CONIFER ROOT NODULES COLONIZED BY ARBUSCULAR MYCORRHIZAL FUNGI IN JURASSIC GEOTHERMAL SETTINGS FROM PATAGONIA, ARGENTINA

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Premise of research. Despite the ecological significance of arbuscular mycorrhizae in modern terrestrial ecosystems, knowledge about their evolution based on the fossil record is still scarce, especially concerning the case of root nodules harboring arbuscular mycorrhizal fungi, as in some extant gymnosperms and angiosperms. Exceptionally preserved conifer nodular roots were found in the Jurassic fossil-bearing chert deposits of the Deseado Massif (Santa Cruz, Argentina), raising the possibility of studying them in association with arbuscular mycorrhizal fungi. The aim of this study is to describe the plant organs and their fungal partners and to discuss the ecological significance of the interactions observed, particularly with respect to their occurrence in the hot spring settings.

Methodology. Thin sections of chert samples from the Cañadón Nahuel locality of the La Matilde Formation, Deseado Massif (Santa Cruz, Argentina) were observed using light microscopy.

Pivotal results. The cortex of the nodules is occupied by several glomeromycotan fungal structures. The structures occur in a specific zone of the cortex—toward its center—and include intracellular hyphal coils and arbuscules. Glomoid spores and coenocytic hyphae possibly penetrating the epidermal cells are also described and analyzed.

Conclusions. The root nodules have affinities with the Araucariales, representing the oldest record of such structures for this conifer clade. This is also the first record of nodules harboring arbuscular mycorrhizal fungi for the Jurassic; it extends our knowledge of the fossil record for this particular type of fungal association.

Keywords: root nodules, arbuscular mycorrhizal fungi, hot spring settings, Araucariales, Jurassic.

Online enhancements: supplemental figures.

Introduction

Arbuscular mycorrhizal fungi (AMF) have a deep fossil record (Pirozynski and Dalpé 1989; Remy et al. 1994; Taylor et al. 2015; Walker et al. 2018 and citations therein) and today develop symbiosis with approximately 80% of vascular plants worldwide, including five major groups of living conifers (Brun and Qui 2006; Smith and Read 2010; Spathofora et al. 2016; Brundrett and Tedersoo 2018). In modern conifers, AMF colonize roots of the Taxaceae, Cupressaceae, Sciadopityaceae, Araucariaceae, and Podocarpaceae (Wang and Qui 2006; Brundrett 2009; Dickie and Holdaway 2011). The members of the Araucariales clade (i.e., members of the Podocarpaceae and Araucariaceae) and Sciadopityis harbor AMF in young fine roots and small spherical differentiated structures called nodules (Spratt 1912; Godoy and Mayr 1989; Mcgee et al. 1999; Breuninger et al. 2000; Russell et al. 2002; Leon et al. 2008; Dickie and Holdaway 2011; Padamsee et al. 2016). Although AMF are relatively well known from the fossil record (Taylor et al. 2015; Strullu-Derrien et al. 2018; Walker et al. 2018), the fossil record of arbuscular mycorrhizal associations with conifers and related groups is sparse.

Structurally preserved AMF have been described in Carboniferous Cordaites from the Massif Central, France (Strullu-Derrien et al. 2009), in volztzalean conifers from the Triassic of Antarctica (Schwendemann et al. 2011; Harper et al. 2015), and in a taxodiaceous Cupressaceae from the Middle Eocene of Canada (Stockey et al. 2001). Nodular roots related to the Araucariales are known from the Cretaceous of Antarctica, Australia,
and India (Cantrill and Douglas 1988; Cantrill and Falcon-Lang 2001; Banerji and Ghosh 2002) and in the Triassic volctialean Notophyllum krauselli (Schwendemann et al. 2011; Harper et al. 2015). This last record is the only one showing anatomical preservation of the rootlets in which AMF colonization has been described.

