Fungi associated with *Glossopteris* (Glossopteridales) leaves from the Permian of Antarctica: A preliminary report

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Abstract

Fungi today occur on virtually every plant part, living and dead, and represent a significant proportion of fungal diversity. Arborescent seed ferns characterized by large, tongue-shaped leaves with reticulate venation (*Glossopteris*) represent the dominant floral element in the Permian of Gondwana. However, documented evidence of fungi associated with the leaves of these plants is exceedingly rare. Partially degraded *Glossopteris* leaves from two upper Permian permineralized peat deposits from Antarctica yield scattered evidence of fungal colonization in the form of hyphae, spores, sporangia, and mycelia. Intact leaves from the same deposits are typically free of fungi, suggesting that the fungi in the degraded leaves were saprotrophs on the forest floor, rather than colonizers of living leaves. We hypothesize that the scarcity of fungi associated with Antarctic *Glossopteris* leaves may be related to structural and physiological adaptions of the plants to the extreme conditions that governed late Paleozoic polar ecosystems.

Key words: Collinson Ridge, Gondwana, late Paleozoic, permineralization, polar forest, saprotrophism, Skaar Ridge

1. Introduction

Although plant leaves constitute a harsh environment due to several abiotic and biotic factors (e.g., temporary nutrient availability, extreme fluctuations in humidity, temperature, gas exchange gradients, and ultraviolet radiation; see Goodman & Weisz 2002), they are inhabited by a remarkable variety of different organisms, including bacteria, fungi, algae, and small animals (Hill 1977; Lindow & Brandl 2003; Lil & Marquis 2004). Many of the studies investigating microbial life on and in the leaves of extant plants focus on fungi because these organisms are particularly abundant, diverse, and often play important roles in the life history biology of their hosts (e.g., Andrews & Harris 2000; Stone et al. 2000; Arnold 2007; Rodriguez et al. 2009; Osono 2014).

The fossil record of leaf-inhabiting fungi is predominantly composed of Cenozoic epiphyllous fungi (e.g., Dilcher 1963, 1965; Selkirk 1972; Lange 1978; Phipps 2001, 2007; Phipps & Rember 2004), while there is relatively little evidence to date of pre-Cretaceous fungi associated with leaves, with the exception of several reports from the Carboniferous (Barthel 1961; Krings 2001; Krings et al. 2009, 2010, 2011; Dotzler et al. 2011). The main difficulty in studying fungi associated with pre-Cretaceous leaves is that the details needed for systematic assignment...
2. Material and methods

Late Permian permineralized peat containing Glossopteris leaves from Skaar Ridge (84° 49' 11.8" S, 163° 20' 37.0" E; 2300 m, 8600 ft) located within the Buckley Formation near the Beardmore Glacier Area, Queen Alexandra Range, central Transantarctic Mountains, Antarctica (for details on the geological setting, refer to Barrett et al. 1986; Isbell 1990; Collinson et al. 1994). Additional material, also late Permian in age, comes from a lens of silicified peat, 6 m across and 0.6 m thick, within a medium-grained sandstone on Collinson Ridge (85° 13' S, 175° 21' W), presumably located in the lower part of the Fremouw Formation, central Transantarctic Mountains, Shackleton Glacier area (details on the geological setting can be found in Collinson & Hammer 1996; McManus et al. 2002).

Specimens were prepared according to the standard thin-section techniques outlined in Hass & Rowe (1999). Pieces of peat were mounted on microscope slides using Hilquist 2-part A-B epoxy compound, and cut to a thickness of ~250 μm. The wafer was then ground to a thickness of ~50–65 μm and analyzed in transmitted light. Digital images were captured with a Leica DC500 digital camera and processed using Adobe Photoshop CS 6. Images of the same specimen were recorded at multiple focal planes and stacked to produce composite images (Pl. 1, Figs 2, 5–7, 9, 10, 13–17 in this study; e.g., Bercovici et al. 2009). Measurements were taken using ImageJ 1.48b software (Abrâmoff et al. 2004). Specimens and slides are deposited in the Paleobotanical Collections, Biodiversity Institute, University of Kansas (KUPB) under accession numbers KUPB 15312–15375, 19967–19969, and 30713–30745.

