Spore wall ultrastructure and development in a basal euphyllophyte – *Psilophyton dawsonii* from the Lower Devonian of Quebec (Canada)

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Running head: SPORE WALL STRUCTURE AND DEVELOPMENT IN A BASAL EUPHYLLOPHYTE

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> Sol Noetinger, Ph.D.; Sandra L. Strayer; Alexandru M.F. Tomescu, Ph.D.
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PREMISE OF THE STUDY: Euphyllophytes – a clade including living ferns, horsetails, and seed plants – have a rich fossil record going down to the Early Devonian. The euphyllophyte spore wall has complex structure, the evolutionary origins of which are incompletely understood. *Psilophyton* is the best characterized basal euphyllophyte genus, thus data on this genus can inform current hypotheses on spore wall structure and development, which propose a bilayered spore wall organization of combined spore and sporangial origin for the ancestral euphyllophyte.

METHODS: We employed cellulose acetate peel sectioning of permineralized Lower Devonian (Emsian) *Psilophyton dawsonii* sporangia, combined with electron microscopy, to document spore wall structure and development.

KEY RESULTS: The *Psilophyton dawsonii* spore wall has bilayered structure. The inner spore wall is homogeneous, probably of lamellar construction. The outer spore wall, loosely attached to the inner wall, covers distal and equatorial spore areas, and has a foveolate base layer upon which stacks of sporopollenin lumps accrete centrifugally, forming the scaffolding for the final apiculate ornamentation.

CONCLUSIONS: This is the most complete account on spore wall structure, allowing developmental interpretations, in a basal euphyllophyte. The bipartite organization of the *Psilophyton dawsonii* spore wall reflects development as a result of two processes: an inner layer laid down by the spore cell and an outer layer of tapetal origin. Providing direct evidence on the spore wall of a basal euphyllophyte, these data confirm previous hypotheses and mark an empirically-supported starting point for discussions of the evolution of spore wall development in euphyllophytes.

Keywords: development, Devonian, euphyllophyte, fossil, sporoderm, trimerophyte, ultrastructure

In the mid-19th century, Sir John W. Dawson published a reconstruction of a vascular plant from the Gaspé region of southeast Canada, under the name *Psilophyton princeps* Dawson (1859) emend. Hueber 1967. Since that initial description, numerous contributions were published on additional *Psilophyton* species. Banks (1968) included *Psilophyton* Dawson emend. Hueber et Banks in Subdivision Trimerophytina, a group including plants characterized by dichotomous to pseudomonopodial branching, lateral dichotomous or trifurcate axes, terminal clusters of fusiform sporangia with longitudinal dehiscence, sporangial walls several layers thick and a large central protostele. Two other genera were included in the Trimerophytina – *Pertica* Kasper & Andrews emend. Doran et al. (1978) and *Trimerophyton* Hopping (1956) –, but *Psilophyton* is the most speciose and most completely characterized member of the group. More recenty, *Psilophyton* has been included in Subdivision Euphyllophytina by Kenrick and Crane (1997).

Psilophyton represents an important component of fossil floras throughout the first half of the Devonian. Of the several *Psilophyton* species described over the last one and a half century, ten are currently recognized as unequivocal members of the genus. These include *Psilophyton burnotense* Kräusel et Weyland (1948) (including *Psilophyton goldschmidtii* Halle); *Psilophyton forbesii* Andrews, Kasper et Mencher (1968) (including *Dawsonites arcuatus* Halle and *Psilophyton arcuatum* Schweitzer; according to Gerrienne, 1997b); *Psilophyton dapsile* Kasper, Andrews et Forbes (1974); *Psilophyton microspinosum* Kasper, Andrews et Forbes (1974); *Psilophyton dawsonii* Banks, Leclercq et Hueber (1975); *Psilophyton charientos* Gensel (1979); *Psilophyton crenulatum* Doran (1980); *Psilophyton parvulum* Gerrienne (1995); *Psilophyton genseliae* Gerrienne (1997a), *Psilophyton primitivum* Hao et Gensel (1998). Along with Dawson's *P. princeps* emend. Hueber (1967), these bring the number of species to a total of 11.

These different species are distributed throughout the northern hemisphere and range in age from Pragian to early Eifelian, ca. 410-390 Ma [(Kasper et al., 1974; Allen and Gastaldo, 2006; Hao and Xue, 2013); all absolute ages rounded up based on data from Cohen et al., 2013 (updated 2017)], although some are possibly as old as late Lochkovian, ca. 412 Ma (Gerrienne, 1993).

A few additional species require consideration. *Psilophyton kräuselii* Obrhel (1959) is known from Givetian (ca. 387-383 Ma) rocks in the Czech Republic (Berry and Fairon-Demaret, 2001), from material in which the fertile specimens are not preserved well enough to demonstrate the arrangement of sporangia (Berry et al., 2000). For the Emsian (ca. 407-394 Ma) *Psilophyton szaferi* Zdebska (1986), several authors have recommended inclusion in a different genus, based on sporangial morphology (Gerrienne, 1997a; Hao and Gensel, 1998; Hao and Xue, 2013). Finally, another Emsian species, *Psilophyton coniculum* Trant et Gensel (1985), has been described exclusively from vegetative axes, although these exhibit anatomy closely similar to other *Psilophyton* species (e.g., *P. dawsonii*). All other *Psilophyton* species described over the years have either been synonymized into other genera, or are based on sterile compression material that does present the minimal set of characters needed for inclusion in the genus.

