula containing two trilete megaspores (rarely one or three) and each microspore massula containing only one trilete microspore.

DISCUSSION

Comparisons with sori of the modern water fern genera

In Marsileaceae, the sori are longitudinally aligned inside sporocarps, a specialized structure with sclerenchymatous walls that protect the reproductive organs against dryness and mechanical damage (Tryon and Tryon, 1982; Nagalingum et al., 2006). Sori are indusiate and heterosporangiate, bearing numerous mega- and microsporangia (Tryon and Lugardon, 1991; Schneider and Pryer, 2002; Nagalingum et al., 2006). Sporocarps in Salviniaceae are quite different. In *Azolla*, two or four sori are borne on short stalks and are enclosed in a sporocarp with parenchymatous wall; the sori are homosporangiate with either a single megasporangium or multiple microsporangia (Lucas and Duckett, 1980; Lumpkin and Plucknett, 1980; Stafford, 2003a; Nagalingum et al., 2006). In *Salvinia*, 4–20 sori are alternately arranged along the inner segments of the submerged root-like leaf (Croxdale, 1978, 1979, 1981; Lemon and Posluszny, 1997; de la Sota, 1999), but they are not enclosed in sporocarps; the sori are homosporangiate with either several megasporangia or multiple microsporangia (Loyal and Grewal, 1966; Stafford, 2003b; Nagalingum et al., 2006).

Macrofossil remains of *Paleoazolla* are unknown and therefore the presence of sporocarps and the position of the sori are unknown. In *Paleoazolla*, the sori are clearly heterosporangiate, like marsileaceous ferns, although in this case there is only one elliptical megasporangium surrounded at one end by three or four spherical-to-ovoid microsporangia. On the other hand, some fossil forms of *Azolla* and *Salvinia* have been described as producing heterosporangiate sori (Hills and Gopal, 1967; Bůžek et al., 1971, 1988; Paradkar and Barlinge, 1984; Collinson, 1991; McIver and Basinger, 1993; Van Bergen et al., 1993; Batten and Collinson, 2001). Although the phylogenetic position of *Paleoazolla* is uncertain, we can hypothesize that the presence of heterosporangiate sori could be the plesiomorphic condition for the order Salviniiales.

Comparisons with spores of the modern water fern genera

Main features of modern heterosporous water fern spores are shown in Table 1. The megasporangium of all extant water ferns contains only one trilete megaspore (monomegasporous) (Tryon and Lugardon, 1991; Nagalingum et al., 2006; Smith et al., 2006). *Paleoazolla* differs from all modern water ferns by producing more than one megaspore per megasporangium. However, the megasporangium of *Paleoazolla* produced a single dispersed unit that resembles the megaspore produced by salviniaaceous ferns.

The basic megaspore apparatus of *Azolla* consists of the megaspore body and the floating or “swimming” apparatus situated on the proximal pole (Tryon and Lugardon, 1991). The floating apparatus