3. Results

3.1 Host leaves

Glossopteris leaves from both localities are mostly preserved in a more or less advanced state of degradation, and thus lack diagnostic characters that could be used to assign them to a species; intact leaves also occur but are comparatively rare. Paradermal sections of the leaves show the large meshes

Plate 1: Fungal remains associated with Permian Glossopteris leaves from Antarctica. (1) Peat containing highly degraded Glossopteris leaves (L) in cross section; brackets denote leaf ends; slide no 30713; scale bar = 250 μm. (2) Vascular bundle of Glossopteris containing septate hypha (arrow); slide no 30713; scale bar = 25 μm. (3) Degraded mesophyll containing chytrid-like structure with minute filament (arrow) and possible discharge opening; slide no. 30713; scale bar = 5 μm. (4) Chytrid-like organism with discharge pore (arrow); slide no. 30714; scale bar = 5 μm. (5) Hypha in cross section on leaf surface (arrow); slide no. 30715; scale bar = 10 μm. (6) Leaf with preserved epidermis (Ep) showing fungal hyphae (arrows) in mesophyll and on adaxial surface; slide no. 30716; scale bar = 50 μm. (7) Hyphae penetrating mesophyll cell wall (arrow); slide no. 30717; scale bar = 25 μm. (8) Degraded Glossopteris leaf (epidermis of leaf = Ep) containing clusters of spores (arrow); slide no. 30714, scale bar = 250 μm. (9) Detail of Pl. 1, Fig. 7, showing cluster of spores (S), each enveloped in a mantle composed of septate, thickened toruloid hyphae (arrows); scale bar = 10 μm. (10) Detail of Pl. 1, Fig. 9, showing hyphal mantle; scale bar = 5 μm. (11) Fungal mycelium (M) in matrix between Glossopteris leaves (vascular bundle = VB); slide no. 30715; scale bar = 100 μm. (12) Detail of Pl. 1, Fig. 9, showing largely unbranched hyphae forming a mycelium; scale bar = 25 μm. (13, 14) Putative chlamydomonas spores; white arrow in Pl. 1, Fig. 11 indicates hyphal attachment, black arrow in Pl. 1, Fig. 11 and white arrow in Pl. 1, Fig. 12 show internal sphaerules (probably reproductive units of mycoparasites); slides no. 30718 and 30719; Scale bars = 25 μm. (15) Small fungal spores (S); slide no. 30720; scale bar = 25 μm. (16) Detail of spore type in Pl. 1, Fig. 15 showing surface ornamentation (arrow); scale bar = 10 μm. (17, 18) Combresomyces-like putative peronosporomycete oogonium; arrows indicate papillations of oogonial wall bearing antler-like extensions; slides no. 30721 and 15366; scale bars = 25 μm.
that represent the second-order venation, and often also the prominent midrib, thus attesting to the affinities of the leaves to *Glossopteris*. In transverse section, the bundle sheath, when preserved, is composed of thin-walled cells that sometimes include dark contents. Stomata are rarely preserved, but when observed, they are sunken. Typically the only remaining components of the leaf that are consistent preserved are the parallel, thick-walled vascular bundles (Pl. 1, Fig. 1).

More than 200 intact leaves and leaf fragments from Skaar Ridge and 50 fragmented leaves from Collinson Ridge were examined. Fungal remains were detected in ~10% of the degraded leaves and leaf fragments from Skaar Ridge, whereas the material from Collinson Ridge yielded only a single microorganism remain. Interestingly, none of the intact *Glossopteris* leaves from either locality yielded evidence of fungal colonization.

