

Plate 4.2 A

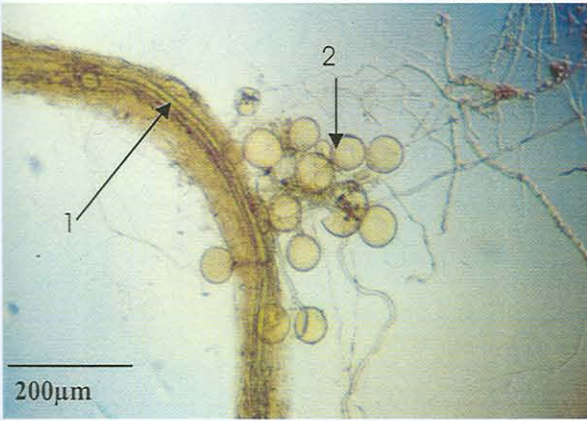


Plate 4.2 B

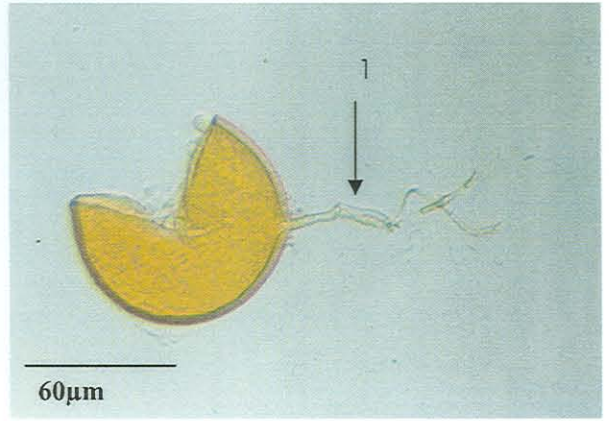


Plate 4.2 C

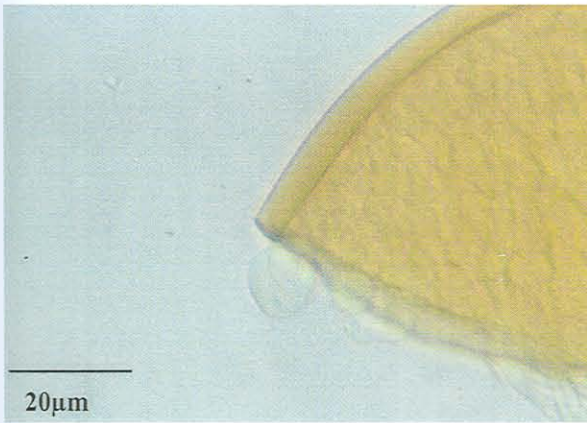


Plate 4.2 D

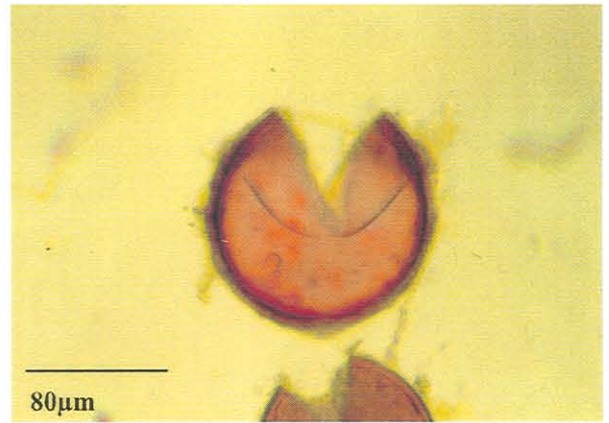


Plate 4.2 Spores of *Glomus* species (A) *Glomus aggregatum*. A loose aggregate of spores (1) a spore in the root (2) aggregate of spores. (B-D) *Glomus etunicatum*. (B) Slightly crushed mature spore with outer layer already sloughed (1) recurved cylindrical subtending hyphae (C) paler more immature spore where sloughing layer and laminae on middle layer has not yet developed (D) spore with sloughing layer patchily stained with Melzer's reagent.

