MYCOTAXON

Volume LX, pp. 129-136 October-December 1996

SCUTELLOSPORA CERRADENSIS: AN ORNAMENTED SPECIES IN THE GIGASPORACEAE (GLOMALES) FROM THE CERRADO REGION OF BRAZIL

JOYCE LANCE SPAIN

3899 NW Jameson Dr.
Corvallis, OR 97330

JEANNE CLAESSEN DE MIRANDA

CPAC/EMBRAPA CAIXA POSTAL 08223
PLANALTINA, D.F. BRAZIL 73301-970

ABSTRACT

A new species of arbuscular mycorrhizal fungi was found in association with plants growing in a dark red latosol in a savanna ecosystem. Pot cultures were established and observations made of papillose hyaline to sub-hyaline spores mounted in polyvinyl alcohol-lactic acid-glycerin (PVLG), water and Melzer's reagent. *Scutellospora cerradensis* was characterized by a four layered spore wall with a pigmented germination shield forming between two flexible inner walls.

RESUMO

Uma espécie nova de fungos micorrízicos arbusculares foi encontrada em associação com plantas cultivadas em um Latossolo Vermelho Escuro em um ecossistema do tipo savana. Culturas em vasos foram conduzidas e observações foram feitas em esporos papilosos e hialinos a sub-hialinos, montados em polivinil-alcool-ácido lático-glicerina (PVLG), água e reagente de Melzer. *Scutellospora cerradensis* foi caracterizada através da parede do esporo, constituída de quatro camadas, com um escudo de germinação pigmentado formado entre duas flexíveis paredes internas.

INTRODUCTION

Scutellospora cerradensis, an early accession (1984) in the collection of native arbuscular endomycorrhizae maintained at the Centro de Pesquisa Agropecuaria dos Cerrados (CPAC), was initially identified as *S. verrucosa*, a spore with similar ornamentation, size and color. A more thorough study revealed a unique spore distinguishable from *S. verrucosa* and other described species.

An innermost flexible layer of the spore wall, not previously described, is discussed. Wall descriptions are based on ontogenetic findings in the study of two *Scutellospora* species by Franke and Morton (1994). A murograph depicts wall morphology.

MATERIALS AND METHODS

Spores, recovered by wet sieving soil and roots from a pot culture, were suspended in water and cleaned by ultrasound for 30 seconds. Color determinations were made by stereomicroscope using fiber optic illumination, color temperature 3200K. Intact spores suspended in water against a black background were compared to the INVAM Color Chart (1993). Color of shields, sporophores, auxillary cells amd wall layer reactions to Melzer's reagent was assessed against a white background.

The spore wall and inner walls of numerous spores were separated in water or PVLG on a slide using a scalpel and dissecting needles. Intact spores, ruptured spores and dissected spores were mounted in PVLG, water or Melzer's reagent and examined with a compound microscope. Melzer's reagent was also diluted with PVLG or distilled water to observe the rate and intensity of histochemical responses. The species diagnosis was based on observations of ruptured and/or dissected spores with shields mounted in PVLG and magnified 1000 X. Type specimens were mounted in PVLG; unmounted spores were preserved in 5% formalin. Observations and measurements were also made of ruptured spores in water.

LATIN DIAGNOSIS

Scutellospora cerradensis Spain & Miranda sp. nov. (Figs. 1-3)

Sporae singulae in solo feruntur in apice aut circa apicem cellulae sporogenae; raro autem sporae duae feruntur a latere unis cellulae sporogenae. Sporae maturae hyalinae vel subhyalinae; senescentes, stramineae (0/0 10/0) vel pallide luteobrunneae (0/10 40/0), globosae vel obovoideum (167-) 230-363 µm X (167-) 230-363 µm, raro irregulares. In PVLG sporae tunica constituitur stratis quatuor: exteriore papilloso <1.0-1.0 µm crasso et adherente in secundo strato rigido, 1.5-2.0 µm crasso et adherente in tertio, subtiliter lamellato, 2-3 µm crasso; quarto membranaceo <0.5-1.0 µm crasso. In PVLG stratum papillosum et stratum secundum disjunguntur. Stratum quartum et stratum tertium disjunguntur, excepta conjunctione cellulae sporogenae. Murus internus primus: stratum primum 1.0 µm crassum adheret secundo 3-5 µm crasso. Murus internus secundus constituitur stratis tribus: primum <0.5-0.5 µm crassum adheret strato secundo 4-6 (-10) µm crasso, sed crassities varietur in PVLG. Stratum tertium, in quo secundum adheret, 0.5-1.0 µm crassum, et raro cum papillis rotundis <1-1.5 µm diametro. Scutellum inter murum primum et secundum formatur.