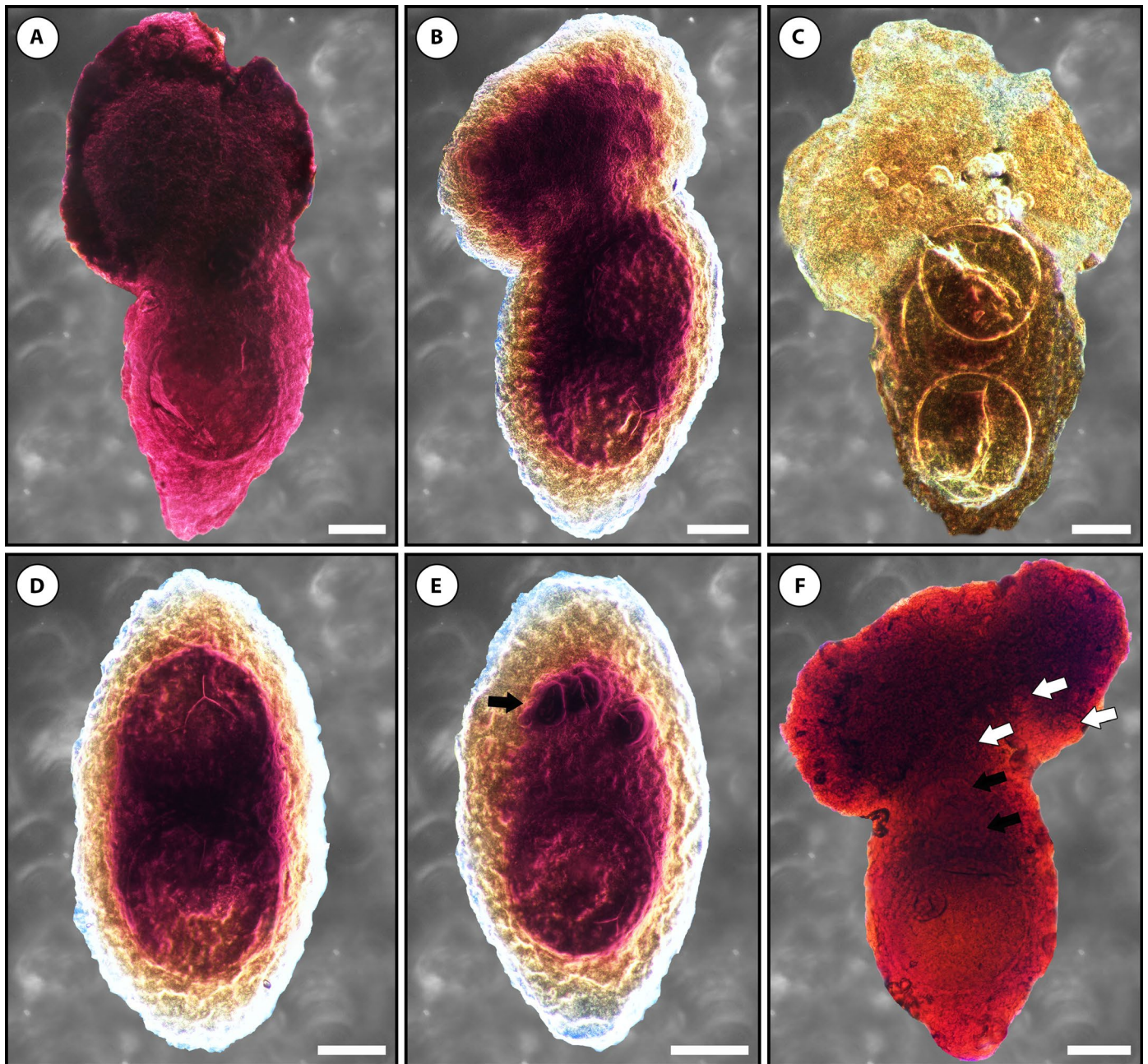


FIGURE 5. *Paleozolla patagonica* Archnagelsky, Phipps, Taylor et Taylor 1999; transmitted light micrographs (A–F). Specimens A and F cleared with Schultze’s reagent. Specimens B–E cleared with a 25% sodium hypochlorite solution.

(A–C) “Heterosporangiate sori” showing the elliptical “megaspore massula” (= megaspore massula) with one (A), two (B), and three (C) megaspores, and the “microsporangia” attached to one end. (A) MPEF-PA 307; (B) MPEF-PA 318; (C) MPEF-PA 301.

(D) Megaspore massula with two megaspores. MPEF-PA 318.

(E) Megaspore massula showing one well-developed megaspore plus three small ovoid aborted megaspores (black arrow). One of these small spores (the central one) is split in two parts and it appears to be four aborted spores. MPEF-PA 318.

(F) Megaspore massula showing one well-developed megaspore plus two slightly developed megaspores above (black arrows). Note the microspores inside the “microsporangia” (white arrows). MPEF-PA 308.

Scale bars (A–F): 100 μ m.

consists of three or nine floats that are attached to the megaspore by the acrolamella (also known as “columella”) and a collar that is a structure, which delimits the periphery of the proximal surface of the megaspore (Saunders and Fowler, 1992, 1993). *Paleozolla*

differs from *Azolla* because it definitely lacks both an acrolamella and a collar.

Paleozolla resembles *Azolla* in its surface appearance (presence of filosum) and the possible presence of floats. In *Azolla*, perinal

TABLE 1. Main characteristics of the spores of modern heterosporous water ferns.