Plate 4.2 E

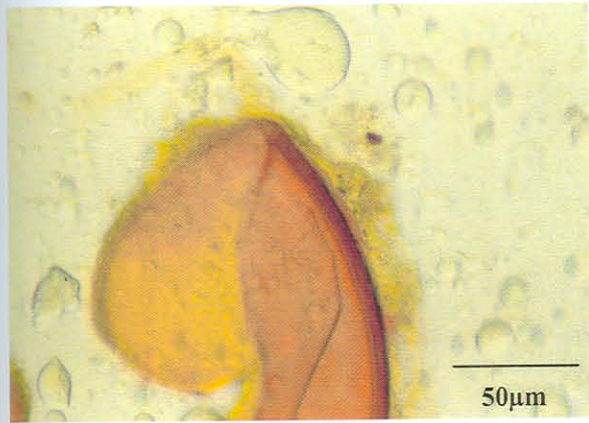


Plate 4.2 F

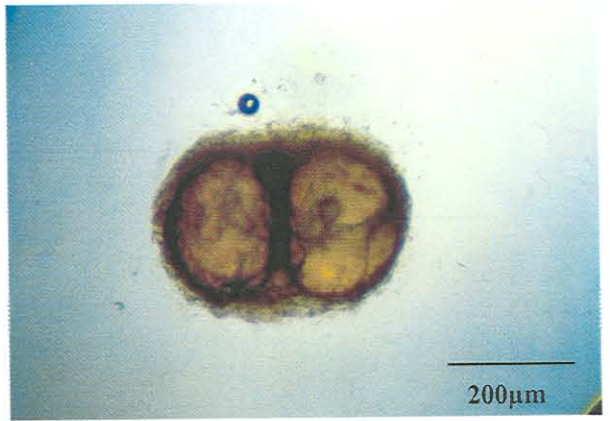


Plate 4.2 G

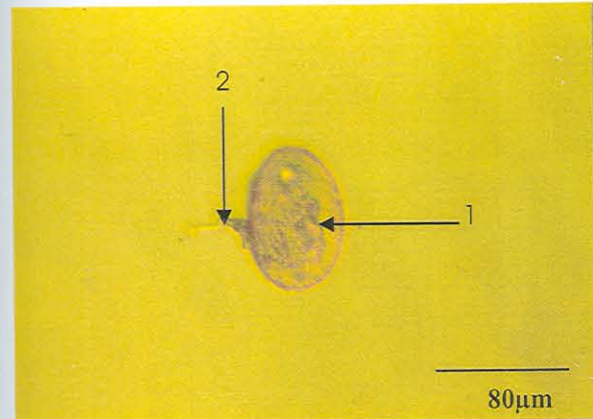


Plate 4.2 H

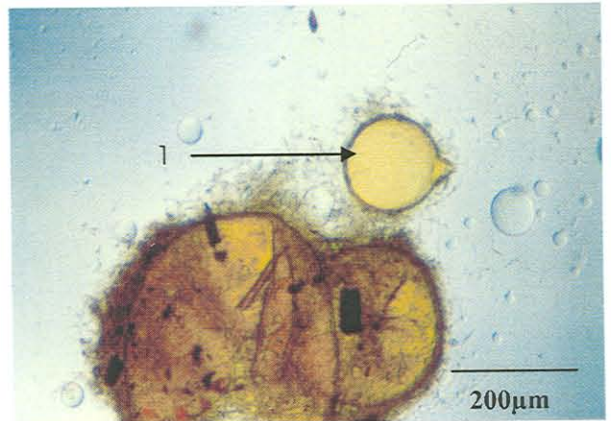


Plate 4.2 Spores of *Glomus* species (E) *Glomus etunicatum* crushed spore in Melzer's reagent showing a stained laminated layer (F-H) *Glomus* sp. (F) Two spores enclosed by a peridial hyphal mantle. (G) Spore with (1) concentric discs and (2) cylindrical hyphal attachment. (H) Vesiculate swelling with wall thickening, with (1) vesiculate spore-like structure appearing as immature spore.