The Early Devonian sequence exposed on the Gaspé Peninsula of Quebec (Canada) hosts rich fossil floras (Gensel and Andrews, 1984; Bourque et al., 2005) that have been influential in understanding not only the morphology of early land plants, but also their ecology (Griffing et al., 2000; Hotton et al., 2001). These floras, extensively studied ever since the 19th century explorations of Logan in 1843 (Hueber, 2001), include the one that yielded Dawson's *Psilophyton princeps*, the type species of genus *Psilophyton*, as well as the first anatomically-preserved species in this genus, *P. dawsonii*, described by Banks et al. (1975). *Psilophyton*

dawsonii is one of only six *Psilophyton* species for which *in situ* spores have been reported. Here, we document spore wall structure in *Psilophyton dawsonii* from material preserved in one of the main rock units hosting plant fossils on the Gaspé Peninsula, the Battery Point Formation, and we provide interpretations on sporoderm development in the species, based on observations of sporoderm variability among spores within a single cluster of quasi-mature sporangia. These data confirm previous hypotheses on spore wall structure and development in basal euphyllophytes. They also lead to an emended diagnosis of *P. dawsonii*.

MATERIAL AND METHODS

The material was collected in 1965 by Dr. Francis M. Hueber, from exposures of the Battery Point Formation on the south shore of Gaspé Bay, close to Douglastown (Quebec, Canada). The Battery Point Formation ranges in age from early Emsian to early Eifelian, based on brachiopod and spore biostratigraphy (Boucot et al., 1967; McGregor, 1973, 1977; Richardson and McGregor, 1986; McGregor and Playford, 1992). The cobble housing the sporangia studied here comes from a locality between Tar Point, where layers of the Battery Point Formation are early Emsian in age, and Douglastown, where the Battery Point Formation have been assigned a late Emsian age (Hoffman and Tomescu, 2013). Consequently, the age of the fossils reported here is mid to late Emsian, ca. 400-395 Ma.

The fossil sporangia containing *in situ* spores are preserved by cellular permineralization with calcium carbonate. The material presented here comes from two contiguous clusters of sporangia (i.e., the sporangia in each cluster are all connected to the same base) containing masses of spores, which are preserved in slabs B and E of the Gaspé cobble deposited in the U.S.

National Museum of Natural History – Smithsonian Institution under collection number USNM 558725. This material was studied in serial anatomical sections obtained using the cellulose acetate peel technique (Joy et al., 1956). For light microscopy, acetate peel material was mounted on slides using Eukitt xylene-soluble medium (O. Kindler GmbH, Freiburg, Germany) and imaged using a Nikon Coolpix 8800VR digital camera (Nikon, Melville, New York, USA), mounted on a Nikon E400 compound microscope. All samples studied in electron microscopy come from the sporangial cluster in slab E of USNM 558725 (peels E bottom). For scanning electron microscopy (SEM), spores were extracted from acetate peel cuttings by acetone dissolution and retrieved on Omnipore membrane filters (MilliporeSigma, St. Louis, Missouri, USA) using a Millipore vacuum micro-funnel. The filters holding fossil material were mounted on aluminum stubs, coated with 100 Å Au on a Desk II sputter coater (Denton Vacuum, Moorestown, New Jersey, USA), and examined using an ABT-32 microscope (Topcon, Paramus, New Jersey, USA). For transmission electron microscopy, acetate peel cuttings containing spores were embedded in ERL 4221 epoxide resin (Polysciences, Warrington, Pennsylvania, USA) and sectioned on a Leica Ultracut R ultramicrotome (Leica Microsystems Inc., Buffalo Grove, Illinois, USA). Sections were stained with uranyl acetate and lead citrate, and imaged on a Philips EM208S microscope (Philips Electron Optics B.V., Eindhoven, Netherlands). Images were processed using Adobe Photoshop software (Adobe, San Jose, California, USA). Measurements were performed using ImageJ 1.49v (U.S. National Institutes of Health, Bethesda, Maryland, USA; Rueden et al., 2017).

SYSTEMATICS

Subdivision—Euphyllophytina Kenrick et Crane

Genus—*Psilophyton* Dawson

Species—*Psilophyton dawsonii* Banks, Leclercq et Hueber emend. Noetinger, Strayer et Tomescu

Emended specific diagnosis *(emendations in bold face)*—Characteristics as for the genus. Vegetative branches three-dimensional and of two sizes; terminated by slender, blunt tips in various attitudes; fertile branches usually dichotomize six times before terminating in clusters of approximately 32 pairs of sporangia 3.0-5.0 mm long by **0.5-**1.5 mm in diameter**; sporangia twisted around each other within each pair; sporangial epidermis of large tubular cells, most of which have differentially thickened outer periclinal walls;** sporangial wall at maturity **consisting of up to 5 cell layers, cells parenchymatous, smaller than those of sporangial epidermis, outermost cell layer (beneath epidermis) often with thickened walls; innermost cell layer with resistant inner periclinal walls that form a membrane around the spore content upon decay of the parenchymatous sporangial wall**; both abortions of sporangia and of limbs of some dichotomies cause variations in total number; each of the first two apparent dichotomies is actually two closely spaced dichotomies each of which should result in three branches but one of the three aborts leaving a stump between the two remaining limbs. Protostele relatively large, ¼ the diameter of the stem; centrarch; becoming massive with several enlarged protoxylem areas and many radially aligned tracheids at the level of vegetative branching; in the area of fertile branching protoxylem branches alternately and distichously foreshadowing a similar arrangement of lateral branches; trace to fertile branch at first terete, soon characteristically rectangular; xylem all tracheids; scalariform pitted to modified