	Megaspore		
	Morphology	Exine	Perine
Marsileaceae:			
Genus^{a,b}			
<i>Marsilea</i> Linnaeus 1753	Spore body prolate (400–740 µm). A disk-shape acrolamella extremely reduced surrounds the laesurae proximally.	Compact, permeated by canals. Surface psilate to slightly rugulate.	Thicker relative to the exine. Two zones: inner smooth to granular; outer alveolate and thicker, grades outward into an open reticulum. With an additional gelatinous perine layer.
Type species: <i>M. quadrifolia</i> Linnaeus 1753			Spore body nearly spherical (31–85 µm). Surface sculpture baculate (papillate) to rugulate. Acrolamella-like structure rarely observed. With an additional gelatinous perine layer.
<i>Regnellidium</i> Lindman 1904	Spore body nearly spherical (425–750 µm). An acrolamella consisting of six to seven twisted leaflike folds surrounds the laesurae proximally.	Compact, permeated by canals. Surface psilate to slightly rugulate.	Spore body nearly spherical (35–55 µm). Surface sculpture rugulate. Acrolamella reminiscent of that present in the megaspores. With an additional gelatinous perine layer.
Type species: <i>R. diphyllum</i> Lindman 1904			
<i>Pilularia</i> Linnaeus 1753	Spore body prolate (384–418 µm). An acrolamella consisting of six to seven straight leaf-like folds surrounds the laesurae proximally.	Compact, permeated by canals. Surface psilate to slightly rugulate.	Spore body nearly spherical (42–58 µm). Surface sculpture rugulate. With an additional gelatinous perine layer.
Type species: <i>P. globulifera</i> Linnaeus 1753			
Salviniaceae^{c,e}			
<i>Azolla</i> Lamarck 1783	Outline elliptical to ovate (200–370 µm), with a spore body spherical and a “swimming” apparatus consisting of pseudocellular “floats” situated on the proximal pole, attached to the megaspore by a central acrolamella and a collar.	With a lacunose/irregularly cavernous structure. Surface psilate to slightly foveolate.	32 or 64 (10–27 µm) aggregated into 3–10 massulae per microsporangium. Massulae subcircular to ovate in outline, which surface, to a greater or lesser extent, is covered by glochidia that can be filamentous or anchor-shaped. Microspore surface psilate to rugulate.
Type species: <i>A. filiculoides</i> Lamarck 1783			
<i>Salvinia</i> Séguier 1754	Spore body ovate (300–500 µm). A delicate acrolamella is located above the laesurae proximally, hidden by three large valves.	Dense to slightly porous. Surface psilate to irregular.	32 or 64 (18–31 µm) aggregated into one massulae per microsporangium. Massulae subcircular to ovate in outline composed of a vacuolated perine. Microspore surface psilate to rugulate.
Type species: <i>S. natans</i> (Linnaeus) Allioni 1785			

^aThe term “acrolamella” is applied here for any extension of the perine at the proximal pole of the megaspores and microspores.

^bDimensions of spores were taken from Tryon and Lugardon (1991).

^cDescriptions of marsileaceous spores are based on Tryon and Lugardon (1991), Schneider and Pryer (2002), and Nagalingum et al. (2006).

^dDescription of *Azolla* spores are based on Lucas and Duckett (1980), Lumpkin and Plucknett (1980), Tryon and Lugardon (1991), Batten and Collinson (2001), Stafford (2003a), and Nagalingum et al. (2006).

^eThe description of *Salvinia* spores are based on Kempf (1973), Tryon and Lugardon (1991), Vanhoorne (1992), Stafford (2003b), and Nagalingum et al. (2006).

hairs (filosum) arise from the acrolamella and the inner surface of the floats, and they are poorly developed on the megaspore surface (Tryon and Lugardon, 1991; Saunders and Fowler, 1992, 1993). However, some fossil *Azolla* species produced megaspore apparatuses completely covered by the filiosum and lacking a collar (Fowler and Stennett-Willson, 1978; Batten and Collinson, 2001; De Benedetti et al., 2018), and thus they are similar to the megaspore massula of *Paleoazolla*.

The megaspore wall of *Azolla* is two-layered, with a homogeneous-to-cavernous exine, and a thick perine divided into an alveolate and/or granulate inner perine and a compact outer perine (Fowler and Stennett-Willson, 1978; Perkins et al., 1985; Tryon and Lugardon, 1991). The megaspore wall of *Paleoazolla* is also two-layered—exine and perine—as that of *Azolla*. The exine of *Paleoazolla* has two zones and has more open spaces than typical *Azolla* exines. The perine of both genera share a vacuolated structure and filamentous filiosum. In *Azolla* species, the inner perine is highly alveolated in the raised areas of the megaspore excrescences (also in the acrolamella and collar), but in the depressions it becomes finely alveolated to granular (Tryon and Lugardon, 1991; Vanhoorne, 1992; Pereira et al., 2001). In *Paleoazolla* the inner perine is fibrous to granular and it is the outer perine that has a spongy vacuolated structure.