SPORES are hyaline to sub-hyaline when mature, straw (0/010/0) to a pale yellow-brown (0/10/40/0) with age; globose to ovoid, (167-) 230-363 µm X (167-) 230-363 µm, rarely irregularly shaped. Usually spores are borne singly in soil at or near apex of sporogenous cell, however, two spores are occasionally borne laterally from one sporogenous cell. Spore contents, opaque white in immature spores, become hyaline with maturity.

The spore wall consists of a weakly laminate layer surrounded by two outer layers and an inner flexible layer, generally separate from the other layers except at point of attachment to sporogenous cell. Unless observed immediately the papillose layer, <1-1 µm thick, papillae 1- 1.5 µm diam. to slightly oblong, up to 1.5 µm high, will be perceived in PVLG as a fine reticulum or simply as a roughened layer which separates from the rigid adjacent layer, 1.5-2.0 µm thick, adherent to laminate layer, 2-3(-5) µm thick. The innermost flexible layer, <0.5-1.0 µm thick, is thicker at connection to sporogenous cell. Inner wall one has an outer layer, 1 µm thick, loosely adherent to an inner layer 3-5 µm thick. Inner wall two has a very thin outer layer, <0.5 µm thick, adherent to a thick layer, 4-6 (-10) µm, over a thin, sometimes papillose (Fig. 3G), layer, 0.5-1.0 µm thick. The germination shield forms between inner walls 1 and 2.

The ornamentation on the outermost layer of the spore wall is discrete in water (Fig. 3A). The spore wall is of four layers: together layer one, with papillae <1-1 µm thick with round, 1- 1.5 µm diam., to slightly oblong up to 1.5 µm high, and layer two are approximately 1 µm thick; layer three, laminated, 2-3 µm thick; layer four, the innermost layer, 0.5-1.0 µm thick, is rarely observed. The outer layer of inner wall one, 0.5-1 µm thick, adheres to the inner layer, 2-3 µm thick. Inner wall two has three adherent layers: a middle layer, 2-4 µm thick, surrounded by an outer layer, 1-1.5 µm thick, and an innermost layer, <1 µm thick, observed as wrinkled or when sparse to more dense papillae, <1-1.5 µm diam and 1-2 µm high, are present (Fig. 3G).

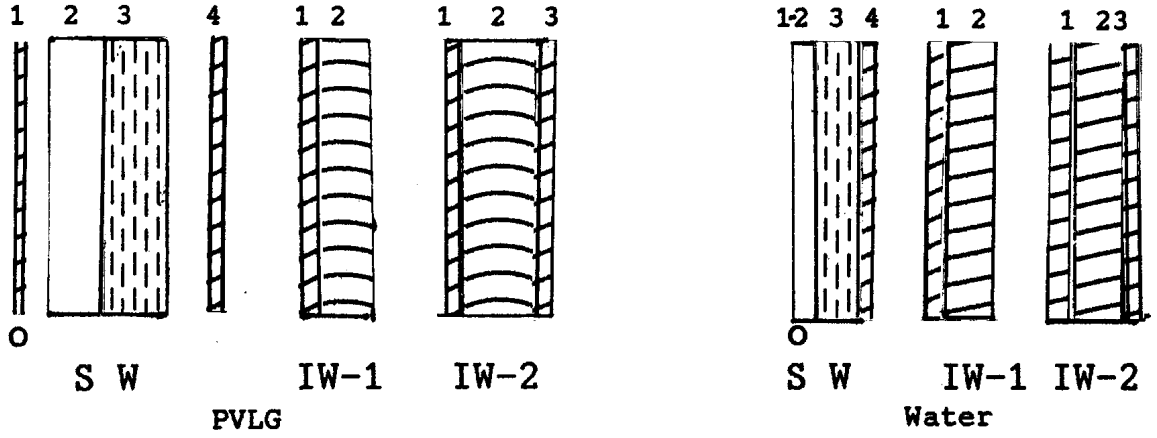

Figure 1. Murograph of *Scutellospora cerradensis*. Spore wall (SW); Inner Wall One (IW-1); Inner Wall Two(IW-2). Open: rigid single layer; vertical dashes: laminae; diagonal lines: flexible layer; hemispheres: plastic; o: ornamented layer.