148 scalariform resembling rudimentary circular pits; phloem and inner cortex parenchymatous,
149 outer cortex collenchymatous with substomatal chambers; epidermis of small boxshaped cells;
150 spores, 40-**86** μm in diameter, **proximal surface** smooth, with darkened areas around
151 commissures, curvaturae; spores resemble the dispersed spore genera *Retusotriletes*,
152 *Phyllothecotriletes* or ***Apiculiretusispora*****; spore wall bilayered; inner spore wall smooth;**
153 **outer spore wall restricted to distal and equatorial surface of spores, easily detached from**
154 **inner layer, at maturity bearing dense spines, many >1 μm tall.**
155
156 **DESCRIPTION**
157 ***Sporangia***—The sporangia are identical in shape, size, and anatomy, to the sporangia of
158 *Psilophyton dawsonii* described and illustrated by Banks et al. (1975). The sporangia form pairs
159 attached to a common stalk. Within each pair, the sporangia are twisted around each other,
160 *Psilophyton*-style (Fig. 1A-C). They are elongated, fusiform, 0.5-0.8 mm thick, and >3.5 mm
161 long (based on observations in serial sections). Each sporangium has a longitudinal dehiscence
162 area along the side that is facing the other sporangium in the pair (Fig. 1C). The sporangial wall,
163 several layers thick, tapers to only one-cell thick in the dehiscence area (Fig. 1B, C). Dehiscence
164 slits are open, but as a result of taphonomic and not physiological processes (see discussion of
165 taphonomy below). The sporangium epidermis consists of tubular cells (Fig. 1B, D), the
166 majority of which have considerably thickened outer periclinal walls (Fig. 1C). Beneath the
167 epidermis, a one- to two-cell thick layer exhibits thinner cell walls. The spores occur as tightly
168 packed masses contained inside a membranous sac (Fig. 1B, C). The empty spaces between the
169 spore masses and the cells of the sporangial walls is interpreted as corresponding to the inner

parenchymatous layers of the sporangial wall probably representing a cellular secretory tapetum, which was not preserved (see discussion of the nature of the membranous sac containing spore masses, below) – incomplete preservation of parenchymatous tissues is a general issue in the assemblage that yielded these fossils (see discussion of taphonomy below).

Spores—The numerous spores are partly collapsed or folded (Figs. 1D, 2, 3). Pyrite framboids (spheroidal aggregation of small pyrite crystals) scattered throughout the material (Fig. 3A) indicate an anoxic environment of burial and preservation. Observation in both light microscopy (LM) (Figs. 1, 2) and scanning electron microscopy (Fig. 3) shows trilete spores with subcircular to subtriangular amb (Figs. 2B, C; 3B, C), 49-86 µm in diameter. The trilete mark extends 50-75% of the spore radius. LM reveals specimens (Fig. 2B, C) with darkened, subcircular to elongated areas extending between 50% and 60% of the interradial zone (polumbra). Based on these features, the spores would correspond to the dispersed spore *Apiculiretusispora brandtii* Streel (1964), albeit with slightly taller sculptural elements. *Apiculiretusispora brandtii* was reported by McGregor (1973) from the Battery Point Formation in the Gaspé Bay area. This species is currently part of the *Apiculiretusispora brandtii* morphon proposed by Breuer and Steemans (2013).

The sporoderm comprises two layers: an inner smooth layer (Figs. 3A-F, I) 0.4-1.6 µm thick (ca. 0.7 µm on average); and an outer ornamented layer restricted to the distal and equatorial surface of spores and usually detached from the inner layer around most of the spore, (Fig. 3A, C-F, I). Several types of sculptural elements are present on the spores: granules, stacked rounded-tabular units, and spines 0.8-1.6 µm long x 0.4-0.8 µm wide, with acute or rounded tips, sometimes curved (Fig. 3D, F, I-L). These sculptural elements are positioned on a

basal layer with overall foveolate relief (Fig. 3G-H, J, K). The basal layer is formed by irregular juxtaposition of several coats of anastomosing sporopollenin scraps that form network-like patterns with lumina up to 1 μm in diameter (Fig. 3G, H, K).

Transmission electron microscopy adds resolution to the structure of the two sporoderm layers (Fig. 4). The inner layer is homogeneous (Fig. 4A-D). The outer ornamented layer, slightly less electron-dense than the inner layer, can be detached from the latter at places (Fig. 4A, B, E, F). The outer layer consists of an aggregation of sporopollenin lumps which anastomose, forming the foveolate basal layer, ca. 0.5 μm thick (Fig. 3G, H, K). This basal layer supports regularly spaced stacks of rounded tabular units 0.1-0.7 μm wide and 0.01-0.2 μm thick, whose size decreases from base to top (i.e. centrifugally with respect to the spore). The stacks, consisting of up to seven superimposed tabular units, can be topped by a fine spine (Figs. 3L; 4E, F). The arrangement of these sculptural elements on the surface of a spore is such that they interdigitate with the corresponding elements of the adjacent spore (Figs. 3F, 4F), indicating tight packing of the mature spores inside sporangia.

DISCUSSION

***Attribution to* Psilophyton dawsonii**—The sporangia presented here represent dispersed clusters that are not connected to larger axes with *Psilophyton* anatomy. Nevertheless, several lines of evidence converge in their support of the attribution of these sporangia to *Psilophyton dawsonii*. First, they come from the same rock unit (Battery Point Formation) and geographic location (south shore of Gaspé Bay, near Douglastown) as the type specimens of *P. dawsonii* (Banks et al., 1975). Second, typical *P. dawsonii* axes are the most abundant type of fossil in the ca. 15 x

10 x 8 cm cobble that has yielded the sporangia; in fact, the only other identifiable fossils in that cobble are one decorticated *Franhueberia* Hoffman et Tomescu (2013) axis and a zosterophyll axis. Third, the sporangia are identical to those described and illustrated by Banks et al. (1975) for *P. dawsonii*; specifically, their anatomy shows the same features of sporangial wall structure, all the way to the features of vascularization at the base. Fourth, the spores contained in these sporangia are identical to those described and illustrated by Banks et al. (1975) for *P. dawsonii*, with a difference in the interpretation in their development (see below).