Azolla produces 32 or 64 microspores per microsporangium, which are aggregated into approximately 3–10 massulae; in most cases, the surface of these massulae are covered by glochidia that can be filamentous or anchor shaped (Lumpkin and Plucknett, 1980; Saunders and Fowler, 1992, 1993). In *Paleoazolla* there are also probably 32 or 64 microspores per microsporangium, but there is only one large microspore per massula and the glochidia are multibarbed.

Megaspores of *Salvinia* are similar to those of *Azolla* but lack floats and filiosum (Tryon and Lugardon, 1991). The proximal end of the megaspore has a delicate acrolamella hidden by three large valves (Kempf, 1973; Tryon and Lugardon, 1991; Gardenal et al., 2008). *Salvinia* is similar to *Paleoazolla* in the structure of the perine. The perine of *Salvinia* is subdivided in three zones: inner fibrous, middle densely vacuolated, and outer strongly vacuolated (Kempf, 1973; Vanhoorne, 1992; or two vacuolated zones according to Gardenal et al., 2008). *Salvinia* produces 32 or 64 microspores per microsporangium, but only one massula is developed per microsporangium and it lacks glochidia (Tryon and Tryon, 1982; Collinson, 1991).

Comparisons with other fossil megaspore genera

As previously mentioned, the spores of extant and fossil heterosporous water ferns are the most complex reproductive structures among pteridophytes. The presence of floats and/or a dense filiosum on the megaspore and glochidia on the surface of the microspore massula has been used as a characteristic to refer some fossil forms to the *Azolla* stem lineage or elsewhere in the phylogeny of Salviniales. It happens with *Ariadnaesporites*, *Azinia*, *Azollopsis*, *Glomerisporites*, *Hallisporites*, *Paleoazolla*, and *Parazolla*, which, interestingly are all practically restricted to the Cretaceous. Main characters, age, and geographic distribution of these genera are shown in Table 2. Among these, *Glomerisporites*, *Hallisporites*, and *Azollopsis* in particular, are the most similar to *Paleoazolla*.

Glomerisporites and *Hallisporites* have a dense mat of very long, intertwined hairs with numerous small irregular floats that obscure the spore body and the acrolamella giving a fusiform or rounded-to-roughly egg shaped to the megaspore, resembling

the megaspore massula of *Paleoazolla* (Hall, 1974; Batten et al., 1998; Nowak and Lupia, 2005; Kutluk and Hills, 2015). Like *Paleoazolla*, the microspores of *Glomerisporites* and *Hallisporites* are not borne in a true massula. In *Glomerisporites* and *Hallisporites* the microspores are located in an apical neck over a basal region of perine and the surface is partially covered by hairs usually coiled (Hall, 1974; Batten et al., 1998), whereas in *Hallisporites* the microspores bear only one distal appendage and tends to be recovered in masses despite retaining separate individual perines (Hall, 1974, 1975; Nowak and Lupia, 2005).

Azollopsis was defined by Hall (1968) to allocate megaspore complexes with numerous small ovoid floats immersed in a dense mat of hairs that obscure the spore body and acrolamella and microspore massulae with multibarbed glochidia from the Upper Cretaceous (Campanian-Maastrichtian) sediments of Montana, United States. It was later emended by Sweet and Hills (1974) to include megaspores with or without floats and microspore massulae with circinate glochidia from Upper Cretaceous and Paleocene strata of central Alberta, Canada. Other records of *Azollopsis* are restricted to only microspore massulae from the Upper Cretaceous and Paleocene sediments of India (Venkatachala and Sharma, 1982; Ramanujam et al., 1995; Nandi and Chattopadhyay, 2003) and South America (Stough, 1968; Sweet and Hills, 1974; Papú et al., 1988; Povilauskas et al., 2006; Volkheimer et al., 2007; Scafati et al., 2009; Barreda et al., 2012).

Principal characteristics, age, and geographic distribution of *Azollopsis* species are shown in Table 3. In particular, the megaspore of *Azollopsis intermedia* Sweet and Hills is very similar in size and appearance to the megaspore massula of *Paleoazolla* (see Figs. 42–44 of Sweet and Hills, 1974 and compare with Fig. 3A–C). According to Sweet and Hills (1974; p. 1628), the perine of *Azollopsis* megaspores can be divided into an outer fibrous layer or filiosum (as in *Paleoazolla*); an inner, dense, relatively homogeneous layer or inner perine (as the inner perine of *Paleoazolla*); and a sculptured or porous layer named experine (as the outer perine of *Paleoazolla*). The floats of *Azollopsis* are embedded in the filiosum and attached by filament entanglement whereas in *Paleoazolla*, when present, they are embedded in the outer perine (see Fig. 3I).