Plate 4.2 I

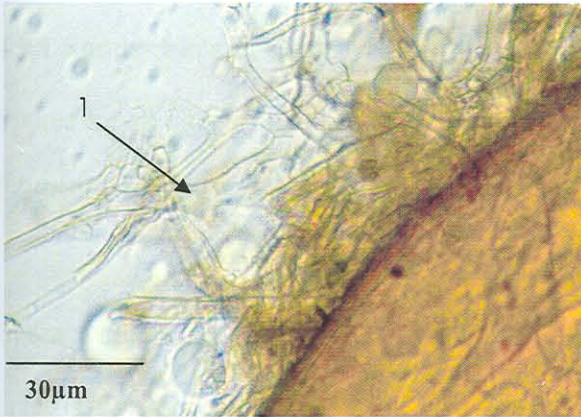


Plate 4.2 J

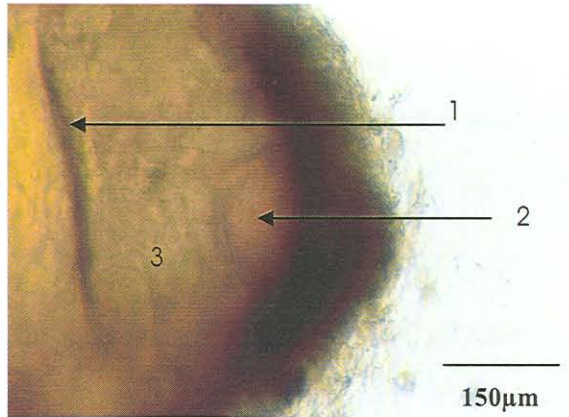


Plate 4.2 K

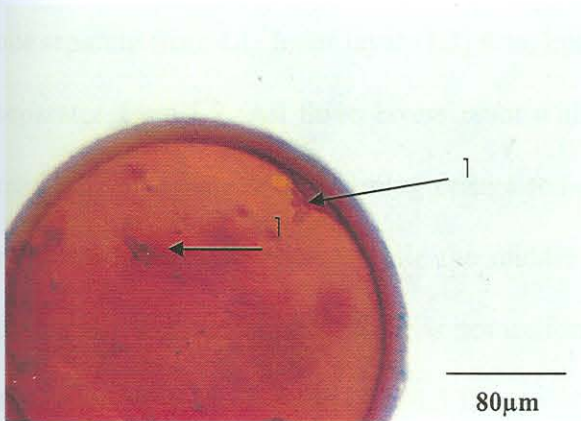


Plate 4.2 L

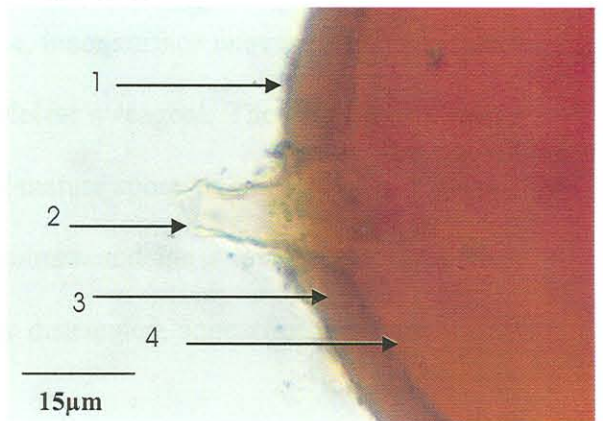


Plate 4.2 Spores of *Glomus* species (I-J) *Glomus globiferum* mantle with (1) septate hyphae (J) Spore with (1) crack in the middle (2) spore-like structure forming on the margin of a large spore.