Taphonomy—The fossil assemblage that includes the *P. dawsonii* sporangia is allochthonous, as demonstrated by the relatively high degree of fragmentation of the plants, and by their randomized positioning in the host rock. This is consistent with the sedimentary structures present in the Gaspé cobbles and, especially, the positional relationships between these structures and plant fragments, such as the case of an axis fragment that had settled at the bottom of a groove formed between two ripples and was subsequently buried in that position. The duration or distance of transport of the plant material preserved in Gaspé cobbles was significant, as suggested by incomplete preservation of parenchymatous tissues. The majority of the permineralized Gaspé plants retain little (if any) of their phloem, parenchymatous inner cortex, or parenchymatous areas of the outer cortex. This is immediately apparent upon consideration of the totality of *Psilophyton dawsonii* material from this rock unit (including that examined by us and that illustrated by Banks et al., 1975), of which only a minute proportion preserves the parenchymatous inner cortex; furthermore, all other plant types examined by us in the unit follow the same pattern of preservation. Also consistent with prolonged transport are the random taphonomic breaks and distortions that occur at high frequencies in the plant material.

The sporangia presented here preserve spores that were nearing, but had not reached, maturity, as indicated by their fully formed inner wall layer and their outer wall layers preserved at different stages of assembly (see below). Therefore, their necrology must have included traumatic removal from living conditions. The incomplete development of the wall of most of the spores contained in the sporangia indicates that although the latter appear dehisced and the innermost parenchymatous layers of the sporangial walls are missing (Figs. 1A-C), these sporangia were not dehisced at the time they entered the taphonomic pathways. Their partial opening likely represents strain due to external mechanical forces during transport. The missing inner parenchymatous layers of the sporangial walls are consistent with the general pattern of incomplete preservation of parenchymatous tissues observed in the assemblage and due to prolonged transport.

Considerations on the nature of the membranous sacs enclosing **Psilophyton dawsonii** ***spores***—Membranous layers enclosing, usually tightly, smaller groups or larger masses of cryptospores and spores have previously been noted in numerous Silurian-Devonian plants (e.g., Wellman et al., 1998; Wellman, 2009). These layers have been interpreted by Wellman (2009) as the remnants of the final secretory products of a tapetal layer of the sporangium wall. In this interpretation, a cellular tapetum surrounding the sporangial locule with spores, degenerates releasing sporopollenin into the locule and later forming the membrane around the spores. Consistent with this interpretation, the membranes documented by Wellman (2009) preserve no cellular patterns.

In *P. dawsonii*, the sporangial wall, well documented by Banks et al. (1975; fig. 44-49), includes an inner parenchymatous layer, several cells thick, which probably represents or

includes a tapetal layer. The fact that this parenchymatous layer is relatively infrequently preserved could have a developmental explanation, i.e., tapetal degeneration. Banks et al. interpret the membranous sacs they document around the spore masses as consisting of the inner tangential walls of the innermost parenchyma of the sporangium wall, left over after breakdown of the parenchymatous layer as a part of sporangium maturation. Consistent with this interpretation, the membranes, when isolated from sporangia with or without their spore content, exhibit conspicuous cellular patterns (Banks et al., 1975; fig. 53-55). These patterns exhibit cells of similar shape and size, oval to rectangular, elongated perpendicular to the long axis of the sporangium and to the direction of elongation of the tubular cells of the sporangial epidermis. These cellular patterns are consistent with a strictly cellular nature of the *P. dawsonii* membranous sacs, i.e. consisting of cell wall material and, thus, different from the membranes discussed by Wellman (2009), which are hypothesized as consisting exclusively of sporopollenin.

What is less clear is whether the origin of the membranous sacs is primarily developmental or preservational. A taphonomic explanation is equally possible, considering the striking anatomical parallelism between the anatomy of *P. dawsonii* axes and sporangia (noted by Banks et al., 1975), and the fact that in the same material containing the sporangia the axes also show frequent incomplete preservation of parenchymatous tissues. Either way, the membrane sacs represent the inner tangential walls of cells that comprised a cellular tapetum. These walls had higher preservation potential than the rest of the parenchymatous component of the sporangial wall. This could have been due to impregnation with sporopollenin or sporopollenin precursors during secretion of the latter in the process of assembly of the outer

sporoderm wall.

Comparisons with* Psilophyton *and trimerophyte spores—Basal euphyllophyte (i.e. trimerophyte) spores have a partially detached outer ornamented layer (Table 1 and references therein) (Wellman, 2009). Depending on the degree of separation in between this outer layer and the inner layer, spores can be attributed to different *sporae dispersae* genera: *Apiculiretusispora* Streel emend. Streel (1967), for those that retain the ornamented layer, and *Retusotriletes* Naumova emend. Streel (1964) or *Calamospora* Schopf, Wilson et Bentall (1944) for the ones that have lost the sculptured layer (Gensel, 1980). Spores of these types are produced by several plants, including species in three trimerophyte genera: *Psilophyton*, *Pertica*, and *Trimerophyton*. The latter comprises only one species, *Trimerophyton robustius* Hopping, which has provided little information for comparisons (Table 1). The *Trimerophyton* material consists of clusters of five to seven spores with an average diameter of 52 μm and smooth walls, with no evidence of wall layer detachment (Hopping, 1956; Gensel, 1980). Genus *Pertica* includes three species, of which only two have yielded information on spores (Table 1). *Pertica varia* Granoff, Gensel et Andrews (1976) and *P. dalhousii* Doran, Gensel et Andrews (1978) spores are quite similar (Gensel, 1980). They both lack a darkened interradial area (polumbra) and have sculptural elements up to but generally smaller than 1 μm tall.