The presence of a single microspore per massula is not restricted to *Paleoazolla*. Sweet and Hills (1974) mentioned one microspore per massula in *Azollopsis pusilla* Sweet and Hills and the possible presence of a “trifolium-like structure” (referred to here as “acrolamella”) in this microspore, both characters observed at *Paleoazolla* microspores. However, *A. pusilla* has circinate glochidia. Microspore massulae of the *Azollopsis* species with multibarbed glochidia enclose commonly more than one microspore, although a single microspore per massula is also occasionally recorded (Sweet and Hills, 1974). However, these massulae can be distinguished from those of *Paleoazolla* by the presence of septa in most of their glochidia (see Table 3). Nonseptate glochidia and the presence of only one microspore per massula are rare in *Azollopsis coccooides* Hall 1968 and *Azollopsis tomentosa* Hall 1968 (Sweet and Hills, 1974). A third species with multibarbed glochidia is *Azollopsis polyancyra* (Stough) Sweet and Hills 1974, only recorded from the Late Cretaceous of southern Argentina and Chile (Stough, 1968; Papú, 2002; Marensi et al., 2004). This species was originally described as *Azolla polyancyra* by Stough (1968) and was established based only on microspore massulae. *Azolla polyancyra* was transferred to the genus *Azollopsis* by Hall (1969b) and latter included in the subgenus *Azollopsis* by Sweet and Hills (1974). The massulae, as described by Stough (1968),

TABLE 2. Fossil megaspore genera related to Salviniaceae: principal characteristics, associated microspores, age, and distribution.

Genus ^a	Megaspore	Microspore	Age/Distribution
Ariadnaesporites Potonié emend. Tschudy 1966 Type species: <i>A. ariadnae</i> (Miner) Potonié emend. Tschudy 1966	Spore body nearly spherical (150–316 µm) with long, primarily distal, ribbon- or thread-like processes few to many times as long as the diameter of the spore. With a prominent membranous leaf-like acrolamella (~200 µm long).	Similar in appearance to the megaspores, differing mainly in size and surface of the outer wall. Typically the long hairs of the microspores become entangled with those of the megaspores. ^b	Albian – Paleocene / Worldwide
Azinia Baluyeva 1964 Type species: <i>A. paradoxa</i> Baluyeva 1964	Outline elliptical to ovate, with a spore body nearly spherical and numerous vertically elongated floats situated on the proximal pole.	Three subcircular to ovate eglochidiate massulae per microsporangium. Each one containing numerous microspores.	Paleocene / Russia
Azollopsis Hall emend. Sweet and Hills 1974 Type species: <i>A. coccooides</i> Hall emend. Sweet and Hills 1974	A dense mat of intertwined hairs and numerous (>32) circular floats (35–80 µm) cover the spore and give it a rounded, fusiform to egg-shaped (540–960 µm long, 360–540 µm wide). Spore 240–400 µm, with a tripartite acrolamellae. Floats attached by filament entanglement and regularly distributed over the surface.	Microspores (30–60 µm) in massulae (60–185 µm long, 50–125 µm wide). Massulae subcircular to ovate in outline with conspicuous multibarbed or circinate glochidia and containing few (1–8) large microspores.	Santonian – Paleocene ^c / North and South America, India
Glomerisporites Potonié 1956 Type species: <i>G. pupus</i> (Dijkstra) Potonié 1956 [Batten et al., 1998]	A dense mat of intertwined hairs covers the spore and give it a rounded to roughly egg-shaped (500–1000 µm long, 300–650 µm broad). Numerous small rugulate floats (15–30 µm) are distributed among the hairs. Spore 350–525 µm, with a tripartite acrolamella (100–225 µm, 100–200 µm broad).	Exine (microspore proper) situated on the "neck" of a vacuolated perine "body" and also surrounded by this material. The surface is partially covered by hairs that are usually coiled. Perine probably forms an acrolamella.	Santonian–Campanian / Netherland and Canada
Hallsiporites Nowak and Lupia 2005 Type species: <i>H. varius</i> (Hall and Peake) Nowak and Lupia 2005	A dense mat of very long hair-like appendages covers completely the spore and gives them a fusiform shaped (1064–1460 µm). Small floats (~10–40 µm) are lodged among the hairs, more proximally than distally. Spore 530–645 µm, with a tripartite acrolamella (492–722 µm long). ^d	Microspores in masses or isolated. Microspore 45–85 µm, with a tripartite acrolamella and sculptured with rugulae or echinae and with a single, long, filamentous appendage arising distally. Total length 98–165 µm.	Cenomanian / United States
Paleoazolla Archangelsky et al. emend. Type species: <i>P. patagonica</i> Archangelsky et al. emend.	Megaspores (240–340 µm) in massulae (440–890 µm long, 270–480 µm wide). Massulae elliptical to ovate, containing 1–3 megaspores, and completely covered by a dense mat of intertwined hairs. Small ovoid floats may be occasionally embedded in the outer perine of the massulae.	Microspores (42–75 µm) in massulae (68–140 µm). Massulae elliptical, circular or triangular in outline with nonseptate multibarbed glochidia and containing only one microspore. With acrolamella.	Maastrichtian / Argentina
Parazolla Hall 1969 Type species: <i>P. heterotricha</i> Hall 1969	Megaspore apparatus consisting of a nearly spherical spore body (310–550 µm) and a swimming apparatus, the later having numerous plate-like floats attached to the proximal end of the megaspore. ^e	Microspores (20–35 µm) in circular to triangular massulae (280–460 µm). Massulae with numerous hairs-like glochidia, knobbed at tip or unstructured, containing 25–35 microspores.	Campanian – Maastrichtian / North America, Europe, India and possibly South America

