AZOLLA SPOROPHYTES AND SPORES FROM THE LATE CRETACEOUS AND PALEOCENE OF PATAGONIA, ARGENTINA

Elizabeth J. Hermsen,[1],* Nathan A. Jud,† Facundo De Benedetti,‡ and Maria A. Gandolfo§

*Paleontological Research Institution, 1259 Trumansburg Road, Ithaca, New York 14850, USA; †Department of Biology, William Jewell College, 500 College Hill, Liberty, Missouri 64068, USA; ‡Museo Paleontológico Egidio Feruglio, CONICET, Trelew, Argentina; and §L. H. Bailey Hortorium, Plant Biology Section, School of Integrative Plant Sciences, Cornell University, Ithaca, New York 14853, USA

Editor: Michael T. Dunn

Premise of research. While *Azolla* has a rich fossil record based on dispersed megaspore apparatuses and microspore massulae, fossil sporophytes are relatively rare. In this contribution, we describe two fossil *Azolla* species based on both sporophytes and spores from Chubut Province, Patagonia, Argentina: *Azolla coloniensis* De Benedetti & Zamaloa, emend. Hermsen et al., and *A. keuja* Jud et al., sp. nov. *Azolla coloniensis* and *A. keuja* are the first fossil species of *Azolla* to be represented by vegetative structures (i.e., leaves, stems, and roots) from both South America and the Southern Hemisphere.

Methodology. We examined sporophyte material of *A. coloniensis* from the Cañadón del Irupé locality, Upper Cretaceous, La Colonia Formation, and *A. keuja* from the Palacio de los Loros locality PL-2, Paleocene, Salamanca Formation. Spores of *A. keuja* were obtained from a sporophyte specimen and its surrounding rock matrix. Material was studied using standard light microscopy, epifluorescence microscopy, and scanning electron microscopy. Fossils are held at the Museo Paleontológico Egidio Feruglio, Trelew, Chubut Province, Argentina.

Pivotal results. *Azolla coloniensis* produced many-floated megaspore apparatuses and microspore massulae with anchor-tipped glochidia, placing it in the fossil *Azolla* section *Florschuetzia*. *Azolla keuja* sporophytes are structurally similar to those produced by the extant African species *A. nilotica* and the Late Cretaceous–Paleocene North American species *A. schopfii* in overall size, growth form, leaf structure, and production of fascicled roots; while all three taxa produce similar microspore massulae, the structure of their megaspore apparatuses differ. *Azolla keuja* cannot be assigned to any section of *Azolla*.

Conclusions. *Azolla coloniensis* and *A. keuja* are important because they provide two new organismal concepts for extinct species of *Azolla*. Our inability to fully classify *A. keuja* to section, in combination with the great morphological diversity of fossil *Azolla*, indicates that a comprehensive reevaluation of phylogeny and taxonomy that incorporates both extant and fossil species is needed.

*Keywords:* Chubut Province, La Colonia Formation, Salamanca Formation, Salviniaceae, Salviniales.

Online enhancements: appendixes.

Introduction

Azolla Lam. includes about six to seven extant species of floating aquatic ferns that grow in tropical and temperate climates (Tryon and Tryon 1982; Schneller 1990; Saunders and Fowler 1992, 1993; Reid et al. 2006; Metzgar et al. 2007). The sporophytes of *Azolla* are highly simplified and often very small; they consist of horizontally growing stems that branch profusely and bear alternate, imbricate, simple leaves in two rows (fig. 1*A*; Strasburger 1873; Schneller 1990; Saunders and Fowler 1992). The leaves are divided into two lobes, a typically inconspicuous ventral lobe and a green dorsal lobe containing a cavity that houses

nitrogen-fixing cyanobacterial symbionts (fig. 1*B*; Strasburger 1873; Peters et al. 1978; Lumpkin and Plucknett 1980; Carrapiço 2010). The roots are adventitious and unbranched, and they typically hang free in the water column (fig. 1*A*; Strasburger 1873; Lumpkin and Plucknett 1980; Schneller 1990; Lumpkin 1993). *Azolla* is heterosporous. Sporangia are enclosed in homosporangiate, indusiate sori, which are sometimes called sporocarps (fig. 1; Nagalingum et al. 2006); each megasporangiate sorus contains one megasporangium that produces a single functional megaspore, whereas each microsporangiate sorus has multiple microsporangia that in turn produce multiple microspores (Nagalingum et al. 2006). Each megaspore is part of a complex structure called a megaspore apparatus, which includes a megaspore proper and a proximal column to which a series of floats are attached (Mettenius 1847; Strasburger 1873; Martin 1976; Fowler and Stennett-Willson 1978; Nagalingum et al. 2006). The micro-
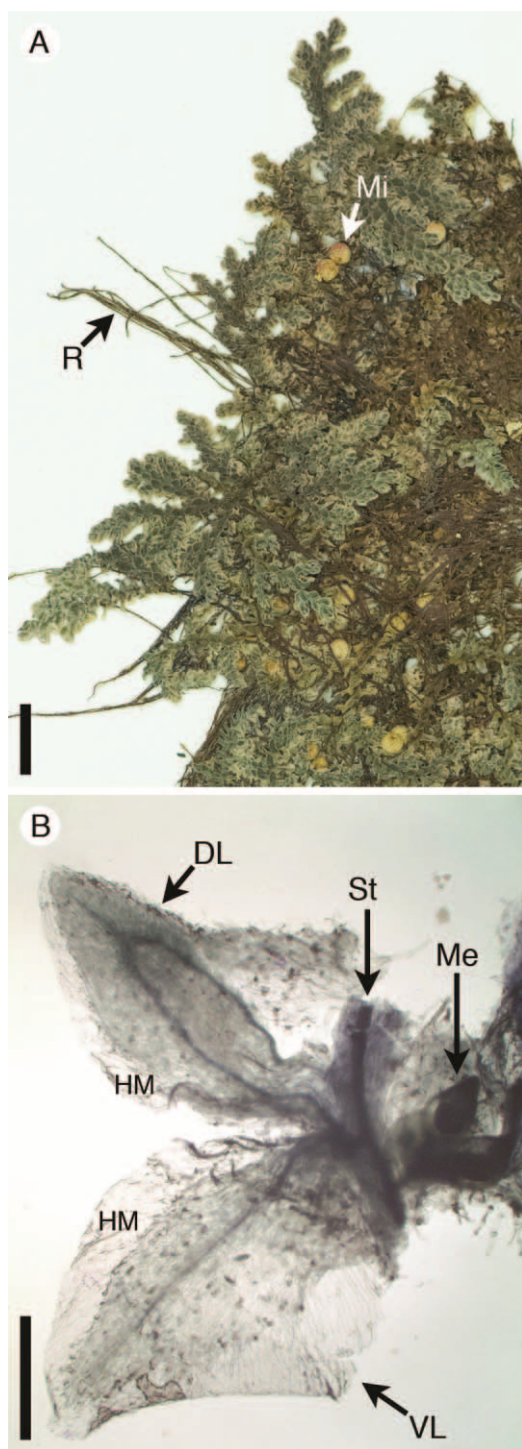
Fig. 1   Fertile sporophytes of *Azolla*. *A*, *Azolla filiculoides* showing branches with imbricate leaves, unbranched roots (R), and microsporangiate sori (Mi); FLAS P9007 (Otto Degener 35,788, Hawaii. Image captured July 2013 and used with permission of the University of Florida Herbarium, joint right-of-use and copyright reserved). *B*, Leaf of *A. nilotica* cleared and stained with toluidine blue to show details of the anatomy. The leaf trace diverges from the stem (St) and immediately branches. One branch becomes the midvein of the ventral leaf lobe (VL). The other branch supplies the dorsal lobe (DL), encircling the leaf cavity and sending off an apical spur. Note the hy-

spores occur in groups surrounded by a common matrix that is typically covered with glochidia (hairs); these units are known as microspore massulae (e.g., Mettenius 1847; Strasburger 1873; Tryon and Tryon 1982; Saunders and Fowler 1992).

Molecular phylogenetic studies of the extant species of *Azolla* support the division of the genus into two monophyletic sister groups that correspond to the traditionally recognized sections *Azolla* and *Rhizosperma* (Reid et al. 2006; Metzgar et al. 2007; see also a recent cluster analysis of data from morphology and molecular markers by Pereira et al. 2011). Section *Azolla* includes the New World species *A. caroliniana*, *A. filiculoides*, and the *A. mexicana-A. microphylla* complex, as well as the Asian to Australasian species *A. rubra* (Reid et al. 2006; Metzgar et al. 2007). Section *Rhizosperma* comprises the widespread *A. pinnata* species group and the African species *A. nilotica* (Reid et al. 2006; Metzgar et al. 2007). These sections can be neatly separated based on the characteristics of their spores. Section *Azolla* is characterized by megaspore apparatuses with three floats and microspore massulae with anchor-tipped glochidia, whereas *Rhizosperma* is characterized by megaspore apparatuses with nine floats and microspore massulae with either simple glochidia (i.e., glochidia lacking anchor-shaped ends) or no glochidia (table 1; see also, e.g., Mettenius 1847; Martin 1976; Tryon and Tryon 1982; Metzgar et al. 2007). Sporophyte characters separating the sections include the absence (section *Azolla*) or presence (section *Rhizosperma*) of trichomes on the stem (Saunders and Fowler 1993; Reid et al. 2006; Metzgar et al. 2007; Pereira et al. 2011), as well as differences in sporophyte shape and leaf structure (Pereira et al. 2011).

The fossil record of *Azolla* begins in the Late Cretaceous and includes more than 50 fossil species, most of which are represented by dispersed spores (Collinson 1980; Kovach and Batten 1989; Batten and Kovach 1990; Vajda and McLoughlin 2005). These fossils suggest that the genus was widely distributed in the past and was sometimes abundant even at very high latitudes (e.g., Hall 1977; Askin 1989, 1990; Collinson 2001; Brinkhuis et al. 2006; Collinson et al. 2009; van der Burgh et al. 2013). Because extinct species often do not fit into the taxonomic framework provided by the sections that encompass extant diversity, several sections of *Azolla* have been proposed based on combinations of spore characters observed solely in fossils (table 1).

Despite *Azolla*'s rich fossil spore record, sporophyte material is relatively rare; there are fewer than 15 species represented by sporophyte macrofossils preserving stems, roots, and/or leaves (table 2; fig. 2). Three of these fossil *Azolla* species are described solely from sporophyte material, whereas the remainder are also known from spores (table 2). Sporophyte records are widespread in the Northern Hemisphere, including occurrences documented from the Indian subcontinent, Siberia, continental Europe, the British Isles, and North America (fig. 2; table 2). By contrast, the only fossil species from the Southern Hemisphere for which sporophyte structures are known is *A. boliviensis*. The specimens of this Cretaceous to Paleocene species include megasporangiate sori (indusia and megasporangia) and

aline margin (HM) on both the dorsal and ventral leaf lobes as well as a megasporangiate sorus (Me); BH 000199372. Scale bars: *A* = 5 mm; *B* = 0.25 mm.

Table 1

Comparison of Sections of *Azolla*

Section	Megaspore apparatus	Glochidia on microspore massula	Age range
Azolla Lam.	3 large floats	Anchor-shaped ends	Late Cretaceous/Paleocene[a]–Recent
Rhizosperma (Meyen) Mett.	9 floats in 2 tiers (3 in upper, 6 in lower)	Spinose or filamentous, may be rare to absent	Oligocene[b]–Recent
Antiqua P.I. Dorof.	9 (or more) floats in 2 tiers (3 in upper, 6–9 in lower); cap present[c]	Unknown	Eocene–Oligocene
Filifera J.W. Hall	Unknown[d]	Filamentous/hairlike with circinate ends	Late Cretaceous
Florschuetzia Kempf[e]	More than 9 floats	Anchor-shaped ends	Late Cretaceous–Eocene
Simplicispora J.W. Hall[f]	Columella float-like or 1 float	Anchor-shaped ends	Late Cretaceous–Eocene
Trisepta K. Fowler	Triseptate columella, 9 floats in 2 tiers (3 in upper, 6 in lower)	Anchor-shaped ends	Eocene[g]

Sources. Dorofeev (1959), Hall (1968), Hall and Swanson (1968), Kempf (1968), Jain and Hall (1969), Hall (1970), Jain (1971), Fowler (1975), and Follieri (1977).

[a] Based on *A. indica* and *A. intertrappea* (Sahni 1941; Sahni and Rao 1943; Surange 1966; Trivedi and Verma 1971).

[b] While *A. deccaniana* from the Late Cretaceous–Paleocene of India was assigned to section *Rhizosperma* (Nambudiri and Chitaley 1991), its megaspore apparatus is unknown; thus, its assignment should be considered equivocal. Dispersed spores assigned to *Rhizosperma* are known beginning in the Oligocene (Dorofeev 1959, 1963; Friis 1977).

[c] Some sources (Jain 1971; Fowler 1975; Follieri 1977) indicate that this section has six to nine floats, but that appears to be a misinterpretation of Dorofeev's (1959) description of *A. antiqua*. Section *Antiqua* could be synonymous with section *Rhizosperma* (see Martin 1976) or *Trisepta*.

[d] Fowler (1975) indicated that megaspore apparatuses in this section have more than nine floats, but other sources (Hall 1968; Hall and Swanson 1968; Jain 1971; Follieri 1977) described the megaspore apparatuses as unknown.

[e] Synonym is *Azolla* section *Kremastospora* R.K. Jain & J.W. Hall (see Martin 1976; Collinson 1980). Both section *Florschuetzia* (originally subgenus *Florschuetzia*) and section *Kremastospora* share the same type, *A. teschiana* Florschütz (Kempf 1968; Jain and Hall 1969).

[f] Collinson (1991) considered this section invalid.

[g] Updated age after Hayes and Collinson (2014).

microsporangia, but no vegetative organs have been found (Vajda and McLoughlin 2005).