Only six of the *Psilophyton* species described to date have yielded *in situ* spores (Table 2). The spores we describe here fit the general description of *Psilophyton* spores provided by Gensel and White (1983). Detailed comparison with *P. crenulatum* (Doran, 1980) and *P. parvulum* (Gerrienne, 1995) reveals significant differences. The former has smaller ornamental elements (only up to 1 μm high), while the latter has stouter, equidimensional elements also only up to 1

μm in size. *Psilophyton princeps* has been described with highly variable spores, both in size and in features of the proximal face, encompassing a range from which some members could fit into other *Psilophyton* species (i.e. *Psilophyton charientos*; see Table 2 and references therein). This could be due, in part, to the taxonomic confusion surrounding this species, discussed by Hueber (1967). The spores of *P. charientos*, *P. forbesii*, and *P. dawsonii* are closely comparable, according to Gensel and White (1983). Nonetheless, *P. forbesii* is the only *Psilophyton* species that has provided ultrastructural information, to date, allowing for comparisons with the *P. dawsonii* spores presented here. *Psilophyton forbesii* spores have ultrastructure similar to that documented here for *P. dawsonii*. However, in the *P. dawsonii* spores we could not observe the lamellae reported by Gensel and White (1983) in *P. forbesii*. For *P. dawsonii*, Gensel and White (1983) report two types of ornamentation, depending on the type of preservation of the material. Thus, *P. dawsonii* spores extracted from shales show discrete coni or grana, whereas spores preserved in calcareous nodules exhibit anastomosing rugulae. Based on our observations of spore wall structure and inferences on spore wall development in *P. dawsonii*, it is possible that this ornamentation-lithology association is coincidental, and that previously reported differences in sporoderm ornamentation represent different stages in spore wall development.

Spore wall development—Current understanding of early tracheophyte spore wall structure and development holds that the plesiomorphic condition in euphyllophytes (i.e., in trimerophytes), as illustrated by *Apiculiretusispora*, consists of an inner lamellate layer, laid down by the spore cell, and an outer layer derived from the sporangial tapetum and present only outside of the contact face (Wellman, 2009). However, as pointed out by Wellman (2002), interpretation of spore wall development in fossils in the absence of appropriate samples documenting successive

developmental stages is problematic. This is because the early stages of structural features often become masked as walls mature, due to sporopollenin accretion. Nevertheless, detailed analysis of the mature ultrastructure of fossil spores, combined with information on spore wall development in extant plants, can and has been used to construct hypothetical developmental pathways for the spores of extinct plants (Taylor, 2000; Wellman, 2002). In fact, this type of reasoning led to the hypothetical pathway of spore wall development proposed for *Apiculiretusispora*, a type of *sporae dispersae*, and for the ancestral trimerophyte.

The wall structure of the *Psilophyton dawsonii* spores we present here is broadly consistent with previous reports on *Psilophyton* spores, in general, and with those of *P. dawsonii* spores (Banks et al., 1975; Gensel and White, 1983). Banks et al. (1975) stated that young *P. dawsonii* spores are surrounded by a minutely ornamented layer, which breaks away with age, implying that the spores were dispersed without the outer wall layer. Our material indicates, instead, that spore maturity involved a fully developed outer layer, consistent with the spore wall developmental pathway proposed for the spores of the ancestral euphyllophyte, in general, and for *Apiculiretusispora*, in particular (Wellman, 2009). This material also provides details interpreted as a developmental sequence of the outer wall layer, and provides a potential explanation for some differences in *P. dawsonii* spore ornamentation reported by Gensel and White (1983).

Wellman and Gensel (2004) characterized the inner layer of *Psilophyton* spore walls as "probably lamellated". Indeed, lamellae are visible in the inner wall of *P. forbesii* spores illustrated by Gensel and White (1983). The inner layer of our *P. dawsonii* spores seems to have homogeneous structure and no lamellae are visible (Fig. 4A-D). Taylor (2000) has pointed out

346 that although lamellae produced by the spore cell are present in the walls of all modern plant
347 spores, at some point during their development, they may be occluded later in development by
348 the addition of sporopollenin from the tapetum cells. Thus, if a lamellar ultrastructure
349 characterizes the inner spore wall layer in all *Psilophyton* species, our *P. dawsonii* specimens
350 represent late developmental stages of this layer.

351 The outer loose layer of the spore wall in *Psilophyton* was initially posited as tapetally-
352 derived by Banks et al. (1975) – in their labeling it as "? perispore", i.e., a layer formed by
353 accumulation of tapetally-derived globules. This interpretation was later upheld by Gensel and
354 White (1983) and by Wellman and Gensel (2004). Our *P. dawsonii* spores observed in electron
355 microscopy come from two sporangia that are part of the same pair in a sporangial cluster and
356 that were close to maturity. This is suggested by the fully formed inner spore layer with what we
357 interpret as a masking of the lamellar ultrastructure of the inner spore wall, as well as by
358 presence of an outer sporoderm layer. The latter displays structural variability between the
359 spores in these two sporangia that, based on their anatomical relationship, were at the same
360 developmental stage. This structural variability could represent either natural within-sporangium
361 spore variability or a developmental series illustrating late stages of sporoderm formation, more
362 specifically of the tapetally-derived outer sporoderm layer. Because the different variations of
363 outer sporoderm wall elaboration can be arranged in a consistent sequence that makes sense from
364 a developmental perspective, we lean toward the latter, developmentally-based interpretation (as
365 detailed below). Nevertheless, we acknowledge that the former, alternative interpretation cannot
366 be empirically rejected. Importantly, irrespective of which of the two interpretations reflects
367 reality, the observations on outer sporoderm wall variability demonstrate centrifugal accretion of

368 constituents of the outer wall layer, consistent with a tapetal origin.

