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SCUTELLOSPORA BIORNATA: A NEW SPECIES IN THE ENDOGONACEAE FROM THE LLANOS ORIENTALES OF COLOMBIA

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SUMMARY

Scutellospora biornata, a mycorrhizal fungus, was isolated from associations with native grasses. Two ornamented walls distinguish it from other species in the genus. Novelties related to the germination shield are discussed as well as the deformation of the laminated wall caused by common mountants.

RESUMEN

Scutellospora biornata, un hongo micorrizógeno que fué encontrado en asociación con gramíneas, tiene esporas con dos paredes ornamentadas que lo distingue de otras especies del género. En la descripción se incluyen aspectos nuevos relacionados con el escudo germinativo. También se discute la deformación de la pared laminada de las esporas en medios de montaje.

INTRODUCTION

Spores from vesicular-arbuscular mycorrhizal associations with native grasses were isolated over a period of years at the Centro Nacional de Investigaciones Agropecuarias (ICA-CIAT) at Carimagua, Meta. Several new species were previously described from this location (Schenck et al., 1984; Schenck et al., 1986). A Scutellospora species having two ornamented walls is described in this paper.

Refer to Spain et al. (1989), for the use of the terms sporogenous cell and sporophore.

DESCRIPTION

Scutellospora biornata Spain, Sieverding & Toro sp. nov. Figures 1-3.

Sporae singillatim in solo ennatae, globosae, (120-) 260-450 (-493) µm diam., flavae vel badiae. Paries sporae e stratis quinque vel sex (primum ad sextum) congregatis in turmis duobus. Turma externa cum stratis tribus (primum ad tertium): stratum primum brunneolo-flavum, solidum, 0.5-1 µm crassum cum ornamentatione e papillis rotundatis (0.5-) 1-3 µm diam. usque ad 2 µm longis; stratum secundum hyalinum lamellatum, (5-) 6-10 µm crassum; stratum tertium membranosum, hyalinum, 0.5-1 µm, cum ornamentatione simile strati primi. Turma interna hyalina cum stratis duobus vel tribus (quartum ad sextum). Stratum quartum membranosum, <0.5 µm crassum, saepe absens; stratum quintum membranosum, <0.5-1 µm crassum; sextum solidum, 1-2 µm crassum. Stratum secundum in solutione cum lactophenolio tumescens. In solutione Melzeri, stratum secundum purpureum colorans, stratum sextum pallide ruber. Cellula sporogenea fusca, (30-) 50-60 (-65) µm diam. Cellulae auxiliares generatim napiformes in fasciculo; cellula auxiliaris cum nudis. Germinatio sporae propria generis: plicatum spatium fuscum, inter stratum quintum et sextum efformatum, (113-) 188-238 X (113-) 188-275 µm diam. Usque ad 20 loci tuborum germinalerum, separati inter se per fissuras nigro-brunneas.

SPORES: borne singly in soil; globose, (120)260-450(-493) µm diam. or sub-globose 282-384 X 341-415 µm (measured in water); translucent, yellowish-brown to brown.

SPORE WALL STRUCTURE (spores ruptured and measured in water, Figs. 1A-E; 2): 6 walls, 8-15 µm thick, in two groups. Group A, 6-12 µm thick, of 3 walls: wall 1 unit wall, 0.5-1 µm thick, brown, ornamented on outer surface with noncontiguous blunt tapering projections from (0.5-)1-3 µm diam. at base up to 2 µm long, fused to wall 2. Wall 2 hyaline to sub-hyaline (up to 15 laminae), (5-)6-10 µm thick, adherent to wall 3. Wall 3, hyaline, membranous, 0.5-1.0 µm thick, ornamented on inside with blunt projections, 0.5-1 µm diam. to 2.0 µm long, generally smaller and more dense than projections on wall 1. Group B, 2-3 µm thick, of hyaline walls 4,5 & 6. Wall 4 membranous, <0.5 µm thick, rarely present; wall 5, membranous <0.5-1 µm thick, less elastic than adherent pliable unit wall 6, 1-2 µm thick.