The goal of this contribution is to present two extinct *Azolla* species from Chubut Province, Patagonia, Argentina, known from both spore and sporophyte specimens, including vegetative and reproductive structures found in organic connection. These are among the southernmost known occurrences of modern and fossil *Azolla* and the only fossil *Azolla* sporophytes retaining vegetative structures from the Southern Hemisphere. They also add to the growing body of research documenting fossil heterosporous water fern (salvinialean) sporophytes from South America. Other reports include marsileaceous ferns from the Early Cretaceous of Colombia (Monje-Dussán et al. 2016) and the Late Cretaceous of Argentina (Cúneo et al. 2013, 2014; Gandolfo et al. 2014; Hermsen et al. 2014; Puebla et al. 2015; Vallati et al. 2017), as well as *Salvinia* (Salviniaceae) from the Paleogene of Colombia (Pérez-Consuegra et al. 2017).

Material and Methods

Geographic and Stratigraphic Context

The *Azolla* fossils described below were collected from the Maastrichtian (~72–66 Ma, Upper Cretaceous) La Colonia Formation and the Danian (~66–61 Ma, lower Paleocene) Salamanca Formation. Both crop out in Chubut Province, Patagonia, Argentina (fig. 2).

Plant macrofossils and associated microfossils (algae, spores, and pollen) are known from the Cañadón del Irupé, Quebrada del Helecho, and Cerro Bosta localities of the La Colonia Forma-

tion (Gandolfo and Cúneo 2005; Cúneo et al. 2013, 2014; Gallego et al. 2014; Gandolfo et al. 2014; Hermsen et al. 2014; De Benedetti et al. 2018). Although *Azolla* spores have been recovered from all of these localities (Cúneo et al. 2014; Gandolfo et al. 2014; De Benedetti et al. 2018), *Azolla* sporophyte material has been found only at the Cañadón del Irupé locality (Cúneo et al. 2014; Gandolfo et al. 2014). The La Colonia Formation is thought to be largely Campanian to Maastrichtian in age (but may also include sediments that are younger and older near the top and bottom, respectively) based on several biostratigraphic markers such as palynomorphs, foraminifera, marine invertebrates, and dinosaurs (see Pascual et al. 2000, as well as citations therein). The horizons yielding the macrofossil flora are considered to be Maastrichtian (N. R. Cúneo, personal communication); they are flat, laminated mudstones interpreted as having been deposited in stagnant waterbodies, possibly coastal lagoons (Pascual et al. 2000; Cúneo et al. 2014). The aquatic component of the fossil flora includes algae (e.g., *Pediastrum*, *Spirogyra*), aquatic ferns (*Azolla*, *Paleoazolla*, and several species of Marsileaceae), monocots (Typhaceae and a floating aroid), and lotus (*Nelumbo*), indicating a low-energy, shallow, freshwater environment (Cúneo et al. 2014).

Fossils were also collected from the Palacio de los Loros-2 locality (PL-2) of the Salamanca Formation in southwestern Chubut Province (fig. 2; Iglesias et al. 2007; Clyde et al. 2014; Comer et al. 2015). The horizon yielding plant fossils at PL-2 comprises flat, laminated to thin-bedded mudstone deposits in tidally influenced fluvial swale-fill channels (Comer et al. 2015). The age assignment of these deposits is constrained to the early Danian using the recently revised chronostratigraphic framework for the Salamanca Formation in the study area,

Table 2

Fossil *Azolla* Species with Vegetative Sporophyte Organs (i.e., Roots, Stems, and/or Leaves)

Taxon	Spores[a]	Age	Location (no. in fig. 2)	Reference(s)
A. coloniensis De Benedetti & Zamaloa, emend. Hermsen et al.	+ (me, mi)	Late Cretaceous	Chubut Province, Argentina (1)	Cúneo et al. 2014; Gandolfo et al. 2014; De Benedetti et al. 2018; this article
A. deccaniana Nambudiri & Chitaley	+ (mi only)	Late Cretaceous–Paleocene[b]	Madhya Pradesh, India (2)	Nambudiri and Chitaley 1991
A. indica Trivedi & C.L. Verma	+ (me, mi)	Late Cretaceous–Paleocene[b]	Madhya Pradesh, India (2)	Trivedi and Verma 1971
A. intertrappea Sahni & H.S. Rao	+ (me, mi)	Late Cretaceous–Paleocene[b]	Madhya Pradesh, India (2)	Sahni 1941; Sahni and Rao 1943; Surange 1966; Hall 1969*a*
A. schopfii Dijkstra	+ (me, mi)	Late Cretaceous–Paleocene[c]	Alberta (3) and Saskatchewan (4), Canada; Montana and South Dakota (not mapped)	Dijkstra 1961; Jain and Hall 1969; Snead 1969; Jain 1971; Sweet and Chandrasekharam 1973; Collinson 1980; McIver and Basinger 1993; Batten and Collinson 2001
A. keuja Jud et al.	+ (me, mi)	Paleocene	Chubut Province, Argentina (5)	This article
A. stanleyi R.K. Jain & J.W. Hall	+ (me, mi)	Paleocene	Alberta (3) and Saskatchewan (not mapped), Canada; North Dakota (6)	Jain and Hall 1969; Sweet and Hills 1976; Melchior and Hall 1983; Hoffman and Stockey 1994
A. velus (Dijkstra) R.K. Jain & J.W. Hall	+ (me, mi)	Paleocene	Saskatchewan, Canada (4); Montana and South Dakota (not mapped)	Dijkstra 1961; Jain and Hall 1969; Martin 1976; McIver and Basinger 1993; Batten and Collinson 2001
A. berryi R.W. Br.	–	Eocene	Colorado, Utah, and Wyoming (7)	Brown 1934; Grande 2013; Hermsen, personal observation, 2013–2014[d]
A. primaeva (Penh.) C.A. Arnold	+ (me, mi)	Eocene	British Columbia, Canada (8, 9)	Penhallow in Dawson 1890; Arnold 1955; Rouse 1962; Hills and Weiner 1965; Hills and Gopal 1967; Dillhoff et al. 2013; Greenwood et al. 2016; Mathewes et al. 2016[e]; Collinson et al. 2017
A. prisca E. Reid & M. Chandler	+ (me, mi)	Eocene	England (10)	Reid and Chandler 1926; Fowler 1975; Collinson 1980; Hayes and Collinson 2014[f]
A. vera Kryst.	–[g]	Eocene–Oligocene	Omsk Oblast (11) and Tyumen Oblast (12), Russia	Kryshtofovich 1952; Akhmetiev et al. 2012; Zaporozhets and Akhmetiev 2013
A. tertiaria E.W. Berry	–[g]	Miocene	Nevada (13)	Berry 1927
A. aff. *A. ventricosa* P. Nikitin sensu P.I. Dorof.	+ (me, mi)	Miocene	Ústecký, Czech Republic (14)	Bůžek et al. 1988

Note.　Localities from which only spores are documented are not mapped in figure 2.

[a] Key: + = spores found on fertile sporophytes, or associated spores considered to be conspecific with fertile sporophytes (me = megaspore apparatuses; mi = microspores/microspore massulae); – = spores unknown.

[b] Age based on information in Smith et al. (2015).

[c] *Azolla schopfii* is known to occur in the Paleocene, although opinions vary about whether it also occurs in the Maastrichtian due to differing stratigraphic interpretations (see Batten and Collinson 2001).

[d] Utah occurrences observed: Denver Museum of Nature and Science, Denver (DMNH EPI.27589, loc. 323); Florida Museum of Natural History, Gainesville (UF 15755-23024, UF 15755-23025); Field Museum of Natural History, Chicago (PP 16743). Wyoming occurrence figured in Grande (2013) as *Azolla*, probably *A. berryi* since it occurs in the Green River Formation.

[e] Identified only as *Azolla*, but probably *A. primaeva* given locality.

[f] Identified only as *Azolla*, but probably *A. prisca* given locality.

[g] Although fertile sporophyte material is known, spores have not been described.
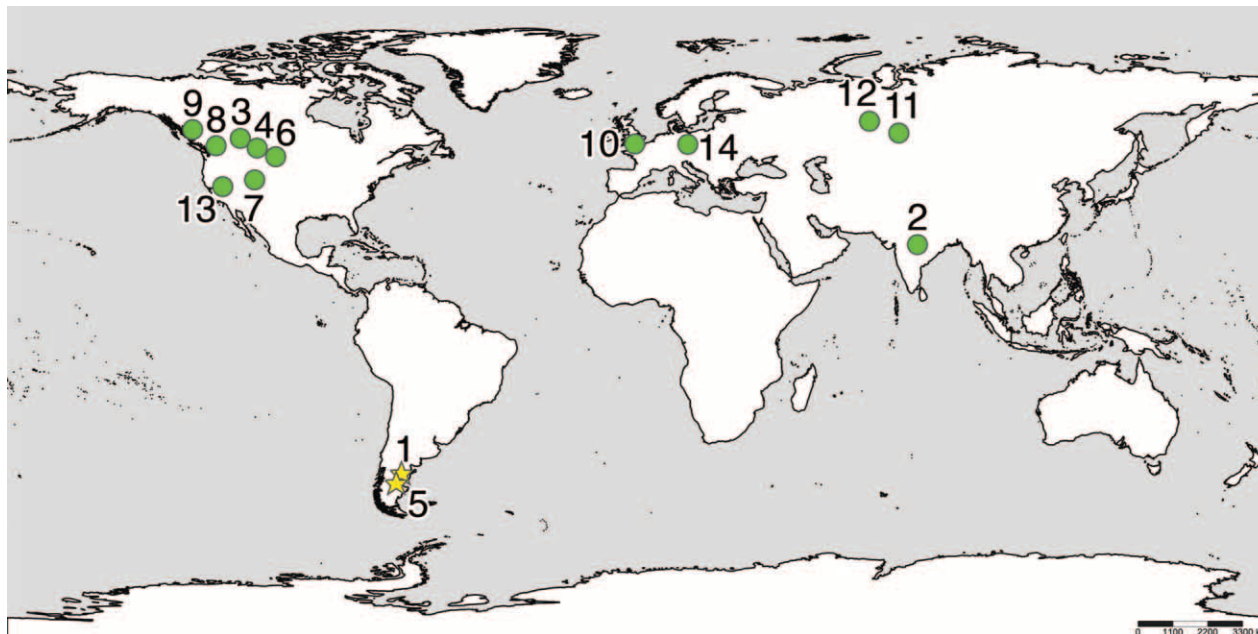
Fig. 2   Distribution map for fossil *Azolla* sporophytes with vegetative structures (roots, stems, and/or leaves) worldwide, Late Cretaceous–Neogene. Numbers on map correspond to numbers in table 2. Note that some localities occurring in the same geographic region have been combined (e.g., Eocene southern Okanagan Highlands localities in British Columbia, Canada; Eocene Green River Formation localities in Colorado, Utah, and Wyoming, etc.). Point map made using Simplemappr (Shorthouse 2010).

which is based on age-diagnostic foraminifera, calcareous nanofossils, dinoflagellate cysts, paleomagnetic data, and radiometric dates from units stratigraphically lower and higher than the fossil-bearing layers (Clyde et al. 2014; Comer et al. 2015). Other elements of the PL-2 assemblage described so far include araucariaceous and podocarpaceous conifers (Escapa et al. 2018; Andruchow-Colombo et al. 2019) and angiosperm reproductive structures assigned to the families Cunoniaceae and Menispermaceae (Jud et al. 2018a, 2018b).

Fossil Specimen Curation and Preparation

The fossils are housed in the paleobotanical and palynological collections of the Museo Paleontológico Egidio Feruglio (MEF; repository acronyms MPEF-Pb for macrofossil collections and MPEF-PA for palynological collections), Trelew, Chubut Province, Patagonia, Argentina. Macrophotographs were taken with a Canon EOS Rebel T2i DSLR camera. Photomicrographs captured under standard illumination were taken using a Nikon SMZ1000 stereoscopic microscope with Nikon DS-Fi1 camera head and DS-L2 camera control unit. Microsporangiate sori were manually removed from one *A. coloniensis* sporophyte (MPEF-Pb 5060) and examined using a JEOL JSM-6460 SEM at Aluar S.A., Puerto Madryn, Chubut Province, Argentina.

All illustrated spores of *A. keuja* were recovered from the same sample. Some were peeled directly from a single specimen with sporophyte remains; the rock from which peels were taken was later disaggregated to recover more spores. The disaggregated rock was treated with hydrofluoric acid (70%) and hydrochloric acid (30%). The resulting residues

were decanted and washed several times, then filtered with 100-μm meshes. Megaspore apparatuses and microspore massulae were picked with needles under a stereomicroscope, cleared with a 10% sodium hypochlorite solution for 2–10 min, stained with safranin, and mounted on slides using glycerin jelly as a mounting medium. Specimens examined using an SEM were not cleared; they were mounted directly on stubs using double-sided tape. Longitudinal sections of spore material were made using a razor blade. Light microscopy (LM) observations were made with a Nikon Eclipse 80i microscope coupled with a Nikon DS-L4 camera at the MEF. SEM observations were made with a JEOL JSM-6460 SEM at Aluar S.A., Puerto Madryn, Chubut Province, Argentina.

Modern Comparative Material

The Patagonian fossils were compared with specimens of modern *Azolla* from the L. H. Bailey Hortorium Herbarium (BH), Plant Biology Section, School of Integrative Plant Science (SIPS), Cornell University, Ithaca, New York. Fragments of shoots cleared for study were soaked for 48 h in water with dilute detergent, then for 3–5 d in a 1:1 water and bleach solution. Afterward, the specimens were stained with toluidine blue O and transferred to temporary wet-mount slides. Cleared specimens were photographed with an Olympus UC90 high-definition digital camera under a Nikon SMZ745 dissecting microscope and an Olympus BX60 compound microscope at the BH. Other specimens of *Azolla* were photographed using a Nikon D7100 DSLR camera at the University of Florida Herbarium (FLAS), Florida Museum of Natural History, Gainesville.