### 3.2 Fungi associated with *Glossopteris* leaves

The most common fungal remains associated with degraded *Glossopteris* leaves are ramifying, septate hyphae (2–4 µm in diameter) that penetrate the walls of individual tracheids within the vascular bundles (Pl. 1, Fig. 2). Additional fungal remains in tracheids include spherical structures that fully occlude the host cells, with attachment points at the base of the spheroidal structure to the host. In the highly degraded mesophyll of several leaves, small circular to pyriform structures occur that range from 8–10 µm in diameter. Only one of these structures (Pl. 1, Fig. 3) shows what appears to be a rhizomycelial attachment or subtending hypha/filament <1 µm in diameter. An additional feature of these structures includes a putative discharge pore (Pl. 1, Fig. 4). Other leaves show fungal hyphae extending along the leaf surface (Pl. 1, Fig. 5). Many of the leaves also contain tenuous hyphae, usually less than 1 µm in diameter, that branch at right angles and are septate (Pl. 1, Fig. 6). Leaves in which the mesophyll tissue is more or less intact may also contain larger septe hyphae that directly penetrate through the cell walls (Pl. 1, Fig. 7).

Clustered spores surrounded by what appears to be a hyphal mantle or mycelium have been found in two transversely sectioned leaves (Pl. 1, Figs 8, 9). Spores are up to 37 µm in diameter, and the interwoven hyphae enveloping the spores are 2 µm in diameter (Pl. 1, Fig. 9, 10). There is also a single specimen of a larger portion of a mycelium composed of interfaced, largely unbranched septate hyphae (Pl. 1, Figs 11, 12).

### 3.3 Fungal remains in the matrix surrounding the *Glossopteris* leaves

Eight morphologically distinct types of fungal reproductive units, as well as several types of remains attributable to the fungus-like *Peronosporomycetes*, have been discovered in the ambient peat matrix surrounding the *Glossopteris* leaves. These have been designated as numbered morphotypes because each lacks sufficient characters to assign it formally to a taxonomic category.

Fungal reproductive units that display a hyphal attachment are all terminal, and there is no evidence of the parental system on which they were produced. Morphotype 1 is ~40 µm in diameter; wall features are not discernible. Some of the units contain between 1 and 3 internal sphereules, each approximately 10 µm in diameter. Morphotype 2 is slightly larger than type 1 (i.e., up to 50 µm in diameter) and the wall is up to 3 µm thick. The subtending hypha is approximately 5 µm in diameter. Internal sphereules present in some of the specimens range from 6 to 10 µm in diameter (black arrow in Pl. 1, Fig. 13). Morphotype 3 is similar to type 2, up to 45 µm in diameter, but the wall is thinner (<2 µm thick). This type is only found within fragments of *Vertebraria* root tissue co-occurring with the leaves in the litter mats. Morphotype 4 is slightly pyriform, approximately 35 µm at the widest point, and possesses a wall 4–5 µm thick; a hyphal attachment may be visible at the narrow end. Morphotype 5 is ~80 µm in diameter and has a wall that is 6–8 µm thick; small spherical structures may be present in the lumen (arrow in Pl. 1, Fig. 14). Morphotype 6 ranges from 15 to 25 µm in diameter and occurs within degrading plant tissues and the matrix, and at varying focal planes reveals some surface ornamentation (Pl. 1, Figs 15, 16). Morphotype 7 is approximately 40 µm in diameter, with a distinctly two-layered wall that reaches 5–7 µm in thickness. Morphotype 8 is also characterized by a two-layered wall up to 8 µm thick, but is up to 50 µm in diameter and shows a blunt attachment point.

Pyriform to ovoid structures up to 80 µm in diameter that occur scattered throughout the peat closely resemble the fossil genus *Combresomyces*, and thus are interpreted as oogonia of members of the fungus-like *Peronosporomycetes* (see Dotzler et al. 2008; Schwendemann et al. 2009; Slater et al. 2013). All are characterized by a surface ornament composed of antler-like extensions positioned on hollow, column-like or broadly triangular papillations of the wall (Pl. 1, Figs 14, 17). Moreover, most occur at the tip of a short segment of the parental hypha; a septum is sometimes visible between the oogonium and subtending hypha. One specimen of this type represents the only microorganism recorded to date from the peat at Collinson Ridge (Pl. 1, Fig. 18).