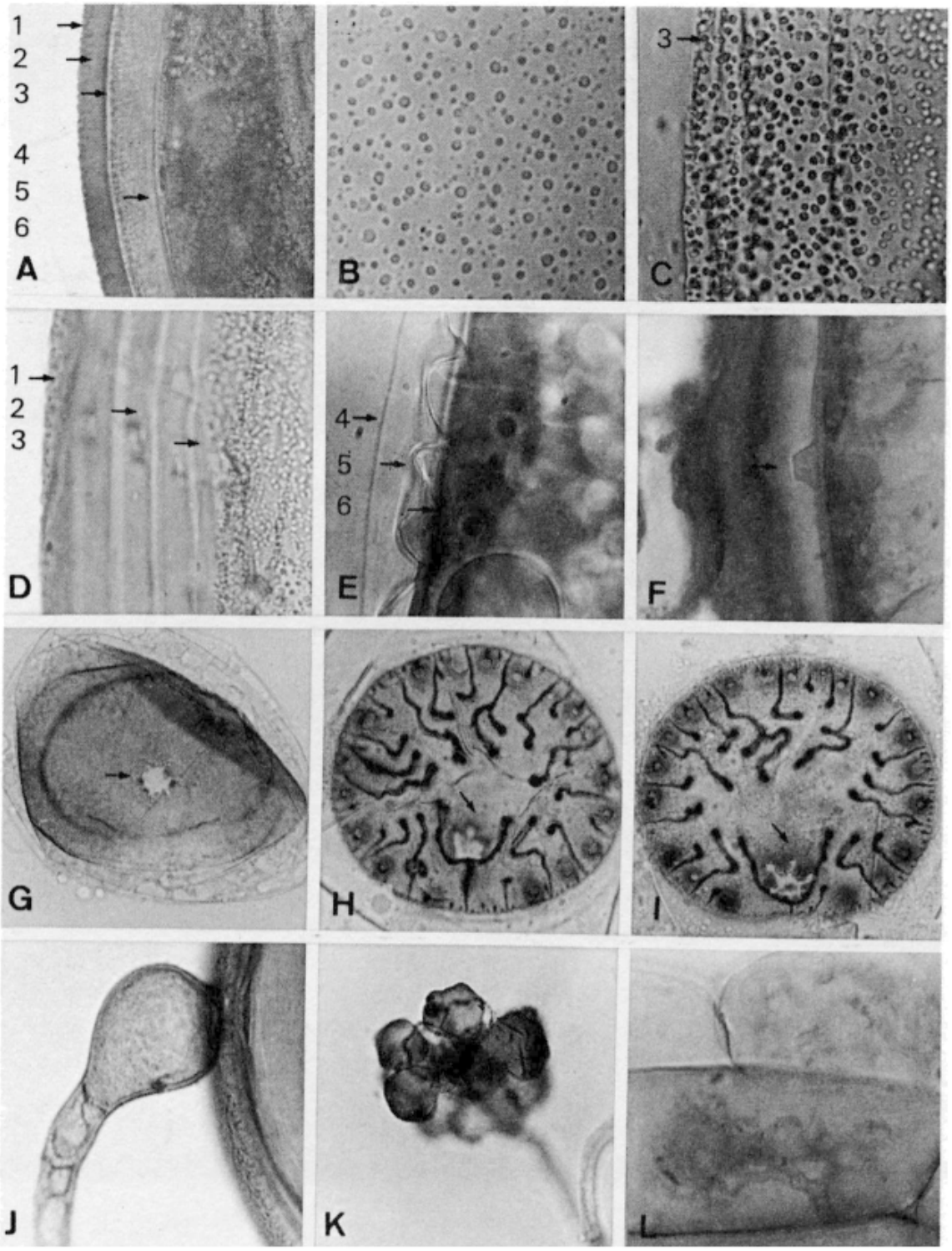
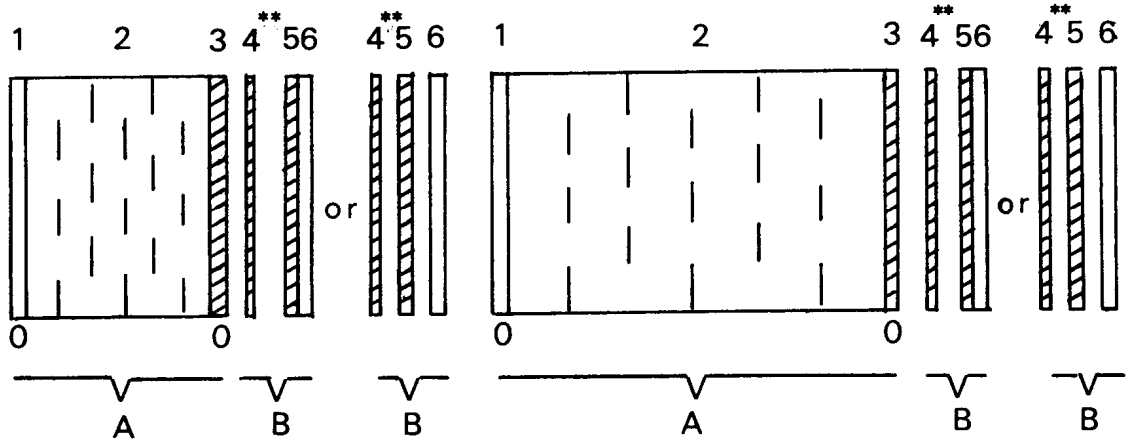