Terminology

The term "sporocarp" has been widely used in the literature for the thin-walled structures that surround the sporangia of *Azolla*. The term "microsporocarp" has typically been used for the structure that surrounds multiple microsporangia, each of which contains several microspore massulae; the term "megasporocarp" has been used for the structure that surrounds one megasporangium, which in turn contains one megaspore apparatus (e.g., Foster and Gifford 1974; Schneller 1990; Nagalingum et al. 2006). Nagalingum et al. (2006), however, argued that the term sporocarp as traditionally used suggests equivalence between structures that are not of the same evolutionary or developmental origin in Marsileaceae and Salviniaceae within water ferns (Salviniales). In Salviniaceae (*Azolla* and *Salvinia*) the structure called a sporocarp is equivalent to a single indusiate sorus (Foster and Gifford 1974; Nagalingum et al. 2006). Herein, we thus use the terms "microsporangiate sorus" and "megasporangiate sorus" for the sporangium-enclosing structures of *Azolla* (see fig. 1*D* in Nagalingum et al. [2006]), as these terms are more precise.

Terminology applied to the megaspore apparatus and microspore massula varies and in some cases is defined inconsistently; terminology in this article follows the usage in De Benedetti et al. (2018) and the references cited therein. Brief definitions of selected terms for reproductive features as used in this article are given in appendix A (apps. A, B are available online) for reference.

Results

Order—Salviniales Link

Family—Salviniaceae Martinov

Genus—Azolla *Lam.*

Section—Florschuetzia *Kempf*

Species—Azolla coloniensis *De Benedetti & Zamaloa, emend. Hermsen, Jud & Gandolfo (Fig. 3A–3H)*

Original species description. *Azolla coloniensis* De Benedetti & Zamaloa in De Benedetti et al. (2018), pages 363 and 366, plates I–II, and figure 15.2 *in* M. Krings, C. J. Harper, N. R. Cúneo, G. W. Rothwell, eds. Transformative paleobotany. Academic Press, London and Oxford, United Kingdom, and San Diego and Cambridge, Massachusetts.

Synonyms. *Azolla*-like megaspore, Cúneo et al. (2014), figure 4*A*; *Azolla* microspore massula, Cúneo et al. (2014), figure 4*B*; *Azolla* sporophytes, Cúneo et al. (2014), figure 5*B*, 5*D*; *Palaeoazolla* [sic] *patagonica*, Gandolfo et al. (2014), figure 2*A*; *Azolla* sp., Gandolfo et al. (2014), figure 3*A*, 3*B*.

Emended diagnosis. Sporophyte stem with at least one dichotomy, sinuous in course, glabrous, bearing alternately to suboppositely arranged lateral branches; lateral branches bearing upturned, imbricate leaves; each leaf obdeltoid in shape, apex bilobed; adventitious roots solitary. Fertile sporophytes with megasporangiate sori or both mega- and microsporangiate sori. Megasporangiate sori ovoid, borne singly or in groups of up to three; microsporangiate sori borne in groups of two. Megaspore apparatus ovoid, surface of exoperine reticulate, collar absent, columella dome-shaped, floats 18–21 (or more) in three (to four) tiers, cap absent; exoperine, columella, and floats with hairs (i.e., infrafilosum and suprafilosum present). Microspore massula with anchor-tipped, aseptate glochidia; about 16 massulae per microsporangium, about four microspores per massula.

Lectotype designated here. MPEF-PA 80, megaspore apparatus illustrated by De Benedetti et al. (2018), plate I, figure 1. Held at the Museo Paleontológico Egidio Feruglio, Trelew, Chubut Province, Argentina.

Paratypes. Other specimens of MPEF-PA 80; MPEF-PA 81–86, 92–95.

Sporophyte specimens examined. MPEF-Pb 5060a, 5060b; 5061; 5081; 5082.

Type locality. Cañadón del Irupé locality, Chubut Province, Patagonia, Argentina (see Cúneo et al. 2014; Gandolfo et al. 2014; De Benedetti et al. 2018).

Stratigraphic position and age. Upper Cretaceous La Colonia Formation, macrofossil flora thought to be Maastrichtian in age (see Cúneo et al. 2014).

Description. Sporophytes are small and usually preserved as fragments (fig. 3*A*–3*D*). Stems are up to 13.5 mm long, 0.2–0.5 mm in width, and have a slightly sinuous course (fig. 3*A*–3*C*). Main stems, which bear either no or few leaves, dichotomize at least once (fig. 3*C*) and give rise to alternately to suboppositely arranged leafy lateral branches up to 2.5 mm in length (fig. 3*A*–3*C*). Some stems clearly show a central vascular strand (fig. 3*A*, 3*B*); no stem trichomes were observed. Leaves are alternate and imbricate; each leaf is simple, sessile, and obdeltoid in shape with a subtly bilobed upturned apex (fig. 3*A*, 3*B*). Leaves are ca. 0.5–1.0 mm long by 0.3–0.4 mm wide and fed by a single vein. No evidence of large root fascicles was found, and roots are interpreted as being borne singly on stems (fig. 3*B*, 3*D*). Roots are up to 2.2 mm long, show a vascular strand, and do not branch (fig. 3*B*, 3*D*).

Fertile sporophytes bear megasporangiate sori or both mega- and microsporangiate sori (fig. 3*C*–3*F*). Megasporangiate sori are ovoid, ca. 0.4–0.6 mm long by 0.2–0.4 mm wide, and borne on short stalks; they occur singly or in groups of up to three (fig. 3*C*, 3*E*). Microsporangiate sori are larger and bear multiple microspore massulae; on the single specimen on which they have been observed, they are apparently borne in a pair (fig. 3*D*, 3*F*). The microsporangiate sori found attached to a sporophyte are ca. 0.7–0.9 mm in diameter (fig. 3*D*, 3*F*, 3*G*); individual microsporangia in these sori are about 130–190 µm in diameter (fig. 3*G*, 3*H*). The megaspore apparatuses and microspore massulae were described as *Azolla coloniensis* De Benedetti & Zamaloa by De Benedetti et al. (2018) based on dispersed structures.