^aMega- and microspores dimensions were taken from the most recent descriptions of the type species of each genus (see additional references in square brackets); when microspores of the type species were unknown, dimensions were taken from another species (specified with a superscript in each case).

^bMicrospores data were taken from *Ariadnaesporites cristatus* Tschudy, because the species *A. ariadnae* is only known as megaspores.

^cWe did not take into account the age range of *Azollopsis polyancyra* because there is not enough information to assign the species to this genus (see text).

^dDimensions were taken from Hall (1975) because Nowak and Lupia (2005) do not mention the size of the spores.

^eAlthough an acrolamella is not mentioned in the original description of the type species, it is recognized by the original author, and others, in subsequent works (see Hall, 1974; Collinson, 1991) and it is also recognized for *Parazolla valcebrensis* Batten, Zavattieri and Collinson (see Batten et al., 2011a).

possess only one trilete microspore and strictly nonseptate multibarbed glochidia as those of *Paleoazolla* (see Table 3). Most of the characters of the microspore massulae of *Azollopsis polyancyra* are coincident with those of *Paleoazolla* (compare plate 2 and Fig. 3 of Stough 1968 with Fig. 4H–R and Fig. 2 of this paper). The only difference is that the massulae of *A. polyancyra* are slightly larger (170 µm) than those described here for *Paleoazolla* (140 µm). On the other hand, Stough did not mention the presence of an acrolamella on *A. polyancyra* but it is very difficult to confirm the presence of this structure in LM (see Fig. 4K–N). Consequently, the assignment of isolated microspore massulae to *Paleoazolla* or to *A. polyancyra* is very difficult, if not impossible. *Azollopsis polyancyra* was included in *Azollopsis* by Hall (1969b) because it was

at that moment the only known genus with multibarbed glochidia; however, it would now be better placed in *Paleoazolla*.

Additionally, Sweet and Hills (1974; p. 1630) mentioned the presence of one to four microsporangia attached to the megaspore complex in *Azollopsis tomentosa*. This is a very interesting feature because, like in *Paleoazolla*, it could also be indicating the possibility that *Azollopsis* produced heterosporangiate sori as well.

In summary, *Paleoazolla* is easily distinguished from all previously described Cretaceous heterosporous aquatic ferns, but it is basically the presence of two megaspores in the microsporangium that allows differentiating it from *Azollopsis*. Based on this characteristic, we can hypothesize that perhaps *Paleoazolla* represents an ancestral condition previous to that of *Azollopsis* in which

TABLE 3. Principal characteristics, age and distribution of *Paleoazolla* and *Azollopsis* species.