Figure 1. Scutellospora biornata. A) Wall groups A (1-3) & B (4-6) in water 200X. B) Wall 1 ornamentation, 1000X. C) Wall 3 ornamentation 1000X. D) Group A (1-3) in lactophenol; laminated wall 2 grossly expanded, 400X. E) Group B (4-6), Melzer's reagent, 1000X. F) Remnant on wall 6 of connection with sporogenous cell, 1000X. G) Aperture in wall 6 for formation of germination shield, Melzer's reagent, 200X. H) Germination shield: 'Y' configuration around aperture, 400X. I) Germination shield: 'U' configuration around aperture, 400X. J) Sporogenous cell of sporophore, 200X. K) Knobby auxiliary cells, 400X. L) Arbuscule in cortical cell of Puereria phaseoloides, 1000X.



WATER LACTOPHENOL

** Rarely present

Figure 2. Murographs of Scutellospora biornata spores in water and lactophenol. Six walls in two groups: walls 1 & 3, group A, ornamented; wall 4**, group B, rarely present. Laminated wall 2, group A, deformed by reaction to lactophenol.

REACTION TO MOUNTANTS AND MELZER'S REAGENT: laminated wall 2, group A, is strongly reactive (swelling) to lactophenol (Figs. 1D; 2) and PVL and turns reddish-purple in Melzer's reagent. Walls 4, 5 & 6, group B, can separate in lactophenol and Melzer's reagent (Figs. 1E; 2); wall 6 turns light to deep pink.

GERMINATION SHIELD (Figs. 1G-I; 3): brown, (113-)188-238 X (113-)188-275 µm, cresent shape in x-section, formed between walls 5 and 6. A spore generally has one shield although two can form. Multiple apertures in wall 6 may be present (internal or external to the developed shield). Greatest pigment concentration around germ tube initials, aperture, Y & U configurations and other fissures. Germ tube initials, numbering (6)12-20 ($\bar{x}$ 17), 6-7 µm diam., generally separated from each other by a long fissure.

GERMINATION TUBE, light brown, coenocytic, 15-20 µm diam. proximate to shield narrowing to 9-14 µm diam., wall 1-1.5 µm thick. A single germ tube usually emerges at germination.