Remarks. Several types of salviniaceous spores are known from the La Colonia Formation: *Azollopsis*, *Azolla coloniensis*, an unnamed species of *Azolla*, and the monotypic taxon *Paleoazolla patagonica* (Archangelsky et al. 1999; Cúneo et al. 2014; Gandolfo et al. 2014; De Benedetti et al. 2018, 2019*a*). Of these, two have been recovered from sediments at the Cañadón del Irupé locality: *A. coloniensis* (De Benedetti et al. 2018) and *P. patagonica* (fig. 3*I*). Although no spores were macerated directly from the La Colonia Formation sporophyte
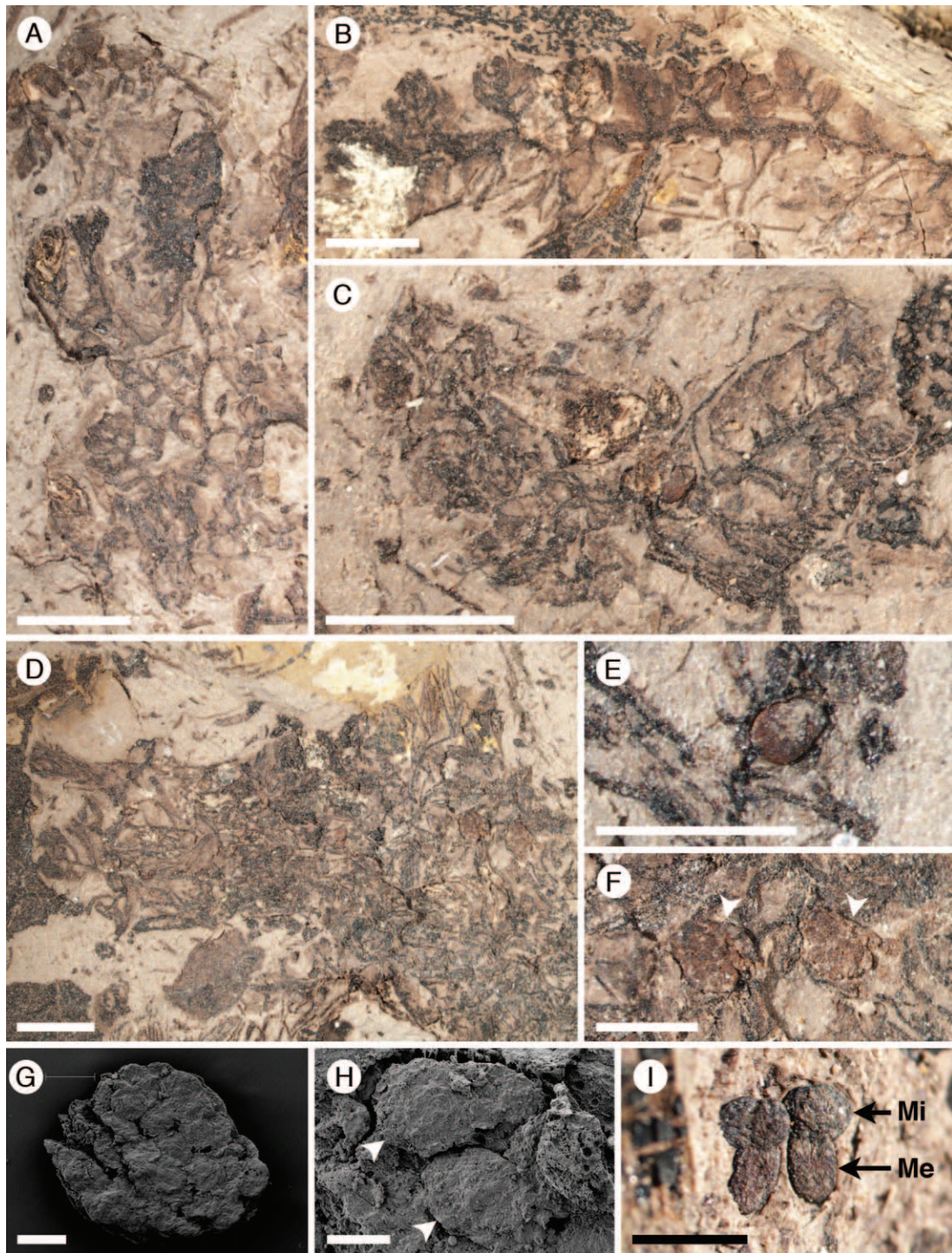
Fig. 3 *Azolla coloniensis* De Benedetti & Zamaloa emend. Hermsen, Jud & Gandolfo and *Paleoazolla patagonica*. *A*, Sterile sporophyte showing sinuous stem, branching pattern, and leaf arrangement and morphology; MPEF-Pb 5061. *B*, Stem bearing short, leafy branches and unbranched roots; MPEF-Pb 5081. *C*, Sporophyte with megasporangiate sorus; MPEF-Pb 5082. *D*, Sporophyte with microsporangiate sori; MPEF-Pb 5060. *E*, Megasporangiate sorus; MPEF-Pb 5060b. *F*, Microsporangiate sori; detail of specimen shown in *D*, MPEF-Pb 5060. Note that these microsporangiate sori were later manually removed for examination with an SEM (see *G*, *H*). *G*, Scanning electron photomicrograph of a microsporangiate sorus with individual microsporangia discernible (circular structures), removed from MPEF-Pb 5060 (sorus at left in *F*). *H*, Detail of microsporangiate sorus showing circular microsporangia (arrowheads), removed from MPEF-Pb 5060 (from sorus at right in *F*). *I*, Two sori of *P. patagonica*, each consisting of one megasporangium (Me) with attached microsporangia (Mi), MPEF-Pb 5075 (these sori were later manually removed and examined under SEM). Scale bars: *A–D* = 2 mm; *E*, *F*, *I* = 1 mm; *G* = 200 μm; *H* = 100 μm.
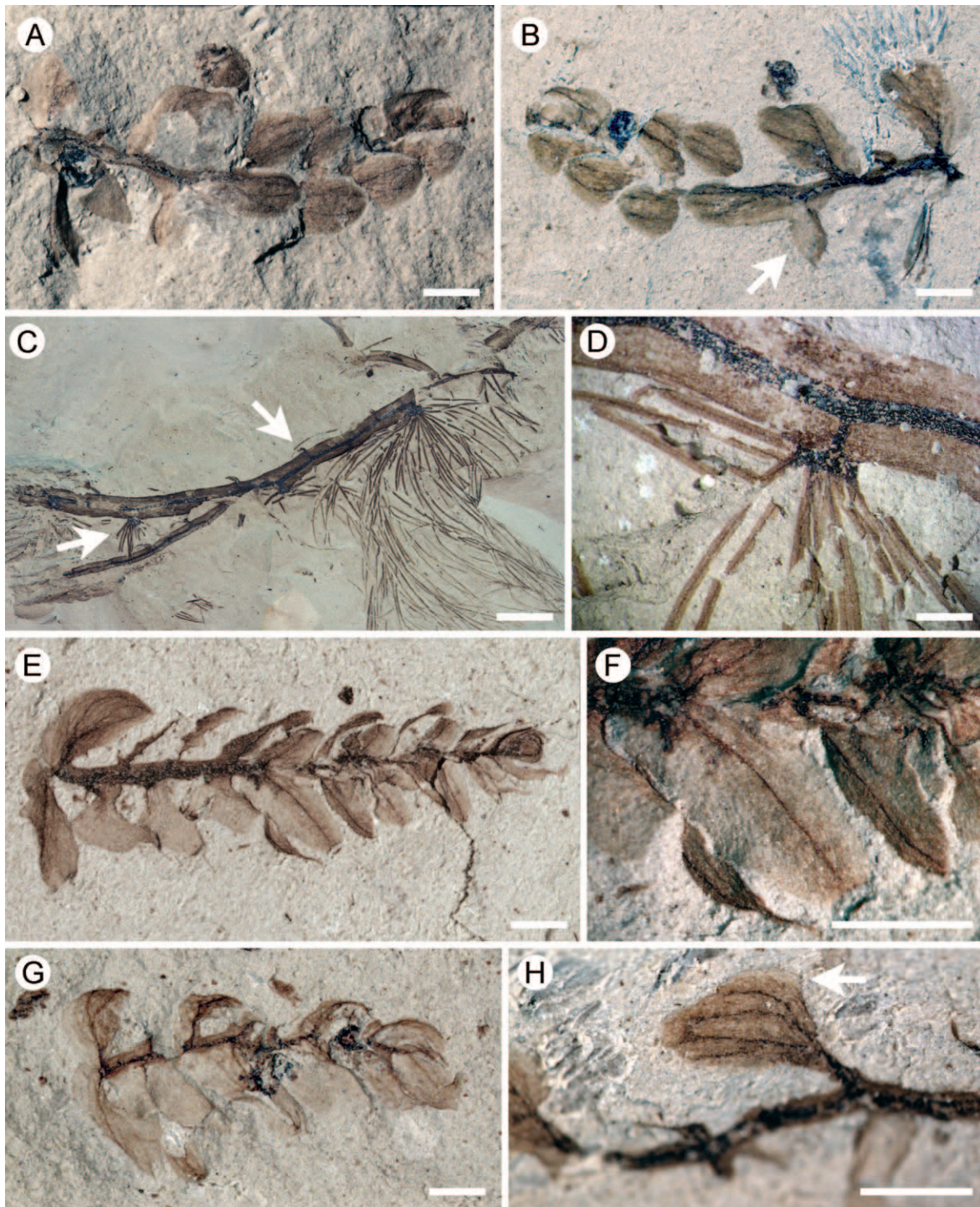
Fig. 4 *Azolla keuja* Jud, De Benedetti, Gandolfo & Hermsen, sp. nov., vegetative structures. *A*, Distal leafy shoot showing imbricate leaves and dichotomous venation of dorsal leaf lobes; MPEF-Pb 9717a (holotype). *B*, Distal leafy shoot showing imbricate leaves and dichotomous venation of the dorsal lobes. One leaf clearly shows the bilobed leaf structure; arrow indicates visible portion of the ventral lobe; MPEF-Pb 9717b (holotype). *C*, Robust stem with root fascicles along ventral side (lower arrow) and leaves attached to dorsal side (upper arrow); MPEF-Pb 9722a (paratype). *D*, Close-up of the root fascicle attached to the main stem, showing stem stele and diverging fascicle trace; MPEF-Pb 9711 (paratype). *E*, Distal shoot showing dorsal and ventral leaf lobes; MPEF-Pb 9716a (paratype). *F*, Close-up of specimen shown in *E*; detail of ventral leaf lobe with a single midvein; MPEF-Pb 9716a (paratype). *G*, Distal shoot showing dorsal and ventral leaf lobes; MPEF-Pb 9714a (paratype). *H*, Close-up of a dorsal leaf lobe showing dichotomous venation and hyaline margin (arrow); MPEF-Pb 9713 (paratype). Scale bars: *A, B* = 1.1 mm; *C* = 6 mm; *D–H* = 1 mm.
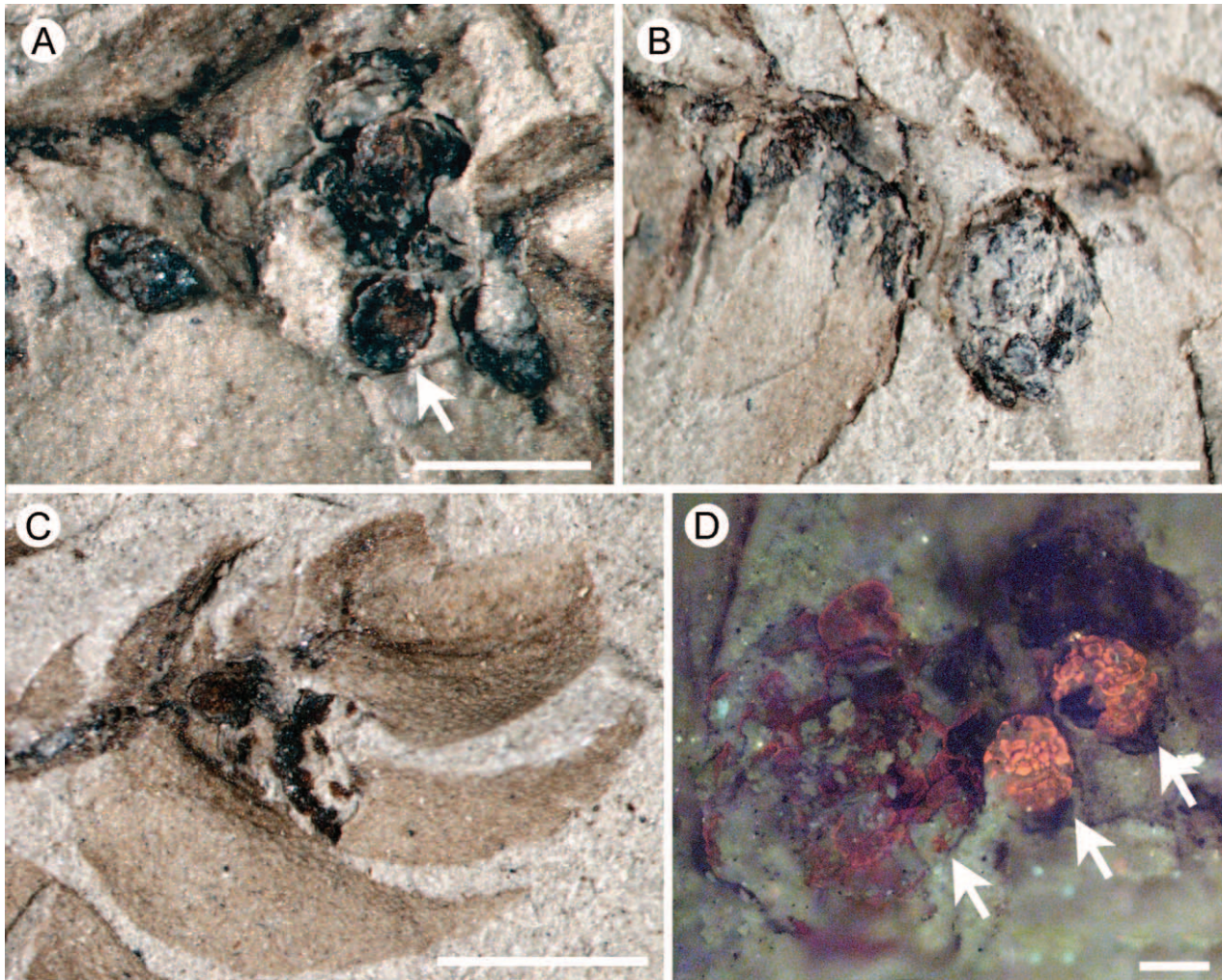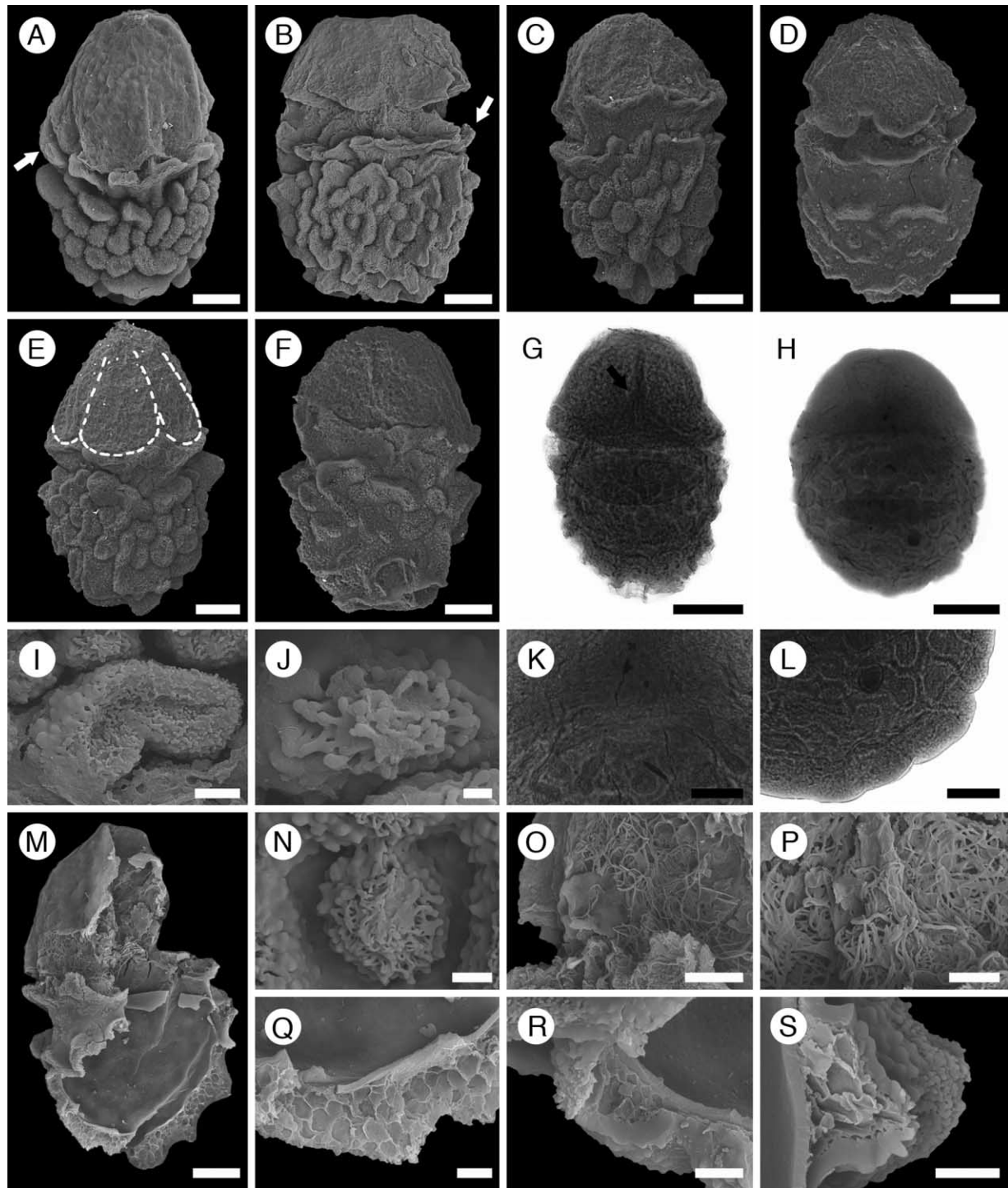
Fig. 5   *Azolla keuja* Jud, De Benedetti, Gandolfo & Hermsen, sp. nov., reproductive structures. *A*, Microsporangiate and megasporangiate (at arrow) sori attached to a distal stem fragment; MPEF-Pb 9714a (paratype). *B*, Microsporangiate sorus attached to distal stem fragment; MPEF-Pb 9712b (paratype). *C*, Shoot apex showing sori among the ventral leaf lobes; MPEF-Pb 9715a (paratype). *D*, Close-up of reproductive structures under epifluorescence (but not same structures shown in *B*). The perinal excrescences of two megasporangiate sori fluoresce brightly in the megasporangiate sori, indicated by the right two arrows, and the larger microsporangiate sorus fluoresces more dimly, indicated by the left arrow; MPEF-Pb 9712a (paratype). Scale bars: *A* = 0.5 mm; *B*, *C* = 1 mm; *D* = 200 μm.

specimens, *Paleoazolla* can readily be eliminated as corresponding to the La Colonia *Azolla* sporophytes. According to a recent reinvestigation of *P. patagonica* by De Benedetti et al. (2019*b*), *Paleoazolla* spores occur in heterosporangiate sori. Each heterosporangiate sorus includes an oblong megasporangium associated with a group of several microsporangia (fig. 3*I*; De Benedetti et al. 2019*b*), making it distinct from the ovoid megasporangiate sorus and roughly circular microsporangiate sorus found on the La Colonia Formation *Azolla* sporophytes (fig. 3*E*–3*G*).

Furthermore, there is correspondence between the dispersed spores of *A. coloniensis* and the sporangia on the La Colonia Formation *Azolla* sporophytes. The megasporangiate sori attached to *Azolla* sporophytes are ovoid in shape (fig. 3*C*, 3*E*), which corresponds to the shape of the *A. coloniensis* megaspore apparatuses (De Benedetti et al. 2018). The overall size of the megasporangiate sori (0.4–0.6 mm long by 0.2–0.4 mm wide) also corresponds to the size of dispersed *A. coloniensis* megaspore apparatuses (360–520 μm long by 260–360 μm wide; De Benedetti et al. 2018). The *A. coloniensis* microspore massulae are considered to have been produced by the La Colonia Formation *Azolla* sporophytes because dispersed *A. coloniensis* microspore massulae are found attached to, and thus correspond to, *A. coloniensis* megaspore apparatuses (see De Benedetti et al. 2018). The diameter of microsporangia found in situ on a sporophyte (up to ~190 μm; fig. 3*D*, 3*F*, 3*G*) is comparable to the diameter of dispersed *A. coloniensis* microsporangia (up to 195 μm; De Benedetti et al. 2018).

De Benedetti et al. (2018) presented the comparative spore morphology and justification for recognizing *A. coloniensis* as a distinct species of *Azolla* on the basis of its spore structure. *Azolla coloniensis* is typified by a megaspore apparatus,

Fig. 6 *Azolla keuja* Jud, De Benedetti, Gandolfo & Hermsen, sp. nov.; megaspore apparatuses. *A–F*, Megaspore apparatuses showing the float system covered by the indusial cap and the distal megaspore with perinal excrescences; arrows indicate the collar. *A*, MPEF-PA 1007. *B*, MPEF-PA 1008. *C*, MPEF-PA 1009. *D*, MPEF-PA 1009. *E*, Dotted line indicates the individual floats; MPEF-PA 1009. *F*, MPEF-PA 1009. *G, H*, Megaspore apparatuses showing the vacuolated structure of the floats, the central columella of the float system, and the distal megaspore. *G*, Note the thick perine and the columella (arrow); MPEF-PA 1002. *H*, MPEF-PA 1000. *I*, Excrescence with a central depression; MPEF-PA 1007. *J*, Excrescences showing the typical ornamentation pattern with fused granules at the apex; MPEF-PA 1007. *K*, Zone between the indusial cap and the megaspore; note the different types of excrescences; detail from *G*, MPEF-PA 1002. *L*, Thick perine and rounded appearance of the excrescences; detail from *H*, MPEF-PA 1000. *M*, Longitudinal section of a megaspore apparatus; MPEF-PA 1007. *N*, Excrescence showing the typical ornamentation pattern with fused granules at the apex; MPEF-PA 1008. *O*, Suprafilosum covering the vacuolated floats; detail from *M*, MPEF-PA 1007. *P*, Perinal hairs of the suprafilosum above the collar; detail from *B*, MPEF-PA 1008. *Q*, Section through mega-

MPEF-PA 80 (De Benedetti et al. 2018). This number refers to more than one megaspore apparatus mounted on a single SEM stub; two specimens from MPEF-PA 80 were figured with the original description of *A. coloniensis*, although neither one was clearly labeled as the holotype. Thus, we have here designated a lectotype to serve as the type for the species.