(K-L) *Glomus geosporum*. (K) Parasitic invaginations. (L) Spore with (1) sloughing outer layer (2) flared subtending hyphae (3) thick middle layer and (4) thin inner layer enclosing the spore contents.

## 4.9 GIGASPORA sp. (Plate 4.3 A-G)

### 4.9.1 Spore characteristics

Spores found singly in soil, borne on a sporogenous cell (Plate 4.3 A), cream (0-0-5-0 to 0-0-20-0) to pale ochraceous (0-10-20-0 and 0-10-40-0), globose, 204-396  $\mu\text{m}$ .

Spore wall characteristics of three layers (**L1-3**) in one group (Plate 4.2 B & C). Outer layer (**L1**) rigid, thin, pale yellow, 1.0-1.5  $\mu\text{m}$ . Middle layer (**L2**) laminate, cream (0-0-5-0 to 0-0-20-0), pale ochraceous to pale yellow (0-10-20-0 and 0-10-40-0), 15-18  $\mu\text{m}$ , does not separate from L1. Inner layer (**L3**) thin, hyaline, inner surface uneven, 0.5-3  $\mu\text{m}$ , rarely separates from L2. All three layers react with Melzer's reagent. The intact spore reacts readily, with young spores turning orange red and mature spores reddish purple. The outer rigid layer reacts to orange while the middle laminate and inner layer reacts to reddish purple in older spores, the colour is not uniformly distributed, appearing as orange brown and reddish brown patches (Plate 4.3 D).

Sporogenous cell borne vertically, 36-48  $\mu\text{m}$  wide, with two layers, outer layer hyaline and inner layer cream (0-0-5-0 to 0-0-20-0) to pale ochraceous (0-10-20-0 and 0-10-40-0); sporogenous cell wall continuous with outer two layers of the spore wall, cell wall at the point of connection with spore 2.5-4.5  $\mu\text{m}$  gradually decreasing to 1.5-3  $\mu\text{m}$  away from the point of attachment; subtending hyphae coenocytic, 15-30  $\mu\text{m}$  wide close to the sporogenous cell and gradually decreasing to 3-9  $\mu\text{m}$  wide away from the sporogenous cell; hyphal wall 3-4.5  $\mu\text{m}$  gradually decreasing to 1-1.5  $\mu\text{m}$ , occlusion closure by plug; colour same with the laminate layer.

Auxiliary cells borne on curved and coiled aseptate hyphae, cells in clumps before development (Plate 4.3 E) and mature cells in aggregates of 4-12 cells; globose, subglobose to clavate,  $27-30 \times 33-36 \mu\text{m}$ , spines cover the entire surface of the cell (Plate 4.3 F), surface with spines  $1.5-4.5 \mu\text{m}$  high,  $<1.5 \mu\text{m}$  wide at the base and  $1.0-4.5 \mu\text{m}$  apart (Plate 4.3 G). Hyphae pale cream (0-0-5-0 to 0-0-15-0);  $4.5-7.5 \mu\text{m}$  wide; wall with two layers,  $0.5-1.5 \mu\text{m}$  thick.

#### 4.9.2 Discussion

The spores of *Gigaspora* sp. were separated from other species by the large size, white to yellowish cream colour and the sporogenous cell. It is distinguished from the three members of *Gigasporaceae* from the site by the dark smooth surface. *Gigaspora* sp. from Malawi was distinguished from the synonym of *G. margarita* also found at the same site by colour, with the colour of the synonym of the *G. margarita*, the species *Gigaspora ramisporophora* Spain, Sieverding & Schenck, distinctly orange to saffron and the *Gigaspora* sp. cream to pale yellow.