The Middle-Late Jurassic chert deposits of the Bahía Laura Complex in the Deseado Massif of Santa Cruz Province, southern Patagonia, Argentina, bear exceptionally preserved organisms in hot spring settings (Channing et al. 2007; Guido et al. 2010; Channing et al. 2011; García Massini et al. 2012, 2016). The best-preserved Jurassic geothermal paleosurfaces can be positively compared, based on their structure, with hot springs at Yellowstone National Park in the United States (Channing and Edwards 2009a, 2009b). These are the only geothermal fossiliferous deposits with an ecosystem-level preservation known for the Mesozoic. Preliminary reports on the fossiliferous content and paleoenvironment reconstruction have been carried out for a few localities (Channing et al. 2007; Guido et al. 2010; García Massini et al. 2012, 2016; Sagasti et al. 2016). Plant remains and fungal and fungus-like vegetative and reproductive structures are abundant (Guido et al. 2010; García Massini et al. 2012, 2013, 2016; Sagasti et al. 2016, 2019) and frequently in association with each other (e.g., García Massini et al. 2016). This provides the opportunity to describe a variety of plant-fungal interactions to characterize the dynamics of the hot springs–associated ecosystems and to broaden our knowledge of fungal evolution and diversity during the Jurassic and, in a broader sense, during the Mesozoic.

In this contribution, we describe particular conifer nodular roots colonized by AMF, preserved as inclusions in the matrix of chert blocks collected from the recently discovered Cañadón Nahuel locality of the La Matilde Formation, Bahía Laura Complex, at the Deseado Massif hot spring deposits. We discuss possible systematic affinities for these roots based on comparisons with extinct and extant examples of conifer nodular roots. We also address the ecological significance of the arbuscular mycorrhizal interaction between the studied conifers and glomeromycotan fungi in the context of the hot springs settings. This finding expands our knowledge of fungal diversity and ecology in the Jurassic and fills a gap in the fossil record of arbuscular mycorrhizal symbiosis in conifers.

**Material and Methods**

**Geological Setting**

The studied samples were collected at the Cañadón Nahuel locality of the La Matilde Formation in the Bahía Laura Complex of the Deseado Massif, Santa Cruz Province, Argentina (fig. 1). The Deseado Massif is an extensive (60,000-km²) region in southern Patagonia in Santa Cruz Province, Argentina. The geologic history of this region is marked by volcanic activity during the Jurassic. Approximately 178–151 million years ago (Pankhurst et al. 2000), this area was subject to bimodal rhyolitic and andesitic volcanism, which led to the formation of the Bahía Laura Complex (Guido 2004). Rocks of the Bahía Laura Complex are part of the Chon Aike silicic large igneous province (which ranges from Patagonia to Antarctica; Pankhurst et al. 1998). This province’s genesis is related to the breakup of Gondwana and the birth of the South Atlantic Ocean (Riley et al. 2001; Richardson and Underhill 2002).

Widespread hydrothermal activity in the Deseado Massif resulted from the mature (quiescent) volcanic phase in this region during the Late Jurassic, which produced metalliferous epithermal deposits (i.e., formed at shallow depths related to the terrestrial surface, in the upper portion of a hydrothermal system under temperatures between 150° and 300°C), extensive silification, and formation of geothermal fields at the surface (Schalamp et al. 1997; Guido and Campbell 2011). Well-preserved fossilized geothermal fields have been found within volcaniclastic fluvio-lacustrine strata, distributed in a 400 × 250-km area of NNW-SSE- and WNW-ESE-oriented belts aligned with major regional structures (Guido and Campbell 2011). Twelve of these geothermal fields preserve fossil assemblages, which represent the only known fossiliferous Mesozoic hot springs. These deposits fill a significant stratigraphic gap between similar Paleozoic and Cenozoic deposits (Channing and Edwards 2013).