Azolla coloniensis megaspore apparatuses have 18 to 21 or more floats, and the microspore massulae have anchor-tipped glochidia (De Benedetti et al. 2018). *Azolla coloniensis* clearly falls outside of the circumscriptions of the modern sections *Azolla* and *Rhizosperma* due to the large numbers of floats on its megaspore apparatuses. The presence of anchor-tipped glochidia on its microspore massulae further excludes it from *Rhizosperma*. The combination of multifloated megaspore apparatuses and microspore massulae bearing anchor-tipped glochidia is considered diagnostic for the extinct section *Florschuetzia* (table 1). Therefore, *A. coloniensis* can be referred to this section along with about 12 other extinct species of *Azolla* documented from both megaspore apparatuses and microspore massulae in the Late Cretaceous to Eocene (see De Benedetti et al. 2018, table 15.1; note that *A. schopfii*, discussed below, was mistakenly included in that table).

Section—Unknown

Species—Azolla keuja *Jud, De Benedetti, Gandolfo, and Hermsen, sp. nov.* (Figs. 4–7)

Specific diagnosis. Larger sporophyte stems straight in course, glabrous, bearing widely spaced leaves and root fascicles. Distal shoots bearing closely spaced, imbricate leaves; each leaf deeply bilobed; dorsal leaf lobe elliptical with dichotomous venation, ventral leaf lobe elliptical with a single midvein, both lobes with a hyaline margin. Fertile sporophytes with megasporangiate sori or both mega- and microsporangiate sori borne laterally in groups of up to four. Megaspore apparatus oval to elliptical, surface of exoperine with tuberculate and rugulate excrescences, collar present; float apparatus thimble-shaped and covered by a persistent cap, inferred to be of six floats in a single tier; exoperine lacking hairs (i.e., infrafilosum absent), floats with hairs (i.e., suprafilosum present). Microspore massula with simple glochidia of spine-like or hairlike morphology; six or more massulae per microsporangium, 6–8 or more microspores per massula.

Holotype designated here. MPEF-Pb 9717a, b (fig. 4*A*, 4*B*). Held at the Museo Paleontológico Egidio Feruglio, Trelew, Chubut Province, Argentina.

Paratypes. MPEF-Pb 9710a, b; 9711; 9712a, b; 9713; 9714; 9715a, b; 9716a, b; 9718a, b; 9720a, b; 9721; 9722a, b, c; 9724.

Spore specimens examined. MPEF-PA 1000–1009.

Type locality. Palacio de los Loros-2 (PL-2), Chubut Province, Argentina (see Clyde et al. 2014; Comer et al. 2015).

*Stratigraphic position and age.* Salamanca Formation, Chron C28n, early Danian (early Paleocene).

Etymology. The specific epithet keuja comes from the Tehuelche word keuj, meaning before/past.

Description. *Azolla keuja* sporophytes are preserved as fragments of two different types: 1. relatively straight, robust stems bearing roots and few leaves and 2. leafy stems interpreted as distal shoots. The largest stems of *A. keuja* are fragments up to 5.85 cm long and characterized by long internodes, widely spaced dorsal leaves, and ventral root fascicles (fig. 4*C*, 4*D*); they vary from 0.6 mm to 2.4 mm wide with a coalified vascular cylinder 0.1 mm to 0.5 mm wide. No trichomes were observed, and stems are interpreted as glabrous. Distal shoots are preserved as unbranched fragments up to 10.6 mm long; these have thinner stems with short internodes and alternate, imbricate leaves occurring at a frequency of 5–7 leaves per linear cm (fig. 4*A*, 4*B*, 4*E*–4*H*). Leaves are sessile, deeply bilobed, and untoothed. Dorsal leaf lobes are elliptical in shape and 1.5–2.5 mm long (measured from the base of the leaf) by 0.9–1.3 mm wide (fig. 3*A*, 3*B*, 3*E*, 3*G*, 3*H*); ventral leaf lobes are elliptical in shape and 2.0–2.8 mm long and 1.0–1.6 mm wide (fig. 3*B*, 3*E*, 3*F*, 3*G*). Lobe apexes are obtuse and rounded and have a hyaline margin ~0.1 mm wide at the midpoint of the dorsal lobe (fig. 4*H*) and 0.2–0.6 mm wide at the midpoint of the ventral lobe (fig. 4*F*); the hyaline margin is wider toward the leaf base. Leaf venation is dichotomous. Each leaf trace diverges from the stem stele and bifurcates near the base of a leaf (fig. 4*B*), with one vein branch entering the ventral lobe and the other the dorsal lobe. In the dorsal lobe, the vein dichotomizes up to three times, producing two to four freely ending veins that widen slightly as they terminate just inside the margin (fig. 4*A*, 4*B*, 4*E*, 4*H*). In the ventral lobe, the vein is unbranched and runs down the middle of the lobe, terminating near the apex just inside the margin (fig. 4*E*, 4*F*). Roots are unbranched and borne in fascicles, with 9–23 roots per fascicle (fig. 4*C*, 4*D*). Fascicles are spaced 5.9–10.3 mm along the stem (fig. 4*C*). Roots are 0.2–0.3 mm wide, with a coalified vascular cylinder 0.03–0.06 mm wide (fig. 4*D*). Root fragments are up to 38.2 mm long. Root hairs were not observed.

The reproductive structures are borne on distal shoot fragments. Sori occur on short stalks in homosporangiate or heterosporangiate groups of 2–4, and they are associated with leaves (fig. 5). Megasporangiate sori are 250–410 μm long by 150–340 μm wide, whereas microsporangiate sori are 470–740 μm across.

The megaspore apparatus is oval to elliptical in outline, composed of a megaspore with a float system situated on its proximal pole (fig. 6*A*–6*H*). When observed on the sporophyte, the megaspore apparatus is oriented so that the megaspore is at the base of the megasporangiate sorus and the float system is near the apex (fig. 5*A*, 5*D*). The megaspore apparatus is 245–410 μm long (average = 313 μm, $n = 36$) by 147–340 μm wide (average = 216 μm, $n = 36$); the float system is 116–195 μm long (average = 148 μm, $n = 21$), and the megaspore is 135–260 μm wide (average = 198 μm, $n = 6$). The megaspore is spherical to subspherical when uncompressed, with a wall composed of an exine and a two-layered perine (fig. 6*R*). The mega-
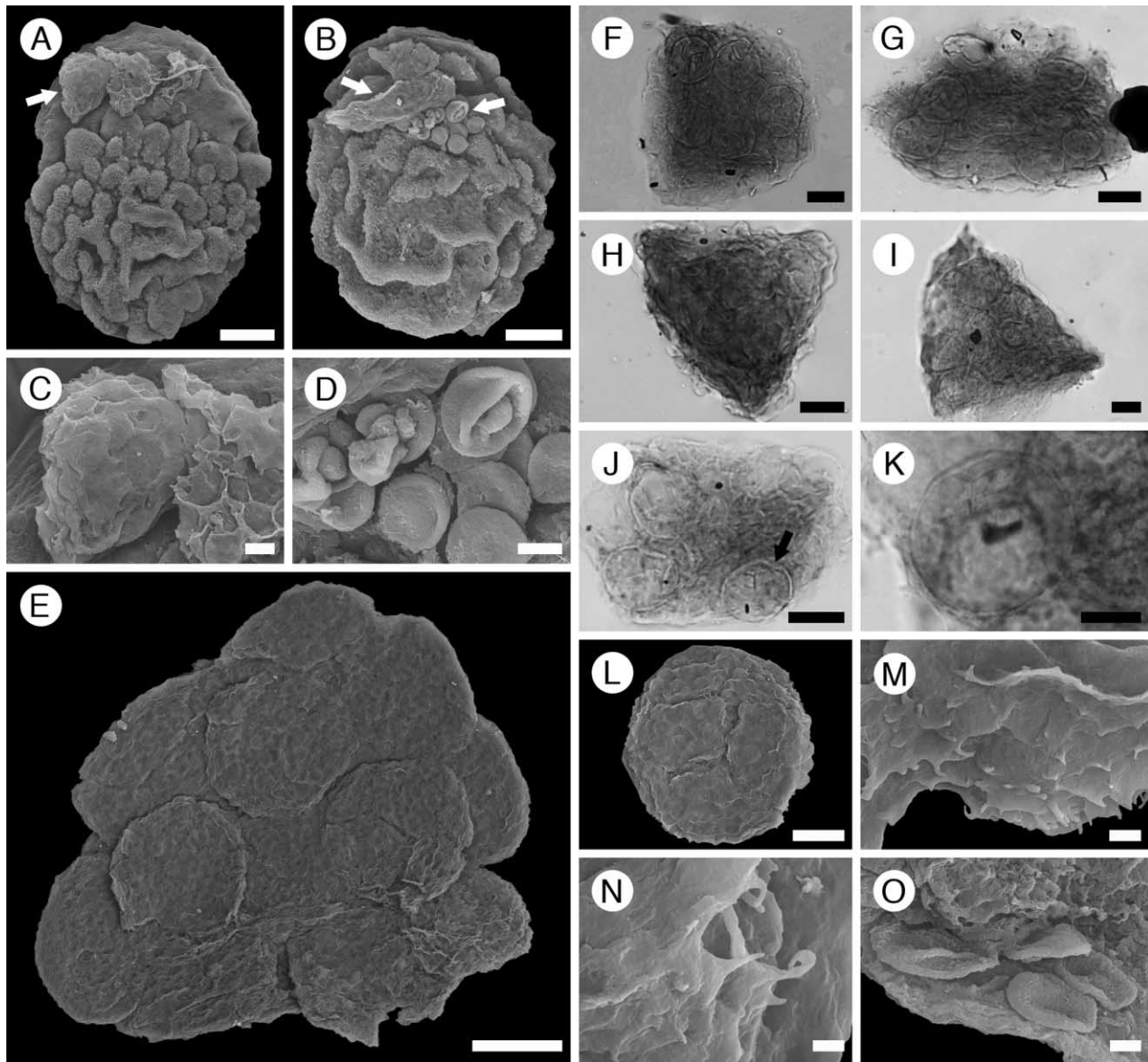
Fig. 7 *Azolla keuja* Jud, De Benedetti, Gandolfo & Hermsen, sp. nov., megaspore apparatuses, microsporangia, and microspore massulae. *A,* Megaspore apparatus with two microspore massulae attached (arrow); MPEF-PA 1007. *B,* Megaspore apparatus with microspores and microspore massulae attached (arrows); MPEF-PA 1007. *C,* Triangular microspore massulae showing the spine to hairlike glochidia (*left*) and the internal vacuolated structure (*right*) attached to a megaspore apparatus; detail from *A,* MPEF-PA 1007. *D,* Psilate to scabrate microspores; detail from *B,* MPEF-PA 1007. *E,* Cluster of microspore massulae thought to represent the partial contents of a microsporangiate sorus. Each spherical to ovoid subunit probably represents the contents of one microsporangium; MPEF-PA 1007. *F–J,* Microspore massulae showing shape, vacuolated structure, and sparse simple (spinose) glochidia. *F,* Irregularly shaped massula; MPEF-PA 1004. *G,* Elliptical massula; MPEF-PA 1003. *H,* Triangular massula; MPEF-PA 1003. *I,* Triangular massula; MPEF-PA 1005. *J,* Irregularly shaped massula; arrow indicates microspore with trilete mark; MPEF-PA 1003. *K,* Trilete microspore, MPEF-PA 1004. *L,* Cluster of microspore massulae interpreted as representing the contents of a microsporangium; MPEF-PA 1007. *M, N,* Details of the surface of microspore massulae showing irregularly distributed spinose to hairlike glochidia. *M,* MPEF-PA 1007. *N,* MPEF-PA 1007. *O,* Broken massula showing four psilate to scabrate microspores; detail from *E,* MPEF-PA 1007. SEM micrographs: *A–E, L–O;* LM micrographs: *F–K.* Scale bars: *A, B, L* = 50 μm; *C, D, K* = 10 μm; *E* = 100 μm; *F–J* = 20 μm; *M, O* = 5 μm; *N* = 2 μm.

spore surface is covered by numerous tuberculate and rugulate excrescences of spongy appearance; these are irregularly distributed and sometimes fused, forming conspicuous folds (figs. 5*D,* 6*A*–6*H*). The excrescences are highly variable in shape and size

(up to 65 μm in maximum width) and sometimes have a central depression (fig. 6*I*). The excrescences are ornamented with granules or verrucae (up to 5 μm in diameter) that usually grade to smaller (up to 1.2 μm diameter) and partially fused granules at

their apexes, sometimes forming an irregular reticulum (fig. 6I, 6J, 6N). Perinal hairs were not found on the surface of the megaspore; therefore, there is no infrafilosum.

Under light microscopy, the exine appears smooth and the spore body is easily distinguished from the perine (fig. 6G, 6H). The perine is thick, up to 60 μm, and composed of an endoperine and an exoperine, which are both involved in the formation of the excrescences (fig. 6I–6L, 6N). In sections under SEM (fig. 6M, 6Q–6S), the exine (up to 2.3 μm thick) and the exoperine are homogeneous, whereas the endoperine is very variable in thickness and has a vacuolated structure with alveoli up to 8 μm in diameter. The endoperine is more developed in the raised areas of the excrescences (fig. 6M, 6Q, 6S).