The morphological features of *Gigaspora* sp. resembles spores of the true type description for *G. margarita* in colour (Schenck & Perez 1990) even though the spore sizes of the *Gigaspora* sp., ( $204-396 \mu\text{m}$ ), ranges both smaller and larger than the true type description of *G. margarita* ( $260-280 \mu\text{m}$ ) and smaller than INVAM specimen description of *G. margarita* ( $260-400 \mu\text{m}$ ). The subglobose shape noted in the true type specimen description of *G. margarita* and INVAM specimen of *G. margarita* and the consistently globose shape of the *Gigaspora* sp. spores are plastic morphological characters that cannot be used to

distinguish species. The sizes were also overlapping and hence based on these three morphological characters, *Gigaspora* sp. could easily be considered a *G. margarita*. The spore wall of the *Gigaspora* sp. from Malawi has three distinct layers observed with composite wall size of 16.5-22.5  $\mu\text{m}$  compared with the type specimen description of *G. margarita* composite wall of 5-24  $\mu\text{m}$  and INVAM specimen of *G. margarita* of 14.6-33.4  $\mu\text{m}$ . The spore wall characters for the new type for *G. margarita* spores described by Bentivenga and Morton (1995) are similar to the specimen from Malawi. The spores of the new type specimen description for *G. margarita* was also described to have three layers, with the outer layer < 1.0-2.5  $\mu\text{m}$  thick, middle layer with variable number of laminae (5-) 14-25  $\mu\text{m}$  and an inner papillate layer 1-5  $\mu\text{m}$  extending into the lumen of the spores. The papillate appearance of the inner layer was observed as uneven surface in the specimen from Malawi. No multiple germ tubes were observed from the uneven surface of the specimen from Malawi. The spore wall characteristics of *Gigaspora* sp. are also similar to the type specimen description of *G. margarita* (Schenck & Perez 1990) and the newly revised description of *G. margarita* (Bentivenga & Morton 1995).

The sporogenous cell (36-48  $\mu\text{m}$ ) diam. and the wall thickness (2.5-4.5  $\mu\text{m}$ ) is within the range of the size of the type specimen description cell diam. (27-58  $\mu\text{m}$ ) and wall thickness (1-5  $\mu\text{m}$ ) of *G. margarita*. The colour of the subtending hyphae being cream to ochraceous and hyphae coenocytic in the *Gigaspora* sp. compared with hyphae being hyaline to pale brown and septate in the type specimen description of *G. margarita*. The hyphal wall thickness in the specimen from Malawi (1.5-4.5  $\mu\text{m}$ ) is within the range of the type specimen description (1-5  $\mu\text{m}$ ). The variations in sporogenous cell are not significant.

The auxiliary cells of the *Gigaspora* sp. are also similar to descriptions of *G. margarita*. Auxilliary cells were described as vesicles formed in soil in the true type specimen description of *G. margarita* with up to 20-35  $\mu\text{m}$  diam. becoming covered with warty projections up to 4  $\mu\text{m}$  high and 5  $\mu\text{m}$  wide. The auxiliary cells of the *Gigaspora* sp. (27-30  $\times$  33-36  $\mu\text{m}$ ) with spines 1.5-4.5  $\mu\text{m}$  high are within the range for the type description of *G. margarita* but the width, <1.5  $\mu\text{m}$  is thinner than in the type specimen description. The characteristics of the spore wall, sporogenous cell and auxiliary cells of the *Gigaspora* sp. are not distinctly different from *G. margarita*.

The spore wall of the *Gigaspora* sp. from Malawi however, reacts differently to Melzers reagent from the spore walls of *G. maragarita* (Schenck & Perez 1990; Bentivenga & Morton 1995). Reactions to Melzer's reagent were observed in all three layers with variable staining intensity in the *Gigaspora* sp. specimen from Malawi compared with reaction of only the middle laminate L2 in the recently revised *G. margarita* specimen described by Bentivenga and Morton (1995) and no reaction was reported in the true type description of the *G. margarita* type specimen (Schenck and Perez 1990).