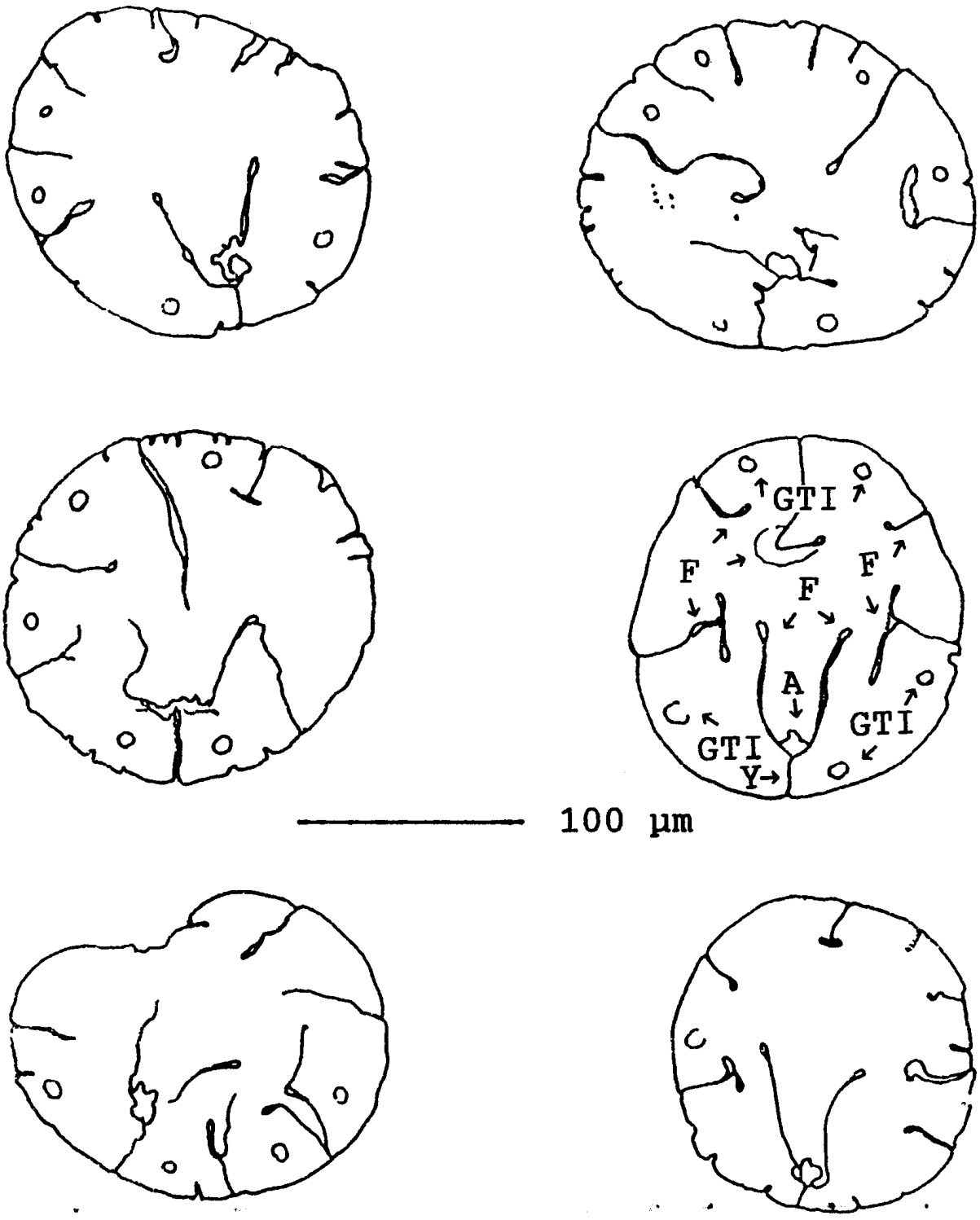

Figure 2. *Scutellospora cerradensis* germination shields, plan view; camera lucida drawings. Aperture (A); ; Germ Tube Initial (GTI); Fissure (F); 'Y' configuration around aperture (Y).