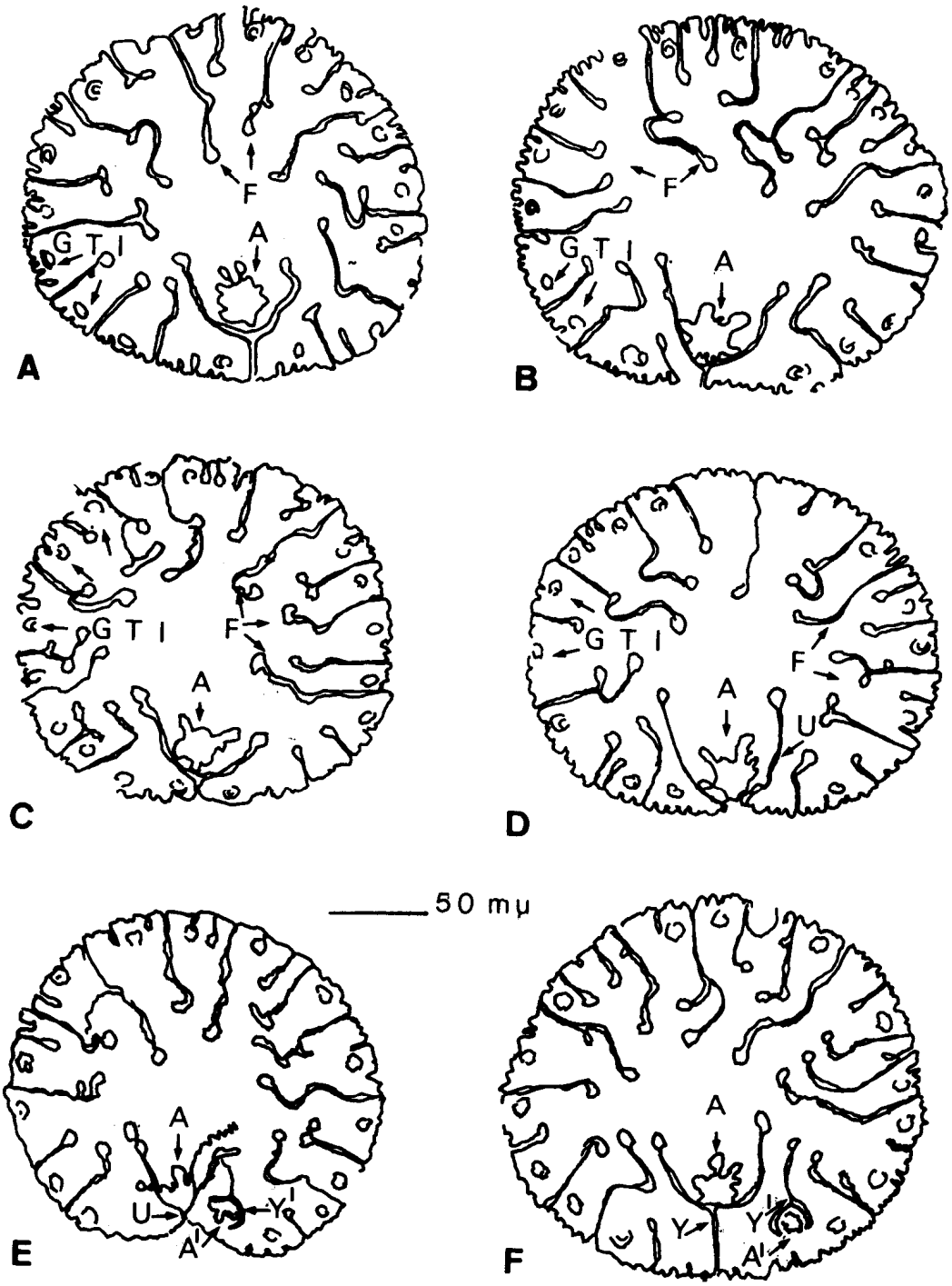


Figure 3. Scutellospora biornata germination shields, plan view (camera lucida drawings). A-F) Germ tube initials (GTI) generally separated by long fissures (F). A-C & F) 'Y' (Y) configuration around aperture (A). D & E) 'U' (U) configuration around aperture (A). E & F) Aborted shield with a small aperture (A') and rudimental 'Y' (Y') configuration within developed shield.

SPOROGENOUS CELL: apical cell of the sporophore, brown, (30-)50-60(-65) µm diam.; 1-3 walls totalling 2-4 µm thick; spore initial site generally apical (Fig. 1J); hyphal branches (pegs) may be present. Projections, somewhat larger than the ornamentation on the outer surface of the spore, may be present on the innermost wall.

SPOROPHORE: brown, septate below the sporogenous cell (Fig. 1J), 15-28 µm diam. narrowing to (7.5-) 10-13 µm in diam. with 1-2 walls up to 2.5 µm thick. Hyphal branches may form below the sporogenous cell.

AUXILIARY CELLS: brown, knobby and generally napiform, 32-48 X 37-48 µm diam., formed in clusters of 10-20 on coiled, thin walled, <1 µm thick, coenocytic hypha 3-5(-8.75) µm diam. (Fig. 1K).

ARBUSCULES and coiled hyphae form in cortical cells of infected roots (Fig. 1L).

TYPE: Colombia, Cali, Centro Internacional de Agricultura Tropical (CIAT), pot culture C-9; holotype GOET; isotypes COL, FLAS, OSC. Viable spores deposited in INVAM.

ETYMOLOGY: biornata, Latin, referring to the two ornamented spore walls.