Species	Megaspore complex ^a					Microspore massulae			Age / Distribution
	Shape/size	Floats	Megaspore	Shape/size ^b	Microspore	Glochidia			
<i>Paleoazolla patagonica</i> Archangelsky, Phipps, Taylor et Taylor emend.	elliptical to ovate; 440–890 µm long, 270–480 µm wide	absent or numerous; embedded in the outer perine; 10–30 µm diameter	240–340 µm. Exine psilate, 4–7 µm thick. Perine: inner dense spongy 4–10 µm thick; outer vacuolated; flosom covering the surface	elliptical, circular or triangular; 68–140 µm diameter	1 per massulae; 42–75 µm; with acrolamella; exine psilate	multibarbed; up to 60 µm long, 1.5–6 µm wide; nonseptate; tip commonly anchor-shaped		Maastrichtian / Argentina	
<i>Azollopsis polyancryra</i> (Stough) Sweet and Hills 1974	—	—	—	irregular; 120–170 µm diameter	1 per massulae; 45 µm; exine psilate	multibarbed; 27–43 µm long, 1.5–4.5 µm wide; nonseptate; tip commonly anchor-shaped		Cenomanian – Maastrichtian / Argentina and Chile	
<i>Azollopsis coccooides</i> (Hall) Sweet and Hills 1974	ovate to pyriform; 540–960 µm long, 360–540 µm wide	130–225 in number; entangled in perinal hairs; 35–80 µm diameter	240–400 µm. Exine punctate to foveolate; 5.5–9.0 µm thick. Perine: inner dense 2–4 µm thick; outer reticulate 2–12 µm thick; flosom 30–60 µm thick	subcircular to ovate; 60–185 µm diameter	1–4 per massulae; 30–60 µm; exine 1.0–2.5 µm thick, psilate to punctate	multibarbed; 35–90 µm long, 3.5–7.0 µm wide; distinctly septate; tip single to double-barbed (usually)		Upper Campanian / USA and Canada	
<i>Azollopsis tomentosa</i> (Hall) Sweet and Hills 1974	subcircular to ovate; 510–900 µm long, 420–620 µm wide	130–225 in number; entangled in perinal hairs; 35–110 µm diameter	240–450 µm. Exine punctate; 5.5–7.0 µm thick. inner dense 2–4 µm thick; outer porous 2–12 µm thick; flosom 35–135 µm thick	subcircular to ovate; 100–220 µm diameter	1–8 per massulae; 30–60 µm; exine 1.0–2.5 µm thick, psilate to punctate	multibarbed; 30–120 µm long, 3–10 µm wide; septate (sometimes nonseptate); tip single (usually) to double-barbed		Maastrichtian – Paleocene ^c / USA and Canada	
<i>Azollopsis pusilla</i> Sweet and Hills 1974	ovate to fusiform; 500–950 µm long, 300–550 µm wide	>128 in number; entangled in perinal hairs; 12–21 µm in diameter	200–450 µm. Exine punctate; 3–5 µm thick. Perine: inner dense 3–4 µm thick; outer porous 9–25 µm thick; flosom 3–30 µm thick	sac-like, pyriform, dumbbell or ovate; 75–150 µm long, 35–75 µm wide	1 per massulae; 37–55 µm; with acrolamella; exine 2–3 µm thick, psilate to punctate	circinate; 12–30 µm long, 0.5–1.5 µm wide; rarely septate; tip coiled 4 µm wide		Lower Campanian / Canada	
<i>Azollopsis intermedia</i> Sweet and Hills 1974	ovate to fusiform; 370–660 µm long, 240–460 µm wide	>256 in number; entangled in perinal hairs; 27–60 µm diameter	180–330 µm. Exine punctate to foveolate; 4.5–10.5 µm thick. Perine: inner dense 2–5 µm thick; outer porous 12–50 µm thick; flosom 2–50 µm thick	ovate, subrectangular to irregular; 120–300 µm diameter	1–7 per massulae; 30–80 µm; exine 1–2 µm thick, psilate	circinate; 20–60 µm long, 0.25–1.0 µm wide; nonseptate; tip coiled 1.5–5 µm wide		Campanian / Canada	

(Continued)

TABLE 3. (Continued)

Species	Megaspore complex ^a				Microspore massulae			Age / Distribution
	Shape/size	Floats	Megaspore	Shape/size ^b	Microspore	Glochidia		
<i>Azollopsis pilata</i> (Snead) Sweet and Hills 1974	ovate to fusiform; 420–660 µm long, 300–490 µm wide	40–57 concave depressions; 30–100 µm diameter and 25–60 µm deep	185–285 µm. Exine psilate, punctate to foveolate; 2.0–4.5 µm thick. Perine: inner dense 1–4 µm thick; outer porous 45–80 µm thick; filorum 5–36 µm thick	subtriangular to irregular; 135–245 µm diameter	1–4 per massulae; 45–60 µm; with acrolamella; exine 1–2 µm thick, psilate	circinate; 15–30 µm long, 0.25–0.5 µm wide; nonseptate; tip coiled 1.5–3 µm wide (or undifferentiated)	Maastrichtian – Paleocene / Canada	
<i>Azollopsis spinata</i> (Hall and Bergad) Sweet and Hills 1974	ovate; 520 µm long, 465 µm wide ^c	numerous concave depressions	276–290 µm. Exine 9.6 µm thick ^c . Perine: inner dense; outer with spinous processes.	—	—	—	Maastrichtian / USA	

^aIn this table, the term “megaspore complex” is applied for both the megaspore apparatus of *Azollopsis* and the megaspore massulae of *Paleoazolla*.