Bentivenga & Morton (1995) did not obviously consider Melzer's staining as distinctive because the true type of *G. margarita* did not stain, yet the description of *G. margarita sensu stricto* (which is not a new type) has L2 staining whereas the true type (Becker & Hall 1976) does not. The synonym of *G. margarita*, *Gigaspora ramisporophora*, has L1 and L2 staining with Melzer's, therefore does not conform to the true type specimen description of *G. margarita*. The fact that the *Gigaspora* sp. from Malawi and the true type

of *G. margarita* and the synonym of *G. margarita*, *G. ramisporophora*, have completely different reactions to Melzer's reagent makes them distinct. However, Bentivenga and Morton (1995) felt differently because they synonymized *G. margarita* and *G. ramisporophora* and described a new type for *G. margarita* with layer (L2) that stains. Melzer's reagent appears to offer the greatest potential to aid in identifying AMF with the dextrinoid colour gradation relating to chemical composition of spore walls and the degree of polymerization of these compounds (Morton 1988). Variability in staining reactions was observed with spore ontogeny (Koske & Walker 1985). With the exception of the inner spore layer, the remaining layers were considered to be variable under different environmental conditions (Morton 1988). Morton and Bentivenga (1995) in their revision of *G. margarita* described reaction with Melzer's in only L2. This explains why they still considered the type specimen described by Becker and Hall (1976) as the same as their species, since staining of L2 is considered to be variable. However, the *Gigaspora* sp. from Malawi had all the three layers reacting with Melzer's reagent. I therefore consider wall staining of the inner layer of the *Gigaspora* sp. taxonomically distinct and hence the *Gigaspora* sp. is distinct from the newly described *G. margarita*.

4.10 GIGASPORA MARGARITA Becker and Hall, Mycotaxon 4: 155-160, 1976. (Plate 4.3 H-J) =*Gigaspora ramisporophora* Spain, Sieverding & Schenck, Mycotaxon 34: 667-677, 1989.

#### 4.10.1 Spore characteristics

Spores found singly in soil, borne on sporogenous cell; saffron (0-30-40-0 to 0-30-100-10 and 0-40-60-0 to 0-40-100-10) to orange and apricot (0-60-60-0 to 0-60-100-10); globose to subglobose, spore size  $187-444 \times 396\mu\text{m}-456 \mu\text{m}$ .

Spore wall characteristics of three layers (L1-3) in one group (Plate 4.3 I & J). Outer layer (L1) thin, rigid, smooth, with debris adhering on the surface, 1-3  $\mu\text{m}$ . Middle layer (L2) laminate, with 3-4 laminae, saffron to orange, 9-10  $\mu\text{m}$ . Inner layer (L3) thin, hyaline, 1-2  $\mu\text{m}$ , inner surface uneven with coalesced spore contents at a localized region (Plate 4.3 J). All spore layers not reacting with Melzer's reagent.

Sporogenous cell borne vertically, sub-hyaline, pale saffron, pale orange to apricot, 36-48  $\mu\text{m}$  wide; cell wall 3-6  $\mu\text{m}$  at point of attachment thinning gradually to 1-3  $\mu\text{m}$  away from the point of attachment; sub-tending hyphae 12-18  $\mu\text{m}$ , sparsely septate, thinning to 9-12  $\mu\text{m}$  away from the sporogenous cell; two hyphal walls 3-4  $\mu\text{m}$  thick gradually narrowing to <1.0  $\mu\text{m}$  away from the sporogenous cell. Spore occlusion is by a plug.

#### 4.10.2 Discussion

The spores of *G. margarita* were separated from other species by the large size, sporogenous cell and the distinct apricot to orange colour. The spores have a smooth shiny surface.