Cañadón Nahuel (GPS coordinates available upon request) is the farthest east of these chert deposits in the Deseado Massif (fig. 1). The locality is located in the northern belt defined by Guido and Campbell (2011). Cherts from Cañadón Nahuel are rather randomly distributed over a large area and show textural and fossiliferous characteristics that relate them to formation in the distal parts or fringes of a siliceous sinter geothermal system. The observed distribution of the cherts suggests that they have been successively removed by recurrent volcanic and phreatic local eruptions or breccias (i.e., by fragmentation) related to the geothermal activity. In some instances, horizontal beds of variable extension are found, and these too are interpreted as having formed in wetlands and shallow saline ponds in the distal parts of geothermal systems.

**Preparation and Imaging of Studied Material**

Thin sections of chert blocks were prepared following standard methodology (Jones and Rowe 1999). Slides were observed using transmitted light microscopy. All images were taken with a Nikon DS-Fi1 camera connected to a Nikon Eclipse 80i microscope. General views of the sections were taken using a Canon 7D camera with a Canon macro lens of 60 mm in a light box with fluorescent tubes. At higher magnifications, multiple images of the same structure of interest were taken at different focal planes to compose an image with greater depth of focus, following an image-stacking technique (Bercovici et al. 2009). We used Helicon Focus software ([http://www.heliconsoft.com/heliconfocus.html](http://www.heliconsoft.com/heliconfocus.html)), with the “Method B” (depth map) parameter. Several partially overlapping images were merged to create high-quality images of critical features. This technique was applied using Adobe Photoshop CS6, and images were processed after to reveal critical characters. The image-stacking technique was not always applicable because in some cases the stacking of images added too much noise or artifacts to the final composition. In such cases, we opted to show different focal planes of the same structure side by side (e.g., fig. 5).

The macrospecimens and thin sections are housed at the Museo Regional Padre Molina of Río Gallegos (Santa Cruz Province, Argentina). The macro- and thin-sections were used to study fossiliferous material and to infer the life conditions of the organisms in these deposits. The fossiliferous material was studied using transmitted light microscopy and scanning electron microscopy (SEM). The SEM images were taken using a FEI Quanta 3D FEG scanning electron microscope at the Department of Earth and Environmental Sciences, Università degli Studi di Milano, Italy. The SEM images were processed using ImageJ software ([http://rsb.info.nih.gov/ij/](http://rsb.info.nih.gov/ij/)).
Province, Argentina) under accession numbers MPM-Pb-16033, 16039–16045.

Results

Description

Fungal specimens occur in association with root nodules that emerge from conifer fine roots. These are preserved in chert and are obliquely cross-sectioned and longitudinally sectioned (fig. 2a). A central vascular cylinder with secondary xylem followed by the cortex and an apparent periderm are observed in oblique cross section of older roots (fig. 2b). In oblique longitudinal section, scarce diagnostic characters of the secondary xylem are observed (fig. 2c–2e). Pits in the radial walls of the tracheids are contiguous, biseriate, or uniseriate with occasional biseriate rows (fig. 2d, 2e).

The young fine nodular roots are 0.5–1 mm in diameter. Some roots are constrained, showing a beaded appearance (fig. 3a). The nodules emerge at a right angle from the parental root (fig. 3c) and are arranged in variable patterns (fig. 2f); some are densely packed while others are scattered, and in some specimens, they are alternately arranged (fig. 3a). They are spherical to spheroidal (i.e., longitudinally elongated; figs. 2a, 2f, 3a–3e) and measure 0.8 (0.45–1.18) mm in diameter.

Anatomically, the nodules are characterized by a central vascular cylinder that is 92 (78–120) μm in diameter surrounded by an endodermis 24 (11–35) μm wide. The endodermis is surrounded by the cortex, ending in an epidermis, and the cortex is 340 (250–445) μm wide (fig. 3c, 3d). Root hairs or their bases are occasionally observed (fig. 3d, 3f). Root hairs are approximately 19.5 μm in diameter; they are wider at the base with an approximate diameter of 25 μm.