REACTION TO MELZER'S REAGENT. Five layers can be observed to react with PVLG/Melzer's reagent (1:1). The laminated layer of the spore wall is reddish yellow (INVAM 0/20 20/0) to reddish brown (20/80 60/0) and clearly distinguished from the outer two layers; the outer layer of inner wall one reacts very weakly and has a tinge of pink (INVAM 0/20 20/0) when folded on itself; the inner layer is pink (INVAM 0/60 20/0). Two layers of inner wall two are observed to react: a light pink (INVAM 0/60 20/0) outer layer over a reddish purple (INVAM 20/80 20/0) layer. In PVLG/Melzer's reagent (3:1) the innermost layer (masked by the reaction of the middle layer at a 1:1 ratio), appears to have a weak dextrinoid reaction. A differential reaction sometimes observed in the middle layer suggests that it may be, in fact, two layers.

GERMINATION SHIELD, light yellowish-brown to brown (INVAM 0/40 80/40 to 0/40 100/0), (87-)125-155 µm X (100-) 137.5-172.5 µm, forms between inner walls one and two; a 'V' or 'Y' configuration develops around aperture (Fig. 7). The largest number of germination tube loci observed was 6; the germination tube widens from a diam. of 5 µm at egress to 12.5 µm near spore.

SPOROGENOUS CELL: light yellowish brown to brown, (INVAM 0/40 80/0 to 0/40 100/0) 35-55µm X (-30)50-75µm (width by distance between apex and first septum of sporophore), often with fine hyaline hyphae coming from one to several peg-like, pigmented hyphae. Papillae are often observed on the innermost layer. A single spore usually develops at apex, however, two spores, generally with a disparity in size, occasionally emerge laterally from a single sporogenous cell. The sporophore is septate and rarely branched (Spain et al., 1989).

AUXILIARY CELLS 20-33 µm diam, smooth, round to knobby, formed in soil on yellowish brown to brown (INVAM 0/40 80/0 to 0/40 100/0) coiled or straight hyphae 3.5-4 µm diam, wall <1µm thick.

MYCORRHIZAL ASSOCIATIONS. Known to associate and develop arbuscules with *Allium porrum*, *Stylosanthes guyanensis* var. vulgaris cv Mineirão and *Sorghum bicolor* L. Moench, host plants for pot culture.

DISTRIBUTION AND HABITAT: Spore known from experimental fields at CPAC/EMBRAPA, Planaltina, D.F. Brazil; 15°35'33" south; 47°42'30" west; 1100 m altitude. This accession was isolated from a dark red latisol (mostly clayey) with poor phosphorus availability. Soil analyses: pH (water 1:2.5):4.8-5.0; phosphorus (ug/g soil): 3.5; aluminum (me/100 g soil): 0.2; calcium + magnesium (me/100 g soil)3.5; potassium (ug/g soil):40. Vegetation at the collection site progressed from predominantly native grasses to pasture to soybeans. A fallow period of about five years was followed by a planting of different legumes for green manure. The isolate was recovered from plots cultivated with *Tephrosia candida*, however, an association with that plant has not been determined.