The float system occupies the proximal two-fifths to proximal half of the megaspore apparatus. The float apparatus is thimbleshaped and in all specimens analyzed was covered by a cap (fig. 6A–6H). A collar subtends the floats, although it is sometimes poorly defined. The collar originates from the perine in the proximal region of the megaspore (fig. 6A–6H) and appears to be formed by coalescence of the perinal excrescences. The floats have a vacuolated structure (fig. 6G, 6H) and are completely covered by the hairs (0.8–1.3 μm diameter) of the suprafilosum (fig. 6O, 6P). Some of these perinal hairs appear to be extensions of the small granules on the surface adjacent to the proximal part of the collar. The floats appear to be attached to a columella in the central region of the float zone (fig. 6G, 6H). The number of floats is uncertain, although six arranged in one tier can be inferred in some specimens based on impressions visible on the surface of the cap (fig. 6E–6G).

Microspore massulae have been found attached to the megaspore apparatuses (fig. 7A–7D). Microsporangiate sori contain at least 20 microsporangia (fig. 7E). Microsporangia are 142–250 μm in diameter (average $= 182$ μm, $n = 22$) and contain six or more massulae (fig. 7E, 7L). Massulae also occur in isolation (fig. 7F–7J). Massulae are triangular, elliptical, or irregular in outline and 67–147 μm (average $= 107$ μm, $n = 28$) in their longest dimension (fig. 7C, 7F–7J). The surface of the microspore massulae has spine-like (up to 3 μm long) to hairlike (up to 5 μm long) glochidia that are sparse and irregularly distributed (fig. 7C, 7M, 7N).

The massulae have a vacuolated structure, with each massula containing 6–8 or more microspores (fig. 7F–7J). The microspores are trilete, 15–30 μm (average $= 24$ μm, $n = 80$) in diameter, and have a psilate to scabrate exine (fig. 7D, 7O). The laesurae extend one-third to one-half the diameter of the spore (fig. 7J, 7K).

Remarks.   Because some *A. keuja* spores were lifted directly from the sporophytes, the spores and sporophytes unequivocally belong to the same taxon. Furthermore, the megaspore apparatuses with their distinctive folded perine were observed on a fertile *A. keuja* sporophyte viewed under epifluorescence (fig. 5D). Megaspore apparatuses of *A. keuja* have an apical cap. Although no floats have been directly observed as part of the apparatus, impressions on the cap suggest the occurrence of six elongated floats (fig. 6E–6G). The number of floats excludes *A. keuja* from all fossil and extant sections of *Azolla* (see table 1).

Discussion

The two Patagonian species described herein are represented by sporophytes having attached megasporangiate and microsporangiate sori and associated dispersed megaspore apparatuses and microspore massulae. Both species were found in the same general region (Chubut Province, Argentina), on either side of the Cretaceous-Paleogene (K-Pg) boundary. They are heterosporous ferns assigned to the genus *Azolla* based on sporophytes with simple and imbricate leaves, unbranched roots, and separate mega- and microsporangiate sori; megaspore apparatuses bearing a proximal float system; and microspores grouped into glochidiate massulae. Although it might be tempting to conclude that *A. coloniensis* and *A. keuja* provide evidence that a single lineage of *Azolla* survived the end-Cretaceous mass extinction in Patagonia, the species show significant differences from each other in sporophyte and spore structure (table 3). These differences suggest that they are not closely related.

The two species may also have been ecologically distinct. Although both were deposited in marginal marine environments, the paleocommunities that they inhabited were very different. *Azolla coloniensis* grew in freshwater lagoons occurring on coastal plains (Cúneo et al. 2014), whereas *A. keuja* was preserved in brackish tidal flats (Clyde et al. 2014; Comer et al. 2015). The La Colonia community is characterized by autochthonous taxa that are salt intolerant, indicating a low-energy freshwater environment with no seawater influence (Cúneo et al. 2014). These include freshwater green algae (e.g., *Botryococcus*, *Pediastrum*, and *Spirogyra*) and a macrofossil flora rich in aquatic to semiaquatic macrophytes, such as several types of water ferns (Salviniales), *Nelumbo*, and the extinct floating monocot *Aquaephyllum* (Gandolfo and Cúneo 2005; Cúneo et al. 2013, 2014; Gallego et al. 2014; Gandolfo et al. 2014; Hermsen et al. 2014). The Palacio de los Loros palynoflora includes freshwater algae (the green algae *Pediastrum* and Zygnemataceae)

Table 3

Comparison of Morphological Features of
Azolla coloniensis* and *A. keuja

Character description	*A. coloniensis*	*A. keuja*
Sporophyte:		
Longer/main stems	Slightly sinuous	Straight
Leaf shape	Obdeltoid, apex bilobed	Deeply bilobed, lobes elliptical
Root arrangement	Solitary, not in fascicles	In fascicles of 9–23
Sori per group	1–3	2–4
Megaspore apparatus:		
Shape	Ovoid	Oval to elliptical
Exoperine sculpture	Reticulate	With tuberculate and rugulate excrescences
Collar	Absent	Present
Floats	18–21 (or more) in 3–4 tiers	6 in 1 tier?
Cap	Absent	Present, covers floats
Infrafilosum	Present	Absent
Microspore massula:		
Massulae/microsporangium	~16	6 or more
Microspores per massula	~4	6–8 or more
Glochidia	Anchor-shaped ends	Hairlike or spine-like

Sources.   De Benedetti et al. (2018); this article.

and dinoflagellate cysts (Clyde et al. 2014); the macrofossil flora was dominated by terrestrial angiosperm taxa (Iglesias et al. 2007; Jud et al. 2018*a*, 2018*b*). Comer et al. (2015) interpreted the macrofossil flora of the Palacio de los Loros PL-2 locality, which yielded the *A. keuja* sporophyte fragments, as parautochthonous, representing vegetation occurring inland from the site of deposition and carried a short distance downstream.

Comparative Sporophyte Morphology of Azolla coloniensis

Apart from *A. coloniensis*, sporophyte organs are known for only three other species of *Azolla* that produced multifloated megaspore apparatuses: *A. schopfii* from the Late Cretaceous–Paleocene of North America (Sweet and Chandrasekharam 1973; McIver and Basinger 1993), *A. stanleyi* from the Paleocene of North America (Melchior and Hall 1983; Hoffman and Stockey 1994), and *A. velus* from the Paleocene of North America (McIver and Basinger 1993; however, see Batten and Collinson 2001 regarding the number of floats on the megaspore apparatuses). An additional unnamed species was also reported from the Wind River Formation of Wyoming (Collinson 1991; Stuart and Erwin 2006). Of these, *A. stanleyi*, *A. velus*, and the unnamed species also have microspore massulae with anchor-tipped glochidia; therefore, these species can be placed in section *Florschuetzia* (Jain and Hall 1969; Sweet and Hills 1976; McIver and Basinger 1993; Hoffman and Stockey 1994; Stuart and Erwin 2006). Like sporophytes of *A. coloniensis*, sporophytes of *A. stanleyi* and *A. velus* are relatively small (up to ca. 2.25 cm and 2.1 cm long, respectively) and show alternate branching; figured specimens show that at least some axes have a sinuous or zigzag course (McIver and Basinger 1993; Hoffman and Stockey 1994). The leaves of *A. coloniensis* and *A. stanleyi* are clearly imbricate; however, *A. stanleyi* differs from *A. coloniensis* in having a deltoid growth form and megasporangiate sori that occur frequently on lateral branches (Hoffman and Stockey 1994). *Azolla velus* differs from *A. coloniensis* in having relatively long lateral branches and in lacking imbricate leaves (McIver and Basinger 1993). Sporophytes of the unnamed Wind River *Azolla* are much larger than those of *A. coloniensis* (6 cm or more in length), and sori occur throughout the lateral branches (Stuart and Erwin 2006; see also fig. 7.6*g* in Collinson 1991).

Comparative Morphology of Azolla keuja

Most extant species of *Azolla* are small, with sporophytes ranging from less than 1 cm up to ca. 4 cm long (e.g., Sweet and Hills 1971; Saunders and Fowler 1992; Lumpkin 1993); several fossil species are also known from similarly small specimens (Brown 1934; Hills and Gopal 1967; Bůžek et al. 1988; McIver and Basinger 1993; Hoffman and Stockey 1994). The robust nature of *A. keuja* axes suggests that whole sporophytes were much larger than their maximum observed length of 5.8 cm (fig. 4*C*). The only extant species with similarly large sporophytes is *A. nilotica* (section *Rhizosperma*), which can be up to 40 cm long (Saunders and Fowler 1992). Other fossil species represented by sporophyte fragments up to 5 cm or more in length include *A. schopfii* from the Late Cretaceous–Paleocene of North America (Sweet and Chandrasekharam 1973), *A. vera* from the Paleogene of Russia (Krys-

tofovich 1952), and an undescribed species from the Eocene Wind River Formation of Wyoming (Stuart and Erwin 2006). Sweet and Chandrasekharam (1973) estimated that whole sporophytes of *A. schopfii* could have reached 15 cm in length, Krystofovich (1952) estimated that *A. vera* sporophytes may have reached 8 to 10 cm long, and the unnamed Wind River *Azolla* species is minimally 6 cm long (Stuart and Erwin 2006).

Azolla keuja is characterized by deeply bilobed leaves with dorsal (upper) and ventral (lower) lobes (fig. 6*E*), similar to extant species of *Azolla* (fig. 1*B*; Strasburger 1873; Peters et al. 1978; Schneller 1990). In extant species of *Azolla*, the dorsal leaf lobe is chlorophyllous and has a single vein that encircles a cavity harboring the cyanobacterial symbiont *Nostoc* (or *Anabaena*) *azollae* (fig. 1*B*; Strasburger 1873; Demalsy 1953; Peters et al. 1978; Lumpkin and Plucknett 1980; Eily et al. 2019). The ventral leaf lobe is typically thinner than the dorsal lobe, has a rounded and obtuse apex, and is achlorophyllous except near its base (Lumpkin and Plucknett 1980; Schneller 1990; Saunders and Fowler 1992). In *A. nilotica*, however, the ventral leaf lobe is more robust, has an acuminate apex, a central chlorophyllous zone, and a broad hyaline (i.e., achlorophyllous) margin (fig. 1*B*; Demalsy 1953; Saunders and Fowler 1992, 1993). *Azolla keuja* differs from all living species in that the venation of its dorsal leaf lobe is dichotomous (fig. 4*A*, 4*B*, 4*E*, 4*G*, 4*H*), as also occurs in the fossil species *A. schopfii* (Sweet and Chandrasekharam 1973, see especially their fig. 6). The ventral leaf lobe in both *A. keuja* (figs. 4*E*–4*G*, 5*C*) and *A. schopfii* (Sweet and Chandrasekharam 1973; table 2 in Saunders and Fowler 1993) is structurally similar to the ventral leaf lobe in *A. nilotica* (fig. 1*B*). Saunders and Fowler (1992, 1993) suggested that the unusually robust chlorophyllous ventral leaf lobe of *A. nilotica* is linked to the relatively ascendant form of its sporophyte, which exposes the ventral leaf lobe to sunlight.

Fascicled roots are documented only in the extant species *A. nilotica* and the fossil species *A. keuja* and *A. schopfii* (fig. 4*C*, 4*D*; Sweet and Chandrasekharam 1973; Saunders and Fowler 1992, 1993). All other extant *Azolla* have roots borne singly (fig. 1*A*; Saunders and Fowler 1992, 1993), as do other fossil species in which this character is documented (e.g., *Azolla primaeva*; Hills and Gopal 1967). Saunders and Fowler (1992) reported that *A. nilotica* plants may have up to 10 roots per fascicle, but we observed many more tangled roots per fascicle on some herbarium sheets (N. A. Jud, personal observation), and Singh and Singh (1987) reported up to 20. *Azolla schopfii* specimens have eight to 15 roots per fascicle, although sometimes fewer (Sweet and Chandrasekharam 1973). *Azolla keuja* has nine to 23 roots per fascicle (fig. 4*C*, 4*D*).

Azolla keuja sporophytes are unusual in that they bear their sori in groups of up to four. Most extant species of *Azolla* produce their sori in pairs, although *A. nilotica* produces them in tetrads (Saunders and Fowler 1992, 1993). Most other fossil species in which this character has been documented have a maximum of two or three sori per group (e.g., *A. stanleyi*: Hoffman and Stockey 1994), although the Miocene taxon *Azolla* aff. *A. ventricosa* also has sori in groups of up to four (Bůžek et al. 1988; number of sori per group is unknown in *A. schopfii*).

Despite the marked morphological similarities in sporophyte structure among *A. keuja*, *A. schopfii*, and *A. nilotica*, the three species have differences in the structure of their megaspore apparatuses. The megaspore apparatus of *A. keuja* has a poorly

defined collar and a persistent cap that covers the floats (fig. 6A–6H); we infer that at least six floats occur in a single tier based on float impressions seen on the cap (fig. 6E). The megaspore apparatus of *A. nilotica* has nine floats in two tiers (an apical tier of three floats and a second tier of six floats), a short cap, and a collar (Martin 1976; Saunders and Fowler 1993). The megaspore apparatus of *A. schopfii* has 15 to 26 floats in three tiers, no cap, and no collar (Dijkstra 1961 as *Triletes schopfii*; Sweet and Chandrasekharam 1973; McIver and Basinger 1993; Batten and Collinson 2001). The structure of the megaspore apparatus in *A. keuja* is unique among fossil and extant *Azolla* both in the number and arrangement of the floats and ornamentation of the spore body.

Azolla keuja has microspore massulae with spinose or hairlike glochidia (fig. 7C, 7M, 7N). Most *Azolla* species have glochidia with anchor-shaped tips on their microspore massulae; those that do not may have coiled, filamentous/hairlike, or spinose glochidia (table 1; see also, e.g., Collinson 1980; Saunders and Fowler 1992). Extant members of *Azolla* section *Rhizosperma* (*A. nilotica* and *A. pinnata*) have spinose or twisted glochidia that lack anchor-shaped ends, although glochidia may be absent on massulae of *A. nilotica* (Saunders and Fowler 1992, 1993; Metzgar et al. 2007). Fossil *Azolla* species characterized by microspore massulae lacking anchor-tipped glochidia first appear in the Late Cretaceous (e.g., Hall 1968; Collinson 1980; Vajda and McLoughlin 2005). Some such fossil species (e.g., *A. nana*, *A. turgaica*, *A. ventricosa*) are assigned to section *Rhizosperma* (Dorofeev 1959; Friis 1977). Those having filamentous glochidia with circinate tips (e.g., *A. circinata*) are assigned to the fossil section *Filifera* (Hall 1968). Others, like *A. keuja* and *A. schopfii*, cannot be assigned to a section.