The description of this specimen was done entirely with spores from field soils hence it was not possible to describe the developmental characters and characters of the auxiliary cells. The specimen resembles the revised species *G. ramisporophora* in all aspects (Bentivenga & Morton 1995; Schenck and Perez 1990). The colour is distinctively orange to apricot (golden yellow to yellowish brown) in the specimen from Malawi as the synonym of *G. margarita*, *G. ramisporophora* (Spain *et al.* 1989). The spore shape and size (globose to subglobose;  $187-444 \times 396-456 \mu\text{m}$ ) of the species from Malawi is within the range of the revised *G. margarita* globose (96-) 200-450(567)  $\mu\text{m}$  to subglobose (143-) 150-400  $\times$  200-450(-501)  $\mu\text{m}$  (Spain *et al.* 1989).

The spore wall of the specimen from Malawi and the synonym of *G. margarita*, *G. ramisporophora* have three layers comprising a slightly thinner outer layer of 1-3  $\mu\text{m}$  compared with 1.4-4.0(-5.7)  $\mu\text{m}$  of the true type description and similar to 1.6-2.4  $\mu\text{m}$  of the description of INVAM specimen, a middle laminate layer with 3-4 laminae and slightly thinner (9-10  $\mu\text{m}$ ) than the true type specimen description (4.0-28  $\mu\text{m}$ ) and the INVAM specimen description of 13-31  $\mu\text{m}$  in PVLG and 14-23  $\mu\text{m}$  in Melzer's reagent. The thickness of inner layer 1-2  $\mu\text{m}$  and the uneven inner surface with coalesced spore contents at a localized region are within the range of the true type description with thickness of L3 of 1.3-2.6  $\mu\text{m}$  with papillae developing prior to germination, but slightly less than the L3 of the INVAM specimen description of warts 1.2-5  $\mu\text{m}$  and 2.5-3  $\mu\text{m}$  wide in germinating spores. In the species from Malawi, the layers did not react with Melzer's reagent, although the orange spore colour could have obscured the reaction, contrary to the reddish purple reaction of L1 and L2 in the type description. The fact that the synonym of *G. margarita*,

*G. ramisporophora*, the true type specimen description of *G. margarita* and the INVAM specimen of *G. margarita* stain differently makes them distinct. However, Morton (1988) considered reaction to Melzer's reagent of outer layers (L1 and L2) to be unstable and only the innermost layer (L3) to be stable. Hence based on Morton's observations on reaction of spore layers to Melzer's reagent, the species from Malawi and the synonym of *G. margarita*, *G. ramisporophora* constitute sufficient evidence to maintain them as the newly described *G. margarita*.

The sporogenous cell was only vertically (apically) attached in the species from Malawi compared with both apical and sub-apically attached in the true type description. The sporogenous cell wall of three layers in both species with the sub-hyaline, pale saffron, pale orange to apricot colours correspond to the colours of the three spore layers. The size (36-48  $\mu\text{m}$ ) of the sporogenous cell of the specimen from Malawi is smaller than the true type specimen ((32-)40-60(-72)  $\times$  (50-)60-80(-83)  $\mu\text{m}$ ). Hyphal protrusions were not observed in the species from Malawi. This may not necessarily indicate that the hyphal protrusions were absent as the material used for description was from the field. The sub-tending hyphae of diameter 12-18  $\mu\text{m}$  and sparse septae of the species from Malawi is from one sporogenous cell compared with the thinner sub-tending hyphae of the type description species with diameter 9.3-13.9  $\mu\text{m}$ , wall thickness 1.4-2.8  $\mu\text{m}$  and distance between septae variable and becoming more frequent close to the sporogenous cell with one or rarely two bulbous cells, some with protrusions. These differences are not sufficient to reclassify the species.

*G. margarita* has been recorded in association with *Vangueria infausta* subsp. *infausta* in the Republic of South Africa (Gaur *et al.* 1999), in association with *Faidherbia albida* in Senegal (Dalpe *et al.* 2000) and in association with *Acacia holosericea* and *Acacia mangium* in Sudan (Ba *et al.* 1996). This is the first record of *G. margarita* in Malawi.

#### 4.11 SCUTELLOSPORA CERRADENSIS Spain & Miranda, Mycotaxon 60: 130, 1996.

(Plate 4.4 A-E).