^bDimensions of the microspore massulae correspond to the major axis.

^cArchangel'sky et al., 1999 described the exine reaching up to 10 µm.

^dThere is an Eocene record based on a single massula (Jain and Hall, 1969), but according to Collinson (1991; p. 140) it may well be reworked.

^eDimensions and thickness of the exine were taken from Hall and Bergad (1971).

only one megaspore develops. There are numerous reports of microspore massulae of *Azollopsis* with multibarbed and circinate glochidia from the Late Cretaceous and Danian of Patagonia (Papú et al., 1988; Poviluskas et al., 2006; Volkheimer et al., 2007; Scafati et al., 2009; Barreda et al., 2012); hence, it is possible that *Azollopsis* could have originated early in this region, from *Paleoazolla* or another related form. Regardless of whether or not *Paleoazolla* represents an ancestral condition previous to that of *Azollopsis*, it is clear that both taxa are very similar and probably closely related.

Monomegasporium

Monomegasporium is defined as “the reduction of the contents of the megasporangium to a single viable megaspore” (e.g., Bateman and DiMichele, 1994, p. 347). This phenomenon is the result of the partial abortion of the megasporocyte, which leaves only one megasporocyte per megasporangium. This process is followed by the abortion of three of the four meiotic products of the last remaining megasporocyte (see Pettitt, 1970; Bateman and DiMichele, 1994).

Heterospory and monomegasporium have been commonly linked, being both nested as synapomorphies of all heterosporous water ferns (Bateman and DiMichele, 1994; Schneider and Pryer, 2002; Nagalingum et al., 2006; Smith et al., 2006). Monomegasporium does not appear to be a synapomorphy for *Paleoazolla* because the common condition is the presence of two megaspores.

Of particular interest are those specimens with one well-developed megaspore and several partially developed megaspores (see Fig. 5E, F). One of these specimens has three small aborted spores (Fig. 5E), and therefore, it is possible that all the spores enclosed in a megaspore massula of *Paleoazolla* correspond to the same tetrad. Because there can be one, two, or three well-developed (possibly fertile) megaspores within the *Paleoazolla* megaspore massula, it is possible that the character monomegasporium was not fixed. Therefore, it could be considered as an attractive hypothetical ancestral condition from which monomegasporium could have diverged. A more likely hypothesis, however, is this lineage could equally likely be characterized by a reversion on character monomegasporium.

Unfortunately, we lack information on the sporophyte of *Paleoazolla*, and therefore there are some hypotheses that should be considered regarding the monomegasporium: (1) *Paleoazolla* could represent an early lineage, previous to the divergence of Salviniaceae and Marsileaceae; or (2) *Paleoazolla* could be more closely related to one of the two families, and thence there are two possible hypotheses: (a) monomegasporium was acquired independently in both families; or (b) there was a reversion in character monomegasporium in *Paleoazolla*.

Regardless of these hypotheses, the reproductive strategy of *Paleoazolla* reveals serious gaps in our current knowledge on the evolution of monomegasporium in heterosporous water ferns, a fact that emphasizes the need for phylogenies including fossils to elucidate patterns of character acquisition.

CONCLUSIONS

This new *Paleoazolla* interpretation reveals a novel set of characters that are essential for understanding the evolutionary transformations among water fern genera. *Paleoazolla patagonica* is characterized by producing heterosporangiate sori composed of one ellipsoidal megasporangium that is surrounded by three to four

oval microsporangia. The megasporangium encloses one hairy megaspore massula with a spongy structure that contains two megaspores, rarely one or three. Each microsporangium is composed of numerous microspore massulae with multibarbed nonseptate glochidia and each massula contains only one microspore. All these characters strongly support the affinity of *Paleoazolla* to Salviniaceae. However, new evidence is needed mainly on macrofossil remains in organic connection with the reproductive structures before making further systematic conclusions.

Among all known modern and fossil heterosporous water fern taxa, *Paleoazolla* is the only one characterized for developing more than one megaspore per megasporangium, offering an attractive hypothetical ancestral condition from which the monomegasporous could have diverged. *Paleoazolla* demonstrates that the long-accepted theory that monomegasporous is a synapomorphy for all aquatic ferns (Marsileaceae and Salviniaceae) should be reevaluated.

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