Concluding Remarks

Azolla and its sister taxon, *Salvinia*, are the only living genera of the floating aquatic fern family Salviniaceae, which is within Salviniales, a broader group of heterosporous ferns (e.g., Smith et al. 2006; Nagalingum et al. 2008; PPG I 2016). The fossil record of *Azolla*, which begins in the Cretaceous, demonstrates that the lineage including extant *Azolla* diverged from the *Salvinia* lineage no later than the Late Cretaceous (e.g., Pryer 1999) and that *Azolla* was widely distributed by the end of the Cretaceous (Batten and Kovach 1990; Vajda and McLoughlin 2005). Several additional dispersed spore genera from the Late Cretaceous and Paleogene, such as *Ariadnaesporites*, *Azollopsis*, *Glomerisporites*, *Hallisporites*, *Paleoazolla*, and *Parazolla*, may also belong to the *Azolla* stem lineage or elsewhere in the phylogeny of Salviniales (Hall 1969b, 1975; Collinson 1991, 1992, 1996; Batten et al. 1998; Archangelsky et al. 1999; Nowak and Lupia 2005). Unfortunately, the majority of fossil *Azolla* species and species within these putative stem genera are known solely from their spores, and only in exceptional cases are spores linked to sporophyte material. For example, *Parazolla* spores are known to have been produced by the extinct taxon *Hydropteridium pinnatum* (G.W. Rothwell & Stockey) Hermsen & Jud, comb. nov. (see app. B), through discovery of *Parazolla*-type spores in the sporocarps of the *Hydropteridium* plant (Rothwell and Stockey 1994). Phylogenetic analyses suggest that *H. pinnatum* may be a stem taxon to crown Salviniaceae (*Azolla* and *Salvinia*) or crown Salviniales (Rothwell

and Stockey 1994; Pryer 1999; Rothwell 1999; Yamada and Kato 2002; Rothwell and Nixon 2006). This case highlights the importance of organismal concepts based on both sporophyte and spore material for understanding the diversity and evolution of the heterosporous aquatic fern clade (see also, e.g., Collinson 1991, 1996; Nagalingum 2007; Hermsen et al. 2014).

The fossil record of *Azolla* now includes 11 species known from vegetative structures (stems, leaves, and/or roots) and spores, and another three known only from sporophyte material (table 2). The Patagonian species *A. coloniensis* and *A. keuja* are the first fossil records of *Azolla* that represent whole plants from the Southern Hemisphere (fig. 2) and, as such, substantially enrich our knowledge of the past worldwide diversity of the genus. Their occurrences on either side of the Cretaceous-Paleogene boundary are congruent with the hypothesis of a major filtering of aquatic fern diversity across the K-Pg boundary, wherein *Azolla* and other extant genera crossed the boundary, whereas most fossil spore genera (e.g., *Paleoazolla*) did not (Collinson 2001; Collinson et al. 2013). Nevertheless, the Patagonian *Azolla* are apparently unrelated species that occurred in roughly the same geographic region on opposite sides of the K-Pg boundary. The Late Cretaceous species *Azolla coloniensis* is typical of the archaic Late Cretaceous to Eocene species of *Azolla* in the morphology of its megaspore apparatus, which is many floated (De Benedetti et al. 2018). It is one of only a few fossil species with many-floated megaspore apparatuses that is also documented from sporophytes preserving vegetative structures. The Paleocene species *A. keuja* has a unique combination of spore and sporophyte character states that make both its classification and affinities difficult to ascertain, although it shows some intriguing similarities to the African species *A. nilotica* and the Late Cretaceous to Paleocene North American species *A. schopfii*.

Molecular phylogenetic studies of *Azolla* support a simple model of relationships in which the sections *Azolla* and *Rhizosperma* are monophyletic sister groups defined by several discrete spore and sporophyte characters (Reid et al. 2006; Metzgar et al. 2007). This model does not consider the diversity of fossil species, meaning that it neglects the vast majority of morphological information available pertinent to the evolution of *Azolla*. Although the fossil sections capture some of this additional diversity, some *Azolla* fossils, like *A. keuja*, cannot be accommodated even within this expanded taxonomy. Furthermore, the fossil sections are likely artificial and may not be monophyletic groups (Batten and Collinson 2001). Few attempts have been made to include fossil species as terminals in phylogenetic analyses (see, however, Saunders and Fowler 1993; Stuart and Erwin 2006). In the future, fossil *Azolla* species and extinct stem taxa, particularly those that are known from both sporophytes and spores, must be incorporated directly into phylogenetic hypotheses so that we can arrive at a robust understanding of infrageneric relationships, character polarity, and character evolution in *Azolla*.

Acknowledgments

Literature Cited

Akhmetiev MA, NI Zaporozhets, VN Benyamovskiy, GN Aleksandrova, AI Iakovleva, TV Oreshkina 2012 The Paleogene history of the Western Siberian seaway: a connection of the Peri-Tethys to the Arctic Ocean. Austrian J Earth Sci 105:50–67.

Andruchow-Colombo A, IH Escapa, RJ Carpenter, RS Hill, A Iglesias, AM Abarzua, P Wilf 2019 Oldest record of the scale-leaved clade of Podocarpaceae, early Paleocene of Patagonia, Argentina. Alcheringa 43:127–145.

Archangelsky A, CJ Phipps, TN Taylor, EL Taylor 1999 *Paleoazolla*, a new heterosporous fern from the Upper Cretaceous of Argentina. Am J Bot 86:1200–1206.

Arnold CA 1955 A Tertiary *Azolla* from British Columbia. Contrib Mus Paleontol Univ Mich 12:37–45.

Askin RA 1989 Endemism and heterochroneity in the Late Cretaceous (Campanian) to Paleocene palynofloras of Seymour Island, Antarctica: implications for origins, dispersal, and palaeoclimates of southern floras. Pages 107–119 in JA Crame, ed. Origins and evolution of the Antarctic biota. Geol Soc Spec Publ 47. Geological Society, London.

——— 1990 Cryptogam spores from the Upper Campanian and Maastrichtian of Seymour Island, Antarctica. Micropaleontology 36:141–156.

Aulenback KR 2009 Identification guide to the fossil plants of the Horseshoe Canyon Formation of Drumheller, Alberta. University of Calgary Press, Alberta.

Batten DJ, ME Collinson 2001 Revision of species of *Minerisporites*, *Azolla*, and associated plant microfossils from deposits of the Upper Palaeocene and Palaeocene/Eocene transition in the Netherlands, Belgium, and the USA. Rev Palaeobot Palynol 115:1–32.

Batten DJ, ME Collinson, APR Brain 1998 Ultrastructural interpretation of the Late Cretaceous megaspore *Glomerisporites pupus* and its associated microspores. Am J Bot 85:724–735.

Batten DJ, WL Kovach 1990 Catalog of Mesozoic and Tertiary megaspores. AASP Contributions Series, no. 24. American Association of Stratigraphic Palynologists Foundation, Dallas.

Berry EW 1927 The flora of the Esmeralda Formation in western Nevada. Proc US Natl Mus 72:1–15.

Brinkhuis H, S Schouten, ME Collinson, A Sluijs, JS Sinninghe Damsté, GR Dickens, M Huber, et al 2006 Episodic fresh surface waters in the Eocene Arctic Ocean. Nature 441:606–609.

Brown RW 1934 The recognizable species of the Green River flora. Prof Pap US Geol Surv 185–C:45–77.

Bůžek Č, M Konzalová, Z Kvaček 1988 *Azolla* remains from the lower Miocene of the North-Bohemian Basin, Czechoslovakia. Tert Res 9:117–132.

Carrapiço F 2010 *Azolla* as a superorganism: its implication in symbiotic studies. Pages 225–241 in J Seckbach, M Grube, eds. Symbioses and stress: joint ventures in biology: cellular origin, life in extreme habitats and astrobiology, vol 17. Springer, Dordrecht.

Clyde WC, P Wilf, A Iglesias, RL Slingerland, T Barnum, PK Bijl, TJ Bralower, et al 2014 New age constraints for the Salamanca Formation and lower Río Chico Group in the western San Jorge Basin, Patagonia, Argentina: implications for Cretaceous-Paleogene extinction recovery and land mammal age correlations. Bull Geol Soc Am 126:289–306.

Collinson ME 1980 A new multiple-floated *Azolla* from the Eocene of Britain with a brief review of the genus. Palaeontology 23:213–229.

——— 1991 Diversification of modern heterosporous pteridophytes. Pages 119–150 in S Blackmore, SH Barnes, eds. Pollen and spores. Systematics Association, special volume no. 44. Clarendon Press, Oxford.

——— 1992 The Late Cretaceous and Palaeocene history of salvinialean water ferns. Pages 121–127 in J Kovar-Eder, ed. Palaeovegetational development in Europe and regions relevant to its palaeofloristic evolution. Proceedings of the Pan-European Palaeobotanical Conference, Vienna, September 15–23, 1991. Museum of Natural History, Vienna.

——— 1996 "What use are fossil ferns?"–20 years on: with a review of the fossil history of extant pteridophyte families and genera. Pages 349–394 in JM Camus, M Gibby, RJ Johns, eds. Pteridology in perspective. Royal Botanic Gardens, Kew, United Kingdom.

——— 2001 Cainozoic ferns and their distribution. Brittonia 53:173–235.

Collinson ME, J Barke, J van der Burgh, JHA van Konijnenburg-van Cittert 2009 A new species of the freshwater fern *Azolla* (Azollaceae) from the Eocene Arctic Ocean. Rev Palaeobot Palynol 155:1–14.

Collinson ME, SY Smith, JHA van Konijnenburg-van Cittert, DJ Batten, J van der Burgh, J Barke, F Marone 2013 New observations and synthesis of Paleogene heterosporous water ferns. Int J Plant Sci 174:350–363.

Collinson ME, JHA van Konijnenburg-van Cittert, F Marone, APR Brain 2017 Reinterpretation of *Azolla primaeva* (Azollaceae, Eocene, Canada) using electron microscopy and x-ray tomographic microscopy. Rev Palaeobot Palynol 240:33–48.

Comer EE, RL Slingerland, JM Krause, A Iglesias, WC Clyde, MS Raigemborn, P Wilf 2015 Sedimentary facies and depositional environments of diverse early Paleocene floras, north-central San Jorge Basin, Patagonia, Argentina. Palaios 30:553–573.

Cúneo NR, MA Gandolfo, MC Zamaloa, E Hermsen 2014 Late Cretaceous aquatic plant world in Patagonia, Argentina. PLoS ONE 9 (8):e104749. doi:10.1371/journal.pone.0104749.

Cúneo NR, EJ Hermsen, MA Gandolfo 2013 *Regnellidium* (Salviniales, Marsileaceae) macrofossils and associated spores from the Late Cretaceous of South America. Int J Plant Sci 174:340–349.

Dawson JW 1890 On fossil plants from the Similkameen Valley and other places in the southern interior of British Columbia. Trans R Soc Can 8:75–91.

De Benedetti F, MC Zamaloa, MA Gandolfo, NR Cúneo 2018 Heterosporous ferns from Patagonia: the case of *Azolla*. Pages 361–373 in M Krings, CJ Harper, NR Cúneo, GW Rothwell, eds. Transformative paleobotany. Academic Press, London.

——— 2019a Diversification of heterosporous water ferns during the Late Cretaceous of Patagonia. Abstract ID 261. Botany 2019, Tuscon, Arizona, July 27–31, 2019.

——— 2019b Late Cretaceous heterosporous water ferns from Patagonia: assessing heterochrony and monomegaspory. Abstract ID 117. Botany 2019, Tuscon, Arizona, July 27–31, 2019.

Demalsy P 1953 Le sporophyte d'*Azolla nilotica*. Cellule 56:5–60.

Dijkstra SJ 1961 Some Paleocene megaspores and other small fossils. Meded Geol Sticht, NS, 13:5–11.

Dillhoff RM, TA Dillhoff, DR Greenwood, ML DeVore, KB Pigg 2013 The Eocene Thomas Ranch flora, Allenby Formation, Princeton, British Columbia, Canada. Botany 91:514–529.

Dorofeev PI 1959 O novykh vidakh *Azolla* Lam. dlja tretichnoj flory SSSR. Bot Zhurn 44:1756–1763.

——— 1963 Tretichnye flory Zapadnoi Sibiri. Akademiya Nauk SSSR, USSR. English translation of pages 62–80, 88–90 by MHW, Department of the Secretary of State Translation Bureau, Multilingual Services Division, Canada, 1975.

Doweld A 2001 Prosyllabus Tracheophytorum. GEOS, Moscow.

Eily AN, KM Pryer, F-W Li 2019 A first glimpse at genes important to the *Azolla–Nostoc* symbiosis. Symbiosis. doi:10.1007/s13199-019-00599-2.

Escapa IH, A Iglesias, P Wilf, SA Catalano, MA Caraballo-Ortiz, NR Cúneo 2018 *Agathis* trees of Patagonia's Cretaceous-Paleogene death landscapes and their evolutionary significance. Am J Bot 105:1345–1368.

Follieri M 1977 Classification and phylogeny of living and fossil water ferns of the genus *Azolla*. Webbia 31:97–104.

Foster AS, EM Gifford Jr 1974 Comparative morphology of vascular plants. 2nd ed. WH Freeman, San Francisco.

Fowler K 1975 Megaspores and massulae of *Azolla prisca* from the Oligocene of the Isle of Wight. Palaeontology 18:483–507.

Fowler K, J Stennett-Willson 1978 Sporoderm architecture in modern *Azolla*. Fern Gaz 11:405–412.