### 4. Discussion

The fungal remains described here represent the first evidence of fungi associated with *Glossopteris* leaves from Antarctica. This discovery is important because documented evidence of fungi residing on...
and in the leaves of glossopterid seed ferns, one of the dominant plant groups in Gondwana during the Permian, is generally scarce. We are aware of only two previous reports on fungal associations with *Glossopteris*, and these are from Indian compression material (Bajpai & Maheshwari 1987; Srivastava 1993).

In general, modern fungal leaf endophytes are defined functionally by their occurrence within asymptomatic (i.e., lacking visible disease symptoms at the moment of detection) tissue of living leaves (Schulz & Boyle 2005). This is problematic when studying fossil material. Krings et al. (2009) therefore offered the suggestion that, with fossils, the designation fungal endophyte should be understood as a strictly descriptive term, and used for all fungi that occur within intact plant cells or tissues in which there are no visible disease symptoms. However, when assessing the nutritional mode(s) of fungi associated with fossil leaves, it is necessary to evaluate as to whether the host leaf was alive and functional when colonized by the fungus or in the process of decay. Colonization of living leaves can be inferred by structural features such as host responses (e.g., callosities). However, without such features the condition of the host at the time of colonization cannot normally be resolved.

Although it is impossible at present to determine the nutritional mode(s) of the fungi associated with the *Glossopteris* leaves reported here, it is likely that the majority, if not all, were saprotrophs that were active in the degradation of organic matter on the forest floor, rather than colonizers of living leaves, or endophytes that acted as latent decomposers (Rajala et al. 2014). Support for this hypothesis is the absence of fungi associated with intact *Glossopteris* leaves. Leaf litter communities today include multiple phyla of fungi that exhibit different types of nutritional modes, including mutualism, saprotrophism, and parasitism (Voňášková & Baldrian 2013). The globose fungal reproductive units that occur in the peat matrix (Pl. 1, Figs 11, 13) are interpreted as chlamydospores produced by glomeromycotan fungi based on morphological similarities to the spores of certain extant Glomeromycota (e.g., Stürmer 2012). Many of the fossil chlamydospores occur in close proximity to narrow-diameter *Vertebraria*-type glossopterid rootlets that regularly co-occur with the *Glossopteris* leaves in the peat. *Vertebraria* rootlets have been shown to harbor endomycorrhizal fungi with affinities to the Glomeromycota (Harper et al. 2013), and it is therefore highly probable that some of the spores described here represent the propagules of these mycorrhizal fungi. Other fungal remains that are spheroidal might represent chytrid-like organisms based on the presence of what appear to be discharge pores (Pl. 1, Fig. 4) and rhizomycelial attachments (Pl. 1, Fig. 3). Finally, the clustered spores/sporangia enveloped in a hyphal mantle (Pl. 1, Figs 7, 8) may have affinities to the Mucoromycotina.

The general scarcity of documented evidence of fungi associated with *Glossopteris* leaves is surprising because glossopterid seed ferns were dominant elements in many forest ecosystems across Gondwana, and have been intensely studied for more than 150 years. One simple explanation for this apparent absence of evidence may be a general lack of interest in fungi by the scientists studying *Glossopteris* leaves. Moreover, the research focus on the leaves might have created a collection bias for well-preserved specimens rather than those that are tattered or degraded. On the other hand, there might also be biological factors that have contributed to the scarcity of fungi on and in *Glossopteris* leaves, especially from Antarctica.

One factor may be the cuticle of *Glossopteris* leaves. The plant cuticle serves two principal functions, i.e., as a hydrophobic barrier that prevents desiccation, and as a structural defense against several adverse abiotic and biotic factors (Martin 1964; Kerstiens 1996; Nawarth 2006). Moreover, some leaf cuticles contain various secondary compounds that are effective as fungal deterrents (Canhoto & Graça 1999; Domínguez et al. 2011). It is therefore possible to hypothesize that perhaps the cuticle of the *Glossopteris* leaves from Antarctica contained especially high levels of substances with fungicidal properties. Geochemical analyses of *Glossopteris* leaves that might assess the chemical composition of the cuticle have not been conducted to date. However, such techniques have been used with geologically younger fungus-infected and non-infected gymnosperms to assess the lipid content in the cuticle (Tu et al. 2000). The results of this study indicate that the fungi in the infected specimens produced specific post-infection degradational compounds, and thus we can infer that biogeochemical data on fungal interactions has the potential to be extracted from fossil plants.