The nodules originate at the protoxylem poles of the roots (fig. 3b). The vascular strand and the endodermis can be followed from the main root up to the middle portion of the nodule in longitudinal section (figs. 2f, 3a–3e). Cortical tissues are composed of 7 (6–8), very occasionally up to 10, layers of cortical cells; cells flatten toward the epidermis (fig. 3d). Resurgent growth can be observed in some nodules, where new cortical tissue grows inside the older cortex (fig. 3e).

The cortex of the root nodules is colonized by AMF (figs. 4–7). Additionally, AMF structures occur in the chert matrix, very close or directly attached to the roots and nodules (fig. 7).
fungi are mainly restricted to the inner layers of cortical tissue, whereas cortical layers below the epidermis remain almost fungal-free. In the inner zone of the cortex, the outer layers are occupied by intracellular coenocytic hyphae that sometimes form coils (fig. 4). The hyphae are irregular in diameter, ranging from 3.4 to 14.5 μm, with an average diameter of 7 μm. The hyphae also develop knobs along their length (fig. 4b) and branch dichotomously (fig. 4c) and at a right angle (fig. 4d). Some coils display possible circular to ellipsoidal scars and are directly associated with narrower hyphae of an intrusive septate fungus.
However, these scars can be preservation artifacts, rendering the metabolic status of the septate fungus saprothrophic or parasitic.

Toward the inner cortex, the hyphae become thinner, developing highly ramified structures that resemble arbuscules (fig. 5a, 5c). Arbuscules are observed partially occupying the cells lumens (figs. 5c, 6; see also appendix, available online). Trunk hyphae of the arbuscules are less than 4 μm in diameter.

Morphologically identical, intra- and extrarhizal glomoid spores are also observed (fig. 7). Intrarhizal spores occur in
the cortex of both young roots and nodules. Extrarhizal spores are observed dispersed in the chert matrix very close to the roots and nodules. Extrarhizal mycelium associated with extrarhizal spores (fig. 7b, 7c) extends parallel to the roots and nodules’ surface. The hyphae measure 2 (1.5–4.5) μm in diameter and present Y branching. In some specimens, coenocytic hyphae emerging from the spores attached to the epidermal surface of nodules (fig. 7b, 7c) and possibly penetrating the epidermal cells are observed (fig. 7d).

The spores are terminal, globose to subglobose, and variable in size, ranging from 26.5 to 43.5 μm in diameter (fig. 7e, 7f). The spore wall appears to comprise two layers (fig. 7e, 7f). The outer layer is hyaline, and the inner layer is opaque. The spores are occluded at their attachment point by a fold of the inner layer in some specimens (fig. 7f) or by a plug in others (fig. 7e). The spores are attached to simple subtending hyphae. The hyphae are coenocytic and 2.0 (1.2–2.9) μm in diameter.

Discussion

Here, we described an ancient plant-fungal association in the root nodules of Jurassic conifers. Coenocytic hyphae forming coils, ramified arbuscules, and spores, attributable to glomero- mycotan fungi, occur inside the cortical tissues of conifer root nodules. We observe that the AMF structures are restricted to the inner layers of the cortex, whereas only a few scattered coenocytic hyphae occur in the outer cortex, close to the entry points below the epidermis. When resurgent growth in the host nodules is present, hyphae are also present in the renewed cortex of the nodules, which positively compares with what has been described in extant Araucariales (Baylis et al. 1963; Russell et al. 2002; Dickie and Holdaway 2011). Analogous general distribution of AMF in host nodules is also observed in extant Araucariales (fig. 5b; table 1; McGee et al. 1999; Russell et al. 2002; Dickie and Holdaway 2011). Similarly, previous records of fossil mycorrhizal plants found identical zoned distribution of AMF in the infected root tissues (Remy et al. 1994; Phipps and Taylor 1996; Strullu-Derrien et al. 2009, 2014; Harper et al. 2015).