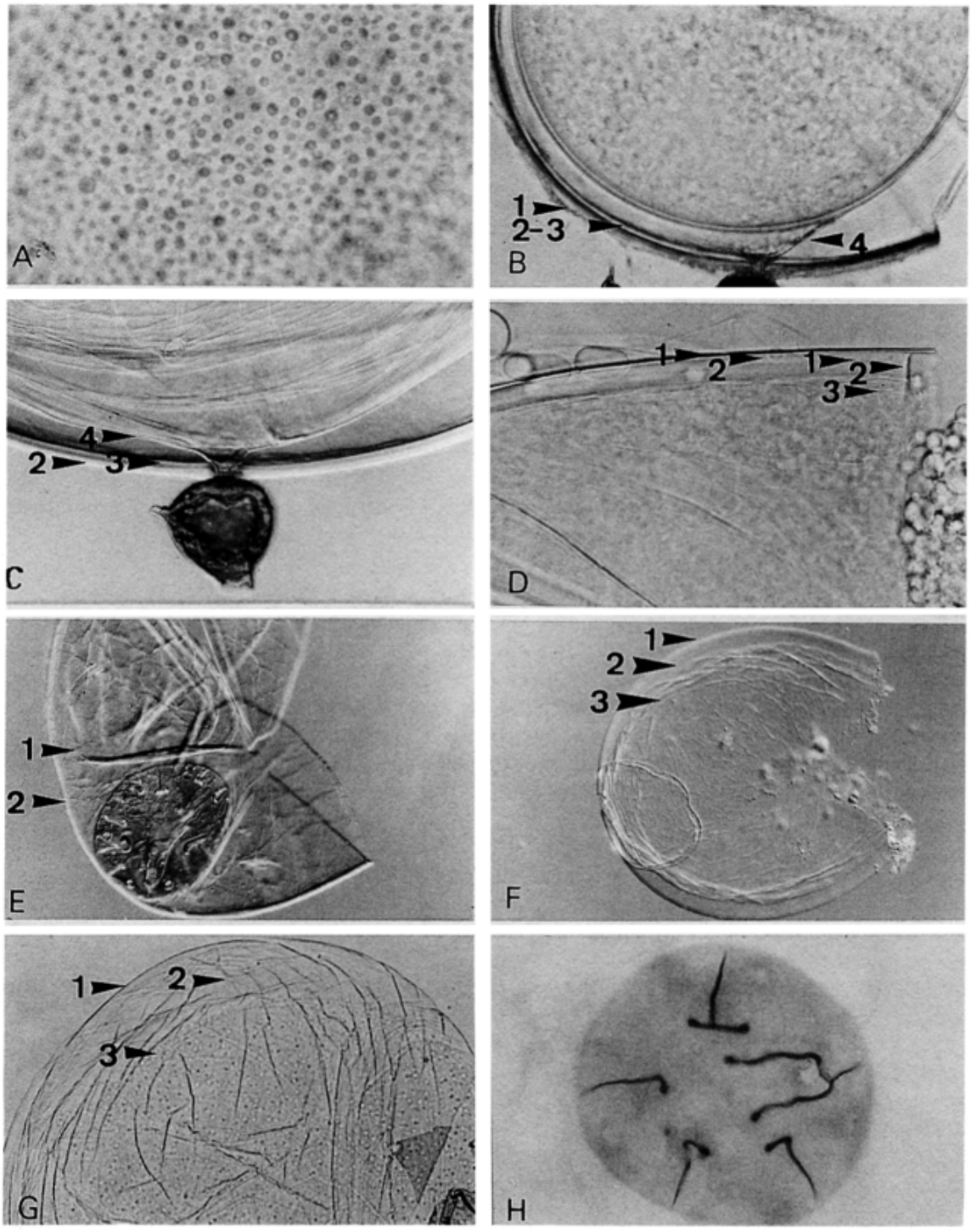

Figure 3. *Scutellospora cerradensis*. A. Spore Wall layer 1 papillose, water, 1000X. B. Spore Wall, layer one detached, PVLG, 200X. C. Spore wall, layer one missing, PVLG, 400X DIC. D. Inner Wall 1, two layers; Inner Wall 2, three layers; Spore Wall layer four visible above Inner Wall 1, PVLG 400X. E. Inner Wall 1, two layers, layer one wrinkled; both reactive to Melzer's reagent, 200X DIC. F. Inner Wall 2, three layers, layer 2 very plastic, PVLG, 200X DIC. G. Inner Wall 2, papillae on layer three rare. PVLG, 400X. H. Germination shield, PVLG 400X.

ETYMOLOGY: Place: cerrado; ensis. Epithet chosen to associate the species with the savannah ecosystem (cerrado) where it was found.

TYPE: From endomycorrhizal association with *Sorghum bicolor* L Moench in pot culture *Scutellospora* sp. CPAC-3 at CPAC/EMBRAPA, Planaltina, D.F. Brazil. Holotype: OSC; isotypes FH, K and IBt. Cultotype, isolate BR 103, deposited at INVAM (West Virginia University, Morgantown, WV).

DISCUSSION

Scutellospora cerradensis and *S. verrucosa* (Koske & Walker) Walker & Sanders have similar ornamentation, size and somewhat similar color when viewed in water against a white background by stereomicroscope, however, the shield is patent only in *S. cerradensis*. *Scutellospora verrucosa*, member of a sub-group including *S. coralloidea, S. fulgida, S. gregaria* and *S. persica* (Morton, 1995), lacks the second inner wall found in *S. cerradensis*. Mounted spores of *S. cerradensis* lacking ornamentation might also be confused with *S. gilmorei* (Trappe & Gerd.) Walker & Sanders, a smooth spore of similar size, color and wall number.