DISTRIBUTION: Scutellospora biornata was first recovered from a sward of native grasses growing in a loamy sand at Hato Alegria, Carimagua, Meta, in the Llanos of Colombia. The soils, Oxisols with pH 4.8, have the following textural and chemical characteristics: sand 68%, silt 23%, clay 9%; exchangeable cations expressed in meq/100 g: Al 0.8, Ca 0.12, Mg 0.06. K 0.04; Al sat'n 82%; P 2.1 mg/kg soil (Bray II) and S 17 mg/kg soil.

MYCORRHIZAL ASSOCIATIONS KNOWN: Andropogon gayanus Kunth, Brachiaria decumbens Stapf, Manihot esculenta Crantz, Pueraria phaseoloides Benth & Zea mays L. and unidentified native grasses.

DISCUSSION

Scutellospora biornata spores can be readily separated from other pigmented Scutellospora species with ornamentation by the presence of a second ornamented wall.

Wall features of some spores in the Endogonaceae can be modified significantly by mountants and fixatives (Morton, 1986). Sward (1981) and others have recognized the need for a fixative and/or mountant which will preserve the integrity of the walls; that need is particularly evident with this spore. Lactophenol and PVL cause gross swelling (to a thickness of 55 µm) of laminated wall 2 (Figs. 1D; 2); apparently phenol is the reactive substance (Morton, 1986). Some or all of the laminae become diaphanous having little, if any, discernible structure; small rod-shape refractive areas are present in the distended wall. The laminae separate slightly at the rupture site in water, however, the wall character is not visibly altered. There was no distension of the laminated wall of spores fixed in FAA and mounted in lactophenol. The reaction time of the laminated wall (wall 2, group A) to Melzer's reagent is delayed. The reaction begins at the rupture sites and progresses slowly due to the non-reactive, tightly adherent, walls 1 and 3.

Wall 1 is fused to the adjacent laminae; observations of developing spores indicate that the initially smooth, sub-hyaline wall 1, continuous with the outer wall of the sporogenous cell, becomes ornamented and pigmented; areas, generally small, free of any ornamentation can occur. Wall 4, an extremely thin membranous wall in group B, observed in water, lactophenol and Melzer's, is rarely present. Walls 5 & 6 have the appearence of a coriaceous wall, a single wall wrinkled externally (Walker, 1986). Wall 5, less elastic than wall 6, wrinkles as it adheres somewhat loosly when the wall group is ruptured; occasionally walls 5 & 6 partially separate in water. Alhough pliable, wall 6, which readily ruptures but does not collapse, is described as a unit wall. A remnant of the connection to the sporogenous cell has been observed on wall 6 (Fig. 1F).

Walker & Sanders (1986) indicated that germination shields may have taxonomic value at the species level. The germination shield of S. biornata is complex compared to the simple shields of S. heterogama and S. calospora and is formed between walls 5 & 6 of group B, rather than being constrained by the inner and outer wall groups as described by Walker and Sanders (1986). It may develop near the sporogenous cell or be unrelated to it. Usually a single aperture develops in wall 6; cytoplasm, apparently confined by the plasmalemma, is extruded through the aperture which

appears as a tear having a very irregular periphery (Figs. 1G; 3A-F); pigment is concentrated around the perimeter. Fissures form a 'U' (Figs. 1I; 3D, E) around the aperture when the shield develops in such a way that the aperture is on the perimeter; shields thus formed are generally less round on the side of the aperture. Fissures form a 'Y' (Figs. 1H; 3A-C, F) when the aperture is sub-marginal to the developed shield. The 'U' and the 'Y' configurations occur with similar frequency. The germ tube initials are generally separated from each other by a long fissure. One or two medium long and numerous short fissures are usually present within these divisions. The mode of development of the fissures is not understood. Walker and Sanders (1986) infer that the "wishbone ('Y') formation occurs when the "...extruding membrane folds back on itself." Until ruptured by an emerging germ tube, the numerous germ tube initials are covered by the outermost ornamented wall. Walls 5 & 6 become rigid and inseparable from the shield; the rigidity and occasionally some pigment may extend slightly beyond the shield.

Two shields of average size have been observed within a single spore; they may overlap slightly or be completely separate. Multiple (up to six have been observed) small apertures, internal and/or external to the shield, may develop in addition to the large aperture. Shield development can be aborted; occasionally a small aperture has a rudimental 'Y' configuration formed around it (Fig. 3E & F); more often there is no evidence that cytoplasm was extruded through the small apertures.

Rare sporophore novelties pertain to the sporogenous cell. One sporogenous cell was bifurcate with no spore development; two sporogenous cells had an attached developed spore and an incipient spore.

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