Two morphological types of arbuscular mycorrhizae have been classically distinguished (Gallaud 1905): Arum type, which includes intercellularly hyphal growth in a longitudinal pattern in the cortex, with short side branches that give rise to arbuscules nearly at a right angle; and Paris type, characterized
by intracellular coiling hyphae with arbuscules forming along the coil (Taylor et al. 2015; Walker et al. 2018). Features of the fungi described here are consistent with the Paris morphological type. However, it has been shown that the morphological types vary on a continuum depending on a series of factors (e.g., host plant tissues, taxa involved, environment; Dickson 2004; Van Aarle et al. 2005; Kubota et al. 2005), rendering this character merely descriptive and of little relative use for

Fig. 5 Thin section of chert block from Cañadón Nahuel locality, MPM-Pb-16039, and thin section of extant Podocarpaceae Podocarpus totara root nodule (images by Larry Peterson and Lewis Melville, from Dickie and Holdaway 2011). a, Longitudinal section of fossil root nodule showing zone of the cortex occupied by arbuscules (delimited by dotted line). b, Longitudinal section of extant root nodule showing zone of the cortex occupied by arbuscules (delimited by dotted line). c, Detail of arbuscules in fossil root nodule. d, Detail of arbuscules in extant root nodule. Scale bars = 100 µm (a, b), 20 µm (c), 50 µm (d).
the classification of AMF. Besides, the distinctions between these morphologies have been used in fossil comparisons (Harper et al. 2015, 2016; Walker et al. 2018).

**Systematic Affinities of the Root Nodules and the Fungi**

The root nodules described here are characterized by a central vascular cylinder surrounded by an endodermis, followed by several layers of cortex surrounded by an epidermis; spherical to spheroidal three-dimensional morphology; and emergence at a right angle from the parental root in variable patterns along the main axis. These are features shared by extant Araucariales, which includes the Podocarpaceae and Araucariaceae (see comparisons in table 1). Additional vegetative and reproductive organs assignable to Araucariaceae and Podocarpaceae are found in the La Matilde Formation (e.g., wood, leafy branches, cones; Zamuner...
and Falaschi 2005; Gnaedinger 2007; Falaschi et al. 2011; Kloster and Gnaedinger 2018). Further refinement of the systematic affinities of the nodules based on the preserved morphological characters, especially considering the morphological variability known to be present in fossil and extant Podocarpaceae and Araucariaceae, is not possible (Spratt 1912; Dickie and Holdaway 2011; tables 1, 2). Considering the presence of both podocarpaceous and araucarian organs in the chert blocks studied and the little morphological variation observed among the mycorrhizal nodules, it remains possible that more than one taxa is present.

Fig. 7  Thin section of chert block from Cañadón Nahuel locality, MPM-Pb-16039. a, Root nodule with intrarhizal glomoid spores. b, Glomoid spore associated with root nodule. Note connecting hypha (arrowhead). c, Glomoid spore with multiple hyphal attachments, some of which connect it to the root nodule. d, Magnification of c showing detail of entry point with penetrating hyphae and development of coil. e, Detail of intrarhizal globose spore in decaying root, showing two-layered wall, simple subtending hypha, and occlusion plug. f, Detail of extrarhizal globose spore, showing two-layered wall, simple subtending hypha, and occlusion.
Table 1

Comparisons of Cañadón Nahuel Locality Specimens of Root Nodules and the Root Nodules of Extant Podocarpaceae and Araucariaceae Species