A second factor that may have contributed to the scarcity of leaf fungi associated with Antarctic glossopterids concerns the physiology of the Antarctic glossopterid seed ferns. Antarctica has remained in the same easterly position during the majority of the Phanerozoic (Torsvik & Cocks 2013). The paleogeographic position of the continent makes the late Paleozoic forest ecosystems of Antarctica a unique setting with no modern analogue. Especially interesting are the light regimes, which would have comprised 4 months of 24-hours light exposure, 4 months of 24-hours total darkness, and 4 months of transitional light regimes (Taylor & Ryberg 2007). One of the many research questions concerning these unique high-paleolatitude forests encompasses leaf longevity in glossopterids, i.e. whether the trees were deciduous or evergreen (Gulbranson et al. 2014). The presence of *Glossopteris* leaf mats has been used to suggest that the trees were deciduous (Axelrod 1984); however, recent studies indicate that the forests contained mixed populations of evergreen and deciduous trees (Gulbranson et al. 2012, 2014). Schwendemann...
(2010) suggests that Glossopteris plants exhibited a mixed C3-C4 intermediate physiology that may have played a vital role as an adaptation to the extreme light regimes. Factors such as seasonality and leaf age are known to greatly influence the abundance and diversity of leaf fungi in modern plants (e.g., Osono 2008). Low foliar fungal diversity in plants from high latitude ecosystems has been suggested to be due in part to the high amount and prolonged exposure to light in these environments (Millberg et al. 2015). It is therefore possible to envision that the extreme conditions which governed the polar Permian ecosystems had a significant effect on fungal colonization of Glossopteris leaves.

A third aspect concerns the fact that the permineralized Glossopteris leaves and leaf mats from Antarctica all represent allochthonous assemblages of plant parts that have been exposed to the peat-forming process for some time and then transported. This may have resulted in leaves being exposed to high levels of mechanical destruction prior to permineralization. The depositional setting for the peat deposits from Skaar Ridge has been interpreted as peat belts that were rafted (prior to permineralization) into the depositional area during a flood and then became stranded on sand bars as flood waters subsided (Taylor et al. 1989). These processes might have removed or destroyed fungi that, if at all present, were only loosely attached to the leaves (e.g., epiphyllous fungi). Moreover, it is possible to speculate that the decayed and fragmented Glossopteris leaves probably represent a late stage in the degradation process in which they no longer provided the necessary nutrients for saprotrophic fungi, and thus were rarely colonized.

5. Conclusion

The evidence presented in this paper suggests that fungi are generally scarce as inhabitants of Permian Glossopteris leaves from Antarctica, and limited to saprotrophs on the forest floor. However, we are currently unable to determine whether this scarcity of evidence reflects a preservation bias, or if living Antarctic Glossopteris leaves were in any way colonized by fungi. Reports of what appear to be microthyriaceous fungi on Glossopteris from India (Bajpai & Maheshwari 1987; Srivastava 1993) suggest that at least some glossopterid leaves in vivo were inhabited by fungi. One interesting speculation suggests that the environmental conditions governing the Antarctic continent during the Permian, along with special adaptations evolved by the plants exposed to these conditions, adversely affected fungal colonization. While there is currently no evidence to support this hypothesis, geochemical analyses (e.g., biomarker analysis) represent a promising new tool with which substances related specifically to the presence of fungi may be identified in fossil leaves, and thus would demonstrate the presence of fungi in the absence of body fossils (Harper et al. 2015). We hope that by reporting the scattered evidence that is available we will further stimulate interest in searching out fungal remains in and on leaves throughout the geologic record, and thus increase the body of data that can be used to document precisely when and how certain types of fungal associations and interactions with the phyllosphere initially evolved.

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