369 The proximal face of the mature *P. dawsonii* spores is smooth, consistent with previous
370 interpretations proposed for the ancestral euphyllophytes (Wellman, 2009) that the deposition of
371 the outer layer takes place while the spores are disposed as tetrads. Development of this spore
372 wall layer starts with the assembly of the basal foveolate layer (Fig. 3G, H, J, K), as a
373 microlayered sequence of anastomosing elongated scraps of sporopollenin (Fig. 3H). This is
374 followed by the addition of globular-tabular sporopollenin lumps in successive layers. The
375 lumps in successive layers have decreasing size, forming tall coni with girdling constrictions
376 (Figs. 3D, J; 4A, B, E, F). Similar projections that may have a stacked structure have been
377 illustrated by Gensel and White (1983) in the outer spore wall layer of *P. charientos*. In our
378 material, the stacked sculptural elements accrete centrifugally (Fig. 3I, J) and subsequently form
379 the scaffolding of what would become the final sculpture of the outer spore wall layer. This is
380 completed by addition of sporopollenin on the stacked sculptural elements, forming the final
381 spines (Fig. 3L) that are similar to those seen in mature dispersed *Apiculiretusispora* specimens.
382 The spore illustrated by Hotton et al. (2001) from the *Trimerophyton* type locality could also
383 represent a *Psilophyton*-type spore in which the final stage of sporopollenin coating to form
384 spines is nearing completion (as suggested by some of the spines on that specimen, which seem
385 to still exhibit a somewhat stepped profile).

386 This model of development is consistent with the ultrastructure on *P. forbesii* spores
387 illustrated by Gensel and White (1983) in transmission electron microscopy. Under this model,
388 the differences in *P. dawsonii* spore ornamentation discussed by Gensel and White (1983) as
389 potentially representing natural variation or being due to preservation, could be explained as

390 distinct stages of development of the outer spore wall layer. Thus, the anastomosing rugulae they
391 reported in *P. dawsonii* material preserved in calcareous nodules could represent early
392 developmental stages of the outer spore wall, corresponding to the foveolate basal layer and
393 early flakes, whereas the material recovered from shales may represent later developmental
394 stages.

395 Another confirmation of the tapetal origin of the outer spore wall comes from evidence of
396 loose sporopollenin globules present in the vicinity of the sporangium wall (Figs. 2D-F, 4B),
397 similar to those documented in the moss *Andreaea* by Brown and Lemmon (1984). Some of the
398 globules are hollow (Fig. 4B) and resemble orbicules (Ubisch bodies) documented in many seed
399 plants (e.g., Taylor, 1978; El-Ghazaly and Rowley, 1997; Hesse et al., 2009). Tapetal
400 contribution to assembly of the outer spore wall layer is reinforced by what seems to represent
401 instances of disruption of the tapetum, releasing sporopollenin globules (e.g., Fig. 2E).
402

403 **CONCLUSIONS**

404 The spores of *Psilophyton dawsonii* have walls with bilayered structure. The inner layer, laid
405 down by the spore cell, is homogeneous and probably lamellar in construction. The outer layer
406 is loosely attached to the inner layer and covers only the equatorial and distal regions of spores.
407 Observed variability in the structural complexity of the outer sporoderm layer of spores within
408 the same quasi-mature sporangia indicates that this layer develops centrifugally, confirming that
409 it is tapetally-derived. Interpreted in a developmental perspective, this variability suggests that
410 assembly of the outer sporoderm wall begins with formation of a foveolate base layer upon
411 which stacks of tabular sporopollenin lumps of decreasing size form protrusions; the later are

412 covered in sporopollenin in the late stages of spore wall development, forming the tall spiniform
413 ornamentation characteristic of mature spores.

414 *Psilophyton dawsonii* is the best characterized of the basal euphyllophytes, and an
415 important and widespread component of Early Devonian floras. This is the most detailed
416 account of spore wall structure in *Psilophyton* and the only detailed description of spore wall
417 development in a basal euphyllophyte. Our data lead to an emended diagnosis of the species.
418 More importantly, these data provide a direct test for hypotheses on the evolution of
419 euphyllophyte spore wall development. They confirm with direct evidence the set of character
420 states hypothesized, based on younger fossils, for the ancestral euphyllophte spores, and provide
421 an empirically supported starting point for the series of evolutionary changes proposed to explain
422 spore wall development along the euphyllophyte lineage (Wellman, 2009).

423 *Psilophyton* sits at the base of the euphyllophyte lineage and very close to the >400
424 million-year-old origin of this clade. Euphyllophytes comprise the majority of extant vascular
425 plants, and represent most of the plant diversity in the fossil record. Their spores (including the
426 pollen of seed plants) cover tremendous ranges of diversity in terms of morphology,
427 ultrastructure, and development. Understanding the origins of all this diversity requires looking
428 at the base of the clade, where *Psilophyton* is now providing answers on the initial conditions of
429 spore wall structure and development preceding the euphyllophyte evolutionary radiation.
430

431 **ACKNOWLEDGEMENTS**

432 We are indebted to William DiMichele, Carol Hotton, and Jonathan Wingerath (U.S. National
433 Museum of Natural History – Smithsonian Institution) for facilitating specimen loans; Cristina

Tellería (CONICET – Laboratorio de Sistemática y Biología Evolutiva, Museo de La Plata and Universidad Nacional de La Plata) for insightful discussions on spore wall development; . Many people provided support at Humboldt State University: Andrew Thran (SEM), Casey Lu (TEM), Marty Reed and Lewis McCrigler (electron microscopy facilities maintenance); Jeffery Barrett (discussions of *Psilophyton* diversity). Comments from two anonymous reviewers greatly improved the manuscript. The collaboration that led to this publication was made possible by CONICET funding (PIP 11220120100182) to SN and a U.S. National Science Foundation grant (IIA-1322504) to AMFT.