| Conifer species | Arrangement | Morphology | Size (mm) | General anatomy | Root hairs | Outer cortex | AMF | Inner cortex |
|-----------------|-------------|------------|-----------|-----------------|------------|--------------|-----|-------------|
| Araucaria angustifolia<sup>a</sup> | ? | Spheroidal | ? | ? | ? | Penetration hyphae | Arbuscules, vesicles |
| Araucaria cunninghamii<sup>b</sup> | ? | Spherical and conical | ? | Central VC connected to MR/En/C/E | ? | Scattered hyphae | Arbuscules |
| Araucaria araucana | PS | Spheroidal | .5 | Central VC connected to MR/En/C/E/Ex (in older nodules) | P (young nodules) | Mainly free | Abundant arbuscules, vesicles |
| Agathis australis<sup>c</sup> | Irregularly spaced | Spherical, beaded nodules | 1 | Central VC connected to MR/En/C/E; VS = half length of the nodule | ? | Scattered hyphae | Coiled hyphae, arbuscules, vesicles |
| Agathis robusta<sup>d</sup> | ? | Spherical and conical | ? | Central VC connected to MR/En/C/E | ? | Scattered hyphae | Arbuscules |
| Wollemia nobilis<sup>e</sup> | ? | Spherical and conical | ? | Central VC connected to MR/En/C/E | ? | Scattered hyphae | Arbuscules |
| Dacrycarpus dacrydioides<sup>e</sup> | 2 or 4 LS | Spherical | ? | Central VC connected to MR/En/C/E; VS = length of the nodule | A | Mainly free; scattered hyphae (below entry point) | Arbuscules, vesicles |
| Dacrydium cupressinum<sup>e</sup> | 2 or 4 LS | Spherical | ? | Central VC connected to MR/En/C/E; VS = half length of the nodule | A | Mainly free; scattered hyphae (below entry point) | Arbuscules, vesicles |
| Dacrydium fonkii<sup>e</sup> | ? | Spherical | ? | ? | ? | ? | Abundant arbuscules, scattered vesicles |
| Podocarpus nubigena<sup>f</sup> | PS | Spherical | 1.2 | ? | P | ? | Abundant arbuscules |
| Podocarpus paniculata<sup>g</sup> | ? | Spherical | ? | ? | ? | ? | Arbuscules, vesicles |
| Podocarpus salignus<sup>h</sup> | 2 or 4 LS | Spherical | .7 | ? | ? | ? | Arbuscules, vesicles |
| Prumnopitys ferruginea<sup>e</sup> | 2 or 4 LS | Spherical | ? | Central VC connected to MR/En/C/E; VS = half length of the nodule | P | Mainly free; scattered hyphae (below entry point) | Arbuscules, vesicles |
| Prumnopitys taxifolia<sup>e</sup> | 2 or 4 LS | Spherical | ? | Central VC connected to MR/En/C/E; VS = half length of the nodule | P | Mainly free; scattered hyphae (below entry point) | Arbuscules, vesicles |
| Prumnopitys andina<sup>i</sup> | ? | ? | 1.1–1.5 | ? | Sc | ? | Arbuscules, vesicles |
| Saxegothaea conspicua<sup>j</sup> | PS | ? | .5–1.0 | ? | P | ? | Arbuscules, vesicles |
| Cañadón Nahuel specimens | Variable | Spherical or spheroidal | .8 | Central VC connected to MR/En/C/E; VS = half length of the nodule | P (occasionally) | Mainly free; scattered hyphae (below entry point) | Coiled hyphae, arbuscules, spores |

Note. AMF = arbuscular mycorrhizal fungi; PS = parallel series; LS = longitudinal series; VC = vascular cylinder; MR = main root; En = endodermis; C = cortex; E = epidermis; Ex = exodermis; VS = vascular strand; P = present; A = absent; Sc = scattered; ? = missing information.

<sup>a</sup> Breuninger et al. 2000.
<sup>b</sup> McGe et al. 1990.
<sup>c</sup> Godoy and Mayr 1989.
<sup>d</sup> Padamsee et al. 2016.
<sup>e</sup> Russel et al. 2002; Dickie and Holdaway 2011.
<sup>f</sup> Leone et al. 2008.
Based on phylogenetic analysis of combined molecular and morphological data, several families have been recognized among the former Glomeromycota (Schüßler et al. 2001; Spatafora et al. 2016): Paraglomeraceae, Archaeosporaceae, Geosiphonaceae, Ambisporaceae, Glomeraceae, Claroideoglomeraceae, Pacisporaceae, Gigasporaceae, Pacisporaceae, Sacculosporaeeae, Acaulosporaceae, and Diversisporaceae (see Schüßler and Walker 2010; Redecker et al. 2013; http://www.amf-phylogeny.com). Differences mainly in spore type formation, spore wall structure, presence of vesicles, and even ecology exclude affinities of the fungi described here with several different groups (see table 3). Particular morphological characters suggest that these fossils are most probably affinities with the Paraglomeraceae.