Friis EM 1977 EM-studies on Salviniaceae megaspores from the Middle Miocene Fasterholt Flora, Denmark. Grana 16:113–128.

Gallego J, MA Gandolfo, NR Cúneo, MC Zamaloa 2014 Fossil Araceae from the Upper Cretaceous of Patagonia, Argentina, with implications on the origin of free-floating aquatic aroids. Rev Palaeobot Palynol 211:78–86.

Gandolfo MA, RN Cúneo 2005 Fossil Nelumbonaceae from the La Colonia Formation (Campanian–Maastrichtian, Upper Cretaceous), Chubut, Patagonia, Argentina. Rev Palaeobot Palynol 133:169–178.

Gandolfo MA, NR Cúneo, EJ Hermsen 2014 Reporte preliminar sobre la paleoflora de la Formación La Colonia (Campaniano-Maastrichtiano, Cretácico tardío), Chubut, Patagonia, Argentina. Bol Soc Geol Mex 66:11–23.

Grande L 2013 The lost world of Fossil Lake: snapshots from deep time. University of Chicago Press, Chicago.

Greenwood DR, KB Pigg, ML DeVore 2016 Eocene paleontology and geology of western North America. Can J Earth Sci 53:543–547.

Hall JW 1968 A new genus of Salviniaceae and a new species of *Azolla* from the Late Cretaceous. Am Fern J 58:77–88.

——— 1969a A reappraisal of the megaspores of two Eocene species of *Azolla*. J Paleontol 43:528–531.

——— 1969b Studies on fossil *Azolla*: primitive types of megaspores and massulae from the Cretaceous. Am J Bot 56:1173–1180.

——— 1970 A new section of *Azolla*. Taxon 19:302–303.

——— 1975 *Ariadnaesporites* and *Glomerisporites* in the Late Cretaceous: ancestral Salviniaceae. Am J Bot 62:359–369.

Hall JW, NP Swanson 1968 Studies on fossil *Azolla*: *Azolla montana*, a Cretaceous megaspore with many small floats. Am J Bot 55:1055–1061.

Hall SA 1977 Cretaceous and Tertiary dinoflagellates from Seymour Island, Antarctica. Nature 267:239–241.

Hayes PA, ME Collinson 2014 The flora of the Insect Limestone (latest Eocene) from the Isle of Wight, southern England. Earth Environ Sci Trans R Soc Edinb 104:245–261.

Hermsen EJ, MA Gandolfo, NR Cúneo 2014 New marsileaceous fossils from the Late Cretaceous of South America and a reevaluation of *Marsileaceaephyllum*. Plant Syst Evol 300:369–386.

Hills LV, B Gopal 1967 *Azolla primaeva* and its phylogenetic significance. Can J Bot 45:1179–1191.

Hills LV, N Weiner 1965 *Azolla geneseana*, n. sp., and revision of *Azolla primaeva*. Micropaleontology 11:255–261.

Hoffman GL, RA Stockey 1994 Sporophytes, megaspores, and massulae of *Azolla stanleyi* from the Paleocene Joffre Bridge locality, Alberta. Can J Bot 72:301–308.

IFPNI International Editorial Board 2014–2018 The International Fossil Plant Names Index. http://www.fossilplants.info.

Iglesias A, P Wilf, KR Johnson, AB Zamuner, NR Cúneo, SD Matheos, BS Singer 2007 A Paleocene lowland macroflora from Patagonia reveals significantly greater richness than North American analogs. Geology 35:947–950.

Jain RK 1971 Pre-Tertiary records of Salviniaceae. Am J Bot 58:487–496.

Jain RK, JW Hall 1969 A contribution to the Early Tertiary fossil record of the Salviniaceae. Am J Bot 56:527–539.

Jud NA, MA Gandolfo, A Iglesias, P Wilf 2018a Fossil flowers from the early Palaeocene of Patagonia, Argentina, with affinity to Schizomerieae (Cunoniaceae). Ann Bot 121:431–442.

Jud NA, A Iglesias, P Wilf, MA Gandolfo 2018b Fossil moonseeds from the Paleogene of West Gondwana (Patagonia, Argentina). Am J Bot 105:927–942.

Kempf EK 1968 Wasserfarne im rheinischen Braunkohlentertiär und ihr stratigraphischer Wert. Z Dtsch Geol Ges 118:161.

Kovach WL, DJ Batten 1989 Worldwide stratigraphic occurrences of Mesozoic and Tertiary megaspores. Palynology 13:247–277.

Kryshtofovich AN 1952 Vodjanoj paporotnik *Azolla* v tretichnykh otlozhenijakh Sibiri. Pages 190–197 in BP Markovsky, ed. Paleontologia i stratigrafija. Gosgeolizdat, Moscow.

Lumpkin TA 1993 Azollaceae Wettstein. *In* Flora of North America Editorial Committee, ed. Flora of North America North of Mexico, vol 2. New York. http://efloras.org/.

Lumpkin TA, DL Plucknett 1980 *Azolla*: botany, physiology, and use as a green manure. Econ Bot 34:111–153.

Martin ARH 1976 Some structures in *Azolla* megaspores, and an anomalous form. Rev Palaeobot Palynol 21:141–169.

Mathewes RW, DR Greenwood, SB Archibald 2016 Paleoenvironment of the Quilchena flora, British Columbia, during the Early Eocene Climatic Optimum. Can J Earth Sci 53:574–590.

McIver EE, JF Basinger 1993 Flora of the Ravenscrag Formation (Paleocene), southwestern Saskatchewan, Canada. Palaeontographica Canadiana 10. Canadian Society of Petroleum Geologists, Geological Association of Canada, Calgary.

Melchior RC, JW Hall 1983 Some megaspores and other small fossils from the Wannagan Creek site (Paleocene), North Dakota. Palynology 7:133–145.

Mettenius G 1847 Ueber *Azolla*. Linnaea 20:259–282.

Metzgar JS, H Schneider, KM Pryer 2007 Phylogeny and divergence time estimates for the fern genus *Azolla* (Salviniaceae). Int J Plant Sci 168:1045–1053.

Monje-Dussán C, C Martínez, I Escapa, S Madriñán 2016 Nuevos registros de helechos y coníferas del Cretácico Inferior en la cuenca del Valle Superior del Magdalena, Colombia. Bol Geol 38:29–42.

Nagalingum NS 2007 *Marsileaceaephyllum*, a new genus for marsileaceous macrofossils: leaf remains from the Early Cretaceous (Albian) of southern Gondwana. Plant Syst Evol 264:41–55.

Nagalingum NS, MD Nowak, KM Pryer 2008 Assessing phylogenetic relationships in extant heterosporous ferns (Salviniales), with a focus on *Pilularia* and *Salvinia*. Bot J Linn Soc 157:673–685.

Nagalingum NS, H Schneider, KM Pryer 2006 Comparative morphology of reproductive structures in heterosporous water ferns and a reevaluation of the sporocarp. Int J Plant Sci 167:805–815.

Nambudiri EMV, S Chitaley 1991 Fossil *Salvinia* and *Azolla* from the Deccan Intertrappean beds of India. Rev Palaeobot Palynol 69:325–336.

Nowak MD, R Lupia 2005 Reinvestigation of *Ariadnaesporites varius* Hall and Peake, 1968 emend. Hall, 1975. Rev Palaeobot Palynol 135:41–59.

Pascual R, FJ Goin, P González, A Ardolino, PF Puerta 2000 A highly derived docodont from the Patagonian Late Cretaceous: evolutionary implications for Gondwanan mammals. Geodiversitas 22:395–414.

Pereira AL, M Martins, MM Oliveira, F Carrapiço 2011 Morphological and genetic diversity of the family Azollaceae inferred from vegetative characters and RAPD markers. Plant Syst Evol 297:213–226.

Pérez-Consuegra N, A Cuervo-Gómez, C Martínez, C Montes, F Herrera, S Madriñán, C Jaramillo 2017 Paleogene *Salvinia* (Salviniaceae) from Colombia and their paleobiogeographic implications. Rev Palaeobot Palynol 246:85–108.

Peters GA, RE Toia Jr, D Raveed, NJ Levine 1978 The *Azolla-Anabaena azollae* relationship. New Phytol 80:583–593.

PPG I (The Pteridophyte Phylogeny Group) 2016 A community-derived classification for extant lycophytes and ferns. J Syst Evol 54:563–603.

Pryer KM 1999 Phylogeny of marsileaceous ferns and relationships of the fossil *Hydropteris pinnata* reconsidered. Int J Plant Sci 160:931–954.

Puebla GG, MB Prámparo, MA Gandolfo 2015 Aquatic ferns from the Upper Cretaceous Loncoche Formation, Mendoza, central-western, Argentina. Plant Syst Evol 301:577–588.

Reid EM, MEJ Chandler 1926 Catalogue of Cainozoic plants in the Department of Geology, vol 1. The Bembridge Flora. Clowes, London.

Reid JD, GM Plunkett, GA Peters 2006 Phylogenetic relationships in the heterosporous fern genus *Azolla* (Azollaceae) based on DNA sequence data from three noncoding regions. Int J Plant Sci 167:529–538.

Rothwell GW 1999 Fossils and ferns in the resolution of land plant phylogeny. Bot Rev 65:188–218.

Rothwell GW, KC Nixon 2006 How does the inclusion of fossil data change our conclusions about the phylogenetic history of euphyllophytes? Int J Plant Sci 167:737–749.

Rothwell GW, RA Stockey 1994 The role of *Hydropteris pinnata* gen. et. sp. nov. in reconstructing the cladistics of heterosporous ferns. Am J Bot 81:479–492.

Rouse GE 1962 Plant microfossils from the Burrard Formation of western British Columbia. Micropaleontology 8:187–218.

Sahni B 1941 Indian silicified plants I. *Azolla intertrappea* Sah. & H.S. Rao. Proc Indian Acad Sci B 14:489–501.

Sahni B, HS Rao 1943 A silicified flora from the Intertrappean cherts round Sausar in the Deccan. Proc Natl Acad Sci India 13:36–75.

Saunders RMK, K Fowler 1992 A morphological taxonomic revision of *Azolla* Lam. section *Rhizosperma* (Mey.) Mett. (Azollaceae). Bot J Linn Soc 109:329–357.

——— 1993 The supraspecific taxonomy and evolution of the fern genus *Azolla* (Azollaceae). Plant Syst Evol 184:175–193.

Schneller JJ 1990 Azollaceae. Pages 57–60 *in* KU Kramer, PS Green, eds. The families and genera of vascular plants. I. Pteridophytes and gymnosperms. Springer, Berlin.

Shorthouse DP 2010 SimpleMappr, an online tool to produce publication-quality point maps. http://www.simplemappr.net.

Singh RP, PK Singh 1987 Morphology and nitrogen-fixing ability of *Azolla nilotica* Decaisne. Curr Sci 56:900–902.

Smith AR, KM Pryer, E Schuettpelz, P Korall, H Schneider, PG Wolf 2006 A classification for extant ferns. Taxon 55:705–731.

Smith SY, SR Manchester, B Samant, DM Mohabey, E Wheeler, P Baas, D Kapgate, R Srivastava, ND Sheldon 2015 Integrating paleobotanical, paleosol, and stratigraphic data to study critical transitions: a case study from the Late Cretaceous–Paleocene of India. Pages 137–166 *in* PD Polly, JJ Head, DL Fox, eds. Earth-life transitions: paleobiology in the context of earth system evolution. Paleontol Soc Pap 21. Paleontological Society.

Snead RC 1969 Microfloral diagnosis of the Cretaceous-Tertiary boundary, central Alberta. Res Counc Alberta Bull 25. Research Council of Alberta, Edmonton.

Strasburger E 1873 Ueber *Azolla*. Ambr. Abel, Leipzig.

Stuart SA, DM Erwin 2006 Fertile sporophytes of *Azolla* from the early Eocene Wind River flora, Wind River Basin, Wyoming, U.S.A. Abstract ID 609. Botany 2006, Chico, California. July 28–Aug. 2, 2006. http://www.2006.botanyconference.org/engine/search/index.php?func=detail&aid=609.

Surange KR 1966 Indian fossil pteridophytes. Botanical Monograph No. 4. Council of Scientific and Industrial Research, New Delhi.

Sweet AR, A Chandrasekharam 1973 Vegetative remains of *Azolla schopfii* Dijkstra from Genesee, Alberta. Can J Bot 51:1491–1496.

Sweet AR, LV Hills 1971 A study of *Azolla pinnata* R. Brown. Am Fern J 61:1–13.

——— 1976 Early Tertiary species of *Azolla* subg. *Azolla* sect. *Kremastospora* from western and arctic Canada. Can J Bot 54:334–351.

Trivedi BS, CL Verma 1971 Contributions to the knowledge of *Azolla indica* sp. nov. from the Deccan Intertrappean Series, M.P., India. Palaeontogr Abt B Palaeophytol 136:71–82.

Tryon RM, AF Tryon 1982 Ferns and allied plants, with special reference to tropical America. Springer, New York.

Vajda V, S McLoughlin 2005 A new Maastrichtian-Paleocene *Azolla* species from of Bolivia, with a comparison of the global record of coeval *Azolla* microfossils. Alcheringa 29:305–329.

Vallati P, A De Sosa Tomas, G Casal, M Calo 2017 Salviniales from the Late Cretaceous of the Golfo San Jorge Basin. Cretac Res 74:45–55.

van der Burgh J, ME Collinson, JHA van Konijnenburg-van Cittert, J Barke, H Brinkhuis 2013 The freshwater fern *Azolla* (Azollaceae) from Eocene Arctic and Nordic Sea sediments: new species and their stratigraphic distribution. Rev Palaeobot Palynol 194:50–68.

Yamada T, M Kato 2002 *Regnellites nagashimae* gen. et sp. nov., the oldest macrofossil of Marsileaceae, from the Upper Jurassic to Lower Cretaceous of western Japan. Int J Plant Sci 163:715–723.

Zaporozhets NI, MA Akhmetiev 2013 The middle and upper Eocene sections of the Omsk Trough, West Siberian Platform: palynological, stratigraphic, hydrologic, and climatic aspects. Stratigr Geol Correl 21:107–130.