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581 **Table 1.** Comparative table of trimerophyte spores. References: (1) Hopping, 1956; (2) Balme, 1995; (3) Allen, 1980; (4) Granoff, Gensel & Andrews, 1976; (5)
582 Doran, Gensel & Andrews, 1978; (6) Gensel, 1980. References for *Psilophyton* spp. Banks, Leclercq & Hueber, 1975; Doran, 1980; Gensel & White, 1983;
583 Gerrienne, 1995. * Refers only to *Psilophyton forbesii*.
584

Species	**Size (µm)**	**Amb**	**Proximal features**	**Ornamentation**	**Ultrastructure**		**Associated *sporae dispersae***
					Inner layer	**Outer layer**	
Trimerophyton robustius	40-63 [(1)]	Circular [(2)]	Curvaturate [(2)]	Smooth [(1)] or finely conate [(2)]	?	?	*Apiculiretusispora* spp. [(2)], *Calamospora atava*; *C. pannucea* [(2)], *Retusotriletes* spp. [(3)]
Pertica spp.	52-90 [(4, 5)]	Subcircular to subtriangular to ovoid [(4, 5)]	Curvaturate Trilete rays simple, 1/3-1/2 of spore radius [(4, 5)]	Coni or grana that may anastomose (1 µm high) [(4, 5)]	?	Ornamented, partially detached from inner layer [(5)]	*Apiculiretusispora plicata* [(4)], *A*. cf. *arenorugosa* [(3)], *A. arenorugosa* [(2)], *A. brandtii* [(6)], *A. plicata* [(5)]
Psilophyton spp.	40-120	Circular to subcircular	Curvaturate; circular polumbra Trilete rays simple, 1/3-1/2 of spore radius	Coni, grana or anastomosing rugulae	May or may not show lamellae*	Continuous with or separated from inner layer Elements ± equidimensional; stacks of rounded units*	*Retusotriletes* spp., *Apiculiretusispora* spp., *Phyllothecotriletes* spp., *Calamospora* spp., *Punctatisporites* spp.
585
586

587 **Table 2.** Comparative table of *in situ Psilophyton* spores. References: (1) Balme, 1995; (2) Allen, 1980; (3) Gensel, 1980; (4) Gensel & White, 1983; (5)

588 Gerrienne, 1995; (6) Banks, Leclercq & Hueber, 1975; (7) Doran, 1980.

589

Species	**Size (µm)**	**Amb**	**Proximal features**	**Ornamentation**	**Ultrastructure**		**Associated *sporae dispersae***
					Inner layer	**Outer layer**	
Psilophyton princeps	40-120 (1)	Circular (1)	Curvaturate; circular polumbra. Trilete rays simple, 1/3-1/2 of spore radius (4)	Coni or warts (≤ 1.5 µm high; 0.5-1 µm wide) (4)	?	Ornamented, often detached from inner layer (4)	*Retusotriletes* spp. (3) *Apiculiretusispora* spp. (3)
Psilophyton forbesii	48-96 (1, 4, 5)	Circular (1)	Curvaturate; polumbra (1)	Apiculate (1)	May or may not show lamellae (4)	Ornamented, often detached from inner layer (4) Elements ± equidimensional Stacks of rounded units (4)	*Apiculiretusispora brandtii* (3)
Psilophyton dawsonii	40-75 (6)	Circular to subcircular (6)	Curvaturate; polumbra Trilete rays simple, 1/2 of spore radius (6)	Discrete coni or grana (compressions); anastomosing rugulae (permineralizations) (4)	?	Ornamented, more often detached from inner layer (6)	*Retusotriletes triangulates* (6) *Phyllothecotriletes* spp. (6) *Retusotriletes* cf. *rotundus* (2) *Apiculiretusispora* (2)
Psilophyton charientos	48-74 (1)	Circular (1)	Curvaturate; polumbra (1)	Apiculate (1)	?	Ornamented, detached after dispersal (1)	*Apiculiretusispora brandtii* (3) *Retusotriletes* spp. (4) *Calamospora* spp. (4) *Punctatisporites* spp. (1)
Psilophyton crenulatum	48-102 (7)	Circular to subcircular (7)	Curvaturae absent (7); polumbra (7) Trilete rays simple, 1/3 to 2/3 of spore radius (7)	Stacks of globules (0.5-1 µm high; 0.1-0.3 µm wide) Reticulate structure beneath sculpture (7)	Smooth	Ornamented, sometimes detached from inner layer (7)	*Apiculiretusispora* cf. *plicata* (7)
Psilophyton parvulum	61-71 (5)	Circular to subcircular (5)	Curvaturae(5); polumbra? (5) Trilete rays simple, 3/4 to 9/10 of spore radius (5)	Cones (1µm high; 1µm wide) (5)	Smooth	Ornamented, sometimes detached from inner layer (5)	*Apiculiretusispora* sp. (5) *Retusotriletes* sp. (5)