Diagnostic characters supporting affinities with the Paraglomeraceae include arbuscules attached to narrow trunks (< 4 mm diameter), development of intrarhizal spores, and frequently coiling intracellular hyphae. However, Paraglomus spores have a three-layered wall, whereas in our specimens, the wall is composed of two layers. Nonetheless, the outer wall of the spores in Paraglomus has been mentioned to disintegrate at senescence, resulting in a two-layered spore wall, and is thus comparable to that displayed by the fossils described here (Morton and Redecker 2001). Alternatively, affinities of the fossil with Glomeraceae are also possible, being different mainly based on the absence of vesicles; additionally, the Glomeraceae commonly develop straight hyphae, whereas the fossil most frequently shows coiled hyphae. These same features also make the fossil somewhat comparable to Diversisporaceae and Ambisporaceae, although these two families develop acaulosporoid spores, which have not been observed in the fossil.

**Fossil Conifers with Arbuscular Mycorrhizal Fungi**

The oldest record of nodular roots colonized by AMF is from the Triassic of Antarctica (Schwendemann et al. 2011; Harper et al. 2015). In this case, both the rootlets and the root nodules

### Table 2

| Age            | Locality          | Preservation | Nodules | Affinities | AMF  |
|----------------|-------------------|--------------|---------|------------|------|
| Middle Triassic| Antarctica        | Permineralization | Prolate, spheroidal | Voltziales | P    |
| Middle Jurassic| Patagonia, Argentina | Permineralization | Spheroidal, spheroidal | Araucariales | P    |
| Cretaceous     | Australia         | Compressions/impressions | ? | ?Taxodiaceae | NP   |
|                | Antarctica        | Compressions/impressions | Spherical | ?Podocarpaceae | NP   |
|                | India             | Molds        | Spherical, oval | ?Podocarpaceae | NP   |

Note. Modified from Schwendemann et al. (2011). AMF = arbuscular mycorrhizal fungi; P = preserved; NP = not preserved; ? = missing information.

### Table 3

| Family                  | Spore type | Glomoid spore wall structure and occlusion | Vesicles | Mycelium | Observations |
|-------------------------|------------|------------------------------------------|----------|----------|--------------|
| Claroideoglomeraceae    | G          | 2 layers (apparent “endospore”);          | ?        | ?        |              |
|                         |            | Occ: septum                              |          |          |              |
| Pacisporaceae           | G          | Two wall groups; Occ: septum              | P (some species) | ? | Association with cyanobacteria |
| Geosiphonaceae          | G          | 3 layers; Occ: amorphous plug            |          | ? |              |
| Ambisporaceae           | G/Ac       | 2 layers; Occ: open-pored or septum      | P/A      | ? |              |
| Diversisporaceae        | G/Ac       | 3 layers                                 | P/A      | ? | With or without auxiliary cell |
| Glomeraceae             | G          | 3 layers; Occ: variable                   | P        | Narrow trunks, coiling rare |              |
| Paraglomeroaceae        | G          | 3 layers; Occ: septum?                    | P/A      | Narrow trunks, coiling frequent | Intrarhizal spores? |

Note. G = glomoid; Ac = acaulosporoid; Occ = occlusion; P = present; A = absent; ? = missing information or not clear.