The spore wall of *S. cerradensis* has four phenotypically distinct layers. The outer layer is unique in its reaction to PVLG; unlike other described ornamented spores, the papillae are not structurally stable (3A). The ornamented layer reacts almost immediately and can appear as a fine reticulum or a roughened surface before detaching from the adjacent smooth layer (3B). Layer three was rarely perceived as laminate; the very thin and flexible innermost spore wall layer was not seen initially in water mounts. Although often appearing in PVLG mounted spores to be associated with the inner walls (3D), it clearly originates from the sporogenous cell (3B & 3C). It is not known whether there is discontinuity in the appearance of this layer as related to the other spore wall layers. This very thin flexible layer, not reported in the ontogenetic study of *S. heterogama* and *S. pellucida* (Franke & Morton, 1994), is present in both species and is not a species level character. Examination of a broader range of taxa in *Scutellospora* will help determine if it is a sub-genus or genus-level character.

The second inner wall is comprised of three layers (3D & 3F). This wall is a highly conserved primary character (Franke & Morton, 1994; Morton, 1995) and *Scutellospora* species described as having a bi-layered second inner wall are hypothesized to be equally complex. Papillae on the innermost layer of some spores (3G) are not considered a taxonomic character. Numerous spores of *S. gregaria*, an isolate native to the cerrado, develop papillae on the innermost layer prior to shield formation (Spain, unpub.). Papillae have also been observed on the innermost layer of an isolate of *S. verrucosa*, a spore with one flexible inner wall. It is not known whether this novelty is genetically induced or a response to biotic or abiotic factors.

Different mountants affect morphology and histochemical reactions of structures in spores of arbuscular fungi (Morton, 1990, Spain, 1990) The subcellular characters were detected and interpretable only with detailed comparisons in both water and PVLG. It is suggested that the example of Berch & Koske (1986) and Walker et al. (1993) be followed: diagnoses should be made from observations of spores mounted in water and PVLG. Although ontogenetic studies were not carried out with *S. cerradensis*, most of the developmental stages described by Franke & Morton (1994) were observed. If found in other habitats *S. cerradensis* should be readily identifiable.

ACKNOWLEDGEMENTS

We wish to thank Valter Lopes, long time assistant of Dr. Miranda at CPAC/EMBRAPA. We are grateful to Dr. Chris Walker for valuable suggestions and Dr. Joseph Morton for the critical review of the manuscript. Father Hugh Feiss OSB kindly provided the Latin translation and Dr. Stephen Alderman assisted with DIC photography. The senior author acknowledges the cooperation kindly given at CPAC/EMBRAPA in Brazil and the lab of Dr. Gabor Bethlenfelvay in Corvallis. Dr. James M. Spain was a valued critic.

LITERATURE CITED

Berch, S.M. and R.E.Koske. 1986. *Glomus pansihalos*: a new species in the Endogonaceae, Zygomycetes. Mycol. 78:838-842.

Franke, M. and J. Morton, 1994. Ontogenetic comparisons of arbuscular mycorrhizal fungi *Scutellospora heterogama* and *Scutellospora pellucida*: revision of taxonomic character concepts, species descriptions, and phylogenetic hypotheses. Can. J. Bot. 72:122-134.

Gerdemann, J.W. & J.M. Trappe. 1974. The Endogonaceae in the Pacific Northwest. Mycologia Memoir No. 5:1-76.

Morton, J.B. 1995. Taxonomic and phylogenetic divergence among five *Scutellospora* species based on comparative developmental sequences. Mycol. 87(1):127-137.

Morton, J.B. 1987. Effects of mountants and fixatives on wall structure and Melzer's reaction in spores of two *Acaulospora* species (Endogonaceae). Mycologia 78:787-794.

Spain, J.L. 1990. Arguments for diagnoses based on unaltered wall structures. Mycotaxon 38:71-76.

Walker, C., V. Gianinazzi-Pearson, and H. Marion-Espinasse, 1993. *Scutellospora castanea*, a newly described arbuscular mycorrhizal fungus. Cryptog. Mycol. 14:279-286.