590 **Figure captions**

591

592 **Figure 1.** *Psilophyton dawsonii* sporangia and spores in light microscopy. **A.** Two pairs of
593 sporangia, one sectioned transversely (left) and the other obliquely (bottom); three of the four
594 sporangia contain spores. USNM 558725 Btop #78. Scale bar = 500 μm. **B.** Pair of sporangia
595 with full spore complements twisted around each other (one sectioned transversely and the other
596 oblique-longitudinally); tubular epidermal cells conspicuous in the transversely sectioned
597 sporangium. USNM 558725 Btop #25. Scale bar = 200 μm. **C.** Transversally sectioned pair of
598 sporangia containing spores; note dehiscence lines on the sides of sporangia facing each other
599 and tapering sporangial wall thickness at dehiscence line. A stoma with substomatal chamber
600 (asterisk) is present in the wall of the lower sporangium. The spores are tightly packed inside
601 membranes corresponding to inner walls of the innermost sporangial wall layer, in A-C; gaps
602 between spore masses and the outer sporangial wall are due to lack of preservation of inner
603 parenchymatous sporangial wall, in A-C. USNM 558725 Btop #13. Scale bar = 200 μm. **D.**
604 Spores inside sporangium. As a result of the cellulose acetate sectioning technique, only the
605 spores at right show inner wall layers (darker brown); most other spores show only the outer
606 wall. Note finely dotted outer walls corresponding to the spiniform ornamentation and strand of
607 tubular cells of the sporangial epidermis (left). USNM 558725 Btop #19. Scale bar = 50 μm.

608

609 **Figure 2.** *Psilophyton dawsonii* sporangia and spores in light microscopy. **A.** Spores inside
610 sporangium; some show both inner wall layer (dark) and outer wall layer (lighter, yellowish),
611 while other show only the outer layer, as a result of the cellulose acetate sectioning technique.

612 USNM 558725 Ebot #149. Scale bar = 53 μm. **B** and **C.** Details of spores showing smooth
613 proximal face with darkened areas around aperture (polumbra). Note finely dotted outer wall
614 corresponding to the spiniform ornamentation, in C (e.g., upper left). USNM 558725 Ebot #97.
615 and #87 (respectively). Scale bar = 26 μm in B; 15 μm in C. **D.** Sporangial wall (left) and spores;
616 note sporopollenin globules (arrows). USNM 558725 Ebot #146. Scale bar = 42 μm. **E.** Detail
617 of D. Clumps of sporopollenin and dispersed globules (arrows). Scale bar = 16 μm. **F.** Tapetal
618 globules in contact with sporangial wall (arrow). USNM 558725 Ebot #87. Scale bar = 13.3 μm.
619
620 **Figure 3.** In situ *Psilophyton dawsonii* spores in scanning electron microscopy. USNM 558725
621 E. **A.** Partially collapsed spores. Outer spore wall layer with sculptural elements (a) partially de-
622 tached from inner, homogeneous layer. Note pyrite framboid (arrow). Scale bar = 10 μm. **B.**
623 Spore with distal half broken off, showing the trilete mark as seen from inside. Scale bar = 10
624 μm. **C.** Spore showing the proximal face (collapsed inside the spore) with a degraded area
625 (box); note detached ornamented outer layer around equatorial area of spore. Scale bar = 10 μm.
626 **D.** Detail of A. Detached ornamented outer layer with prominent sculptural elements (a) and
627 view of the base of the ornamented layer (b) – compare to G and H. Scale bar = 5.5 μm. **E.** Con-
628 tact between the ornamented outer layers of two adjacent developing spores; the inner smooth
629 homogeneous layer is visible in one of the spores (arrow). Scale bar = 5 μm. **F.** Detail of E. In-
630 ner homogeneous layer (arrow) and detached outer ornamented layer with sculptural elements
631 consisting of stacks of rounded tabular units (a). Note tightly interdigitating arrangement of
632 sculptural elements from the adjacent ornamented layers of the two spores (running vertically, to
633 the right of a). Scale bar = 1 μm. **G, H.** Base of the ornamented outer layer as seen from inside

634 the spore; note the foveolate structure. Scale bar = 1.4 μm in G; 0.9 μm in H. **I**. Early stage of the
635 developing sculptural elements, with few tabular units deposited on the foveolate basal layer of
636 the outer spore wall layer. Scale bar = 2 μm. **J.** Later developmental stage with taller stacks of
637 tabular units (a). Scale bar = 2.5 μm. **K.** Detail of J. Note the foveolate base layer forming the
638 foundation of the stacked sculptural elements. Scale bar = 0.6 μm. **L.** Mature spore wall with or-
639 namentation consisting of spines (arrow) formed by deposition of sporopollenin coating the
640 stacked sculptural elements. Scale bar = 4 μm.
641
642 **Figure 4.** In situ *Psilophyton dawsonii* spores in transmission electron microscopy. USNM
643 558725 E. **A.** Spore wall in the vicinity of the sporangial wall (a) and tapetum remnants (b); the
644 inner homogeneous layer (d) is partly folded onto itself and the outer spore layer (c) shows
645 prominent sculptural elements. Scale bar = 2.3 μm. **B.** Detail of A with tapetal orbicule (arrow).
646 Scale bar = 1.3 μm. **C.** Continuity between the inner spore wall layer and the outer ornamented
647 layer; this could represent the equatorial spore area but is difficult to assess due to multiple fold-
648 ing of spore wall and incomplete material. Scale bar = 2.3 μm. **D.** Homogeneous electron dense
649 inner layer with only incipient development of outer ornamented layer; note knife chatter lines
650 not informative of wall structure. Scale bar = 1.25 μm. **E.** Ornamented outer spore wall layer
651 with regular disposition of prominent sculptural elements consisting of stacks of rounded tabular
652 units (best examples at arrows) forming shapes reminiscent of the conifer growth habit with
653 pointed tips. Scale bar = 2.3 μm. **F.** Detail of sculptural elements of the ornamented layers of
654 two adjacent spores (not shown, but corresponding to the bottom and top of the image); note the
655 interdigitating arrangement wherein the sculptural elements on the surface of a spore fit in-be-

656 tween the corresponding elements of the adjacent spore (arrowheads). Scale bar = 0.8 μm.

657 2.3 μm in A,2.3 μm in A, Scale bar = 0.8 μm.