a Schüßler and Walker 2010.
b Redecker et al. 2013.
c Oehl and Sieverding 2004.
d Schüßler 2002.
e Walker et al. 2007.
f Walker and Schüßler 2004.
g Morton and Benny 1990.
h Morton and Redecker 2001.
of Notophytum krauselli are colonized by several fungal structures (i.e., hyphal coils, arbuscules, extrarhizal hyphae, vesicles, and spores; Schwenendemann et al. 2011; Harper et al. 2015). However, fungal structures found in the nodules are different from those found in the secondary rootlets, suggesting that different arbuscular mycorrhizal symbiosis occurs in different organs of the same plant root system (Harper et al. 2015). Other examples of conifers showing root nodules are from different Cretaceous localities from the Southern Hemisphere (Cantrill and Douglass 1988; Cantrill and Falcon-Lang 2001; Banerji and Ghosh 2002). The lack of anatomical preservation in these nodules found as impressions, compressions, and molds prevents any fungal association to be observed. Numerous affinities were discussed for these Cretaceous conifer nodule-bearing roots based on the association with other organs, including affinities with the Podocarpaceae, Araucariaceae, Sciadopityaceae, and even Cupressaceae. This new record of arbuscular mycorrhizal in root nodules with affinities with the Araucariaceae from the Jurassic of Patagonia fills a gap in the geological history of AMF and further documents morphological diversity and ecological preferences of glomeromycetes through time.

Mycorrhizal Nodules and Ecology

The exceptional preservation of the conifer nodular roots of the Cañadón Nahuel locality can be related to the mode of preservation typical of hot spring settings, where plants and other organisms become immersed in a mineral-saturated solution that gradually transforms them into fossils (Channing and Edwards 2004; Hellawell et al. 2013). The presence of fungal structures inside the cortex of these underground organs indicates that the fossilization process of these organisms must have occurred while they were alive or soon after their death (Channing and Edwards 2004). Such a fossilization process consisted in permineralization by silica, which is a characteristic previously observed in La Bajada and San Agustín hot spring localities, also of the La Matilde Formation (García Massini et al. 2016). Moreover, based on their distribution and textural and fossiliferous content, the plant-rich cherts of the Cañadón Nahuel locality are related to the distal areas of a siliceous sinter geothermal system, potentially analogue to the modern geothermally influenced environments in Yellowstone National Park in the United States (Channing and Edwards 2009a, 2009b). Root nodules have also been preliminarily described in the San Agustín and La Bajada localities (see appendix; García Massini et al. 2013, 2016). These root nodules share the general morphology and anatomy of the specimens described here, though the best-preserved examples of arbuscular mycorrhizal colonization are from Cañadón Nahuel.

Presence of AMF is not needed for the development of the root nodules in extant Araucariales, as they are indeed part of the root system ontogeny (Baylis et al. 1963; Baylis 1969); however, these organs are hypothesized to increase the volume of fungal colonization at a lower energy cost than having an extensive root system and therefore maximize efficiency of mineral nutrient acquisition (Russell et al. 2002; Dickie and Holdaway 2011). Although the role of the mycorrhizal nodules in the extant Araucariales is not yet completely understood, these organs are thought to be ecologically significant with respect to uptake of water and nutrients, especially phosphorous and nitrogen; additionally, establishment of seedlings in nutrient-poor environments has also been suggested to be influenced by the development of mycorrhizal nodules (Russo et al. 2002; Dickie and Holdaway 2011 and citations therein; Padamsee et al. 2016). Systematic and taxonomical refinement to the family level of the conifers of Cañadón Nahuel and further characterization of the paleoenvironments where these plants used to live should enrich what can be grasped from these fossils on the evolution and significance of the AM symbiosis in the Jurassic Araucariales of Patagonia.

Conclusions

We present the first record of structurally preserved AMF-colonizing root nodules from the Jurassic, partially filling a gap in the Mesozoic record of conifer mycorrhizal nodules and extending the record geographically.

The mycorrhizal nature of the fungus is demonstrated by the presence of several fungal structures in a delimited zone of the nodule’s cortex, especially important is the presence of hyphal coils, arbuscules, and glomoid spores.

The nodules have affinities with the Araucariales clade of conifers; however, it is not possible to constrain the affinities of these organs further based on the preserved anatomical evidence. Nonetheless, the presence of these structures is important, as they expand our knowledge of the morphology and ecology of these conifers during the establishment of the modern conifer families in the Jurassic. They also open the possibility to work on whole-plant concept reconstructions in the future.

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