##### 4.11.1 Spore characteristics

Spores found singly in soil and in roots as ovoid spores; borne on sporogenous cell (Plate 4.4 A & 4.4 B), hyaline, white, straw (0-0-5-0 to 0-0-15-0) to yellowish cream 0-0-20-0 to 0-0-40-0) in mature spores, globose to subglobose  $126-246 \times 186-300 \mu\text{m}$ .

Spore wall characteristics of five layers (**L1-5**) in three groups (groups A, B and C) (Plate 4.4 D). Spore wall breaks easily under slight pressure. Group A a bi-layer, outer sub-layer (**L1**) hyaline, thin,  $1.5-3.0 \mu\text{m}$ , with warts, warts more conspicuous in young spores appearing highly spiny (Plate 4.4 B),  $0.5-3.0 \mu\text{m}$  high and  $<1.0$  wide and  $0.5-3.0 \mu\text{m}$  apart. Inner sub-layer (**L2**), unit, rigid, straw to yellowish cream,  $3-6 \mu\text{m}$ . Group B a bilayer, outer sub-layer (**L3**), laminate, slightly separated from L4. Inner sub-layer (**L4**) a unit layer completely detached from L5. Group C of a single layer (**L5**), thin, membranous layer,  $<1.5 \mu\text{m}$ . L2 stains lightly to reddish orange, and L4 and L5 stains readily to purplish red in Melzer's reagent, other layers not reacting (Plate 4.4 D); sporogenous cell is borne laterally or vertically (Plate 4.4 A & 4.4 B), cinnamon (0-30-50-10 to 0-30-70-10) to pale brown (0-

40-50-10 to 0-40-70-10), cell 21-30  $\mu\text{m}$  wide; cell wall two layers, 1.5-4.5  $\mu\text{m}$  at the point of attachment with spore thinning to  $<1.0 \mu\text{m}$ , cell wall continuous with the spore outer layer; subtending hyphae pale brown (0-40-100-0 to 0-40-100-10), width at point close to sporogenous cell 15-25  $\mu\text{m}$  thinning to 6-9  $\mu\text{m}$  away from the cell, hyphal wall 0.5-1.5  $\mu\text{m}$ ; germination shield located beneath the sporogenous cell underneath L3, rusty tawny to date brown (20-60-60-0 to 20-80-100-10), subglobose; 21-52  $\times$  30-60  $\mu\text{m}$ , rough margin, 8 fissures with Y configuration, aperture present (Plate 4.4 A); auxiliary cells 5-9, borne on coiled hyphae (Plate 4.4 E), rusty tawny to date brown (20-60-60-0 to 20-80-100-10), hyphal wall  $<1.5 \mu\text{m}$ , cells lobed/knobbed, 15-24  $\mu\text{m}$ , lobes 2-4 per cell, 9-12  $\mu\text{m}$  wide, 3-6  $\mu\text{m}$  high, sometimes lobes are so low that they are indistinguishable.

#### 4.11.2 Discussion

The spores are distinguished from other species by the white, yellowish cream to straw colour, the sporogenous cells, large size, and the globose, subglobose to irregular shaped spores. The surface appearance of the spore is finely granular and the distinct brown germination shield is visible under the stereomicroscope.

The species from this site resemble the type specimen description in most aspects with very slight variations observed (Spain & Miranda 1996). The spore size (126-246  $\times$  186-300  $\mu\text{m}$ ) of the specimen from Malawi is within the range of sizes (167-)230-363 of the type specimen and 220-380  $\mu\text{m}$  of INVAM specimen description, though at the small end of the size distribution. Five layers in three groups, in PVLG and in PVLG and Melzer's reagent, were observed in the specimens from Malawi compared with four layers and two inner

walls in the type description and four layers and two bi-layered inner flexible walls in the INVAM specimen description. The type specimen has a laminate layer surrounded by two outer layers and an inner flexible layer that generally separates from the other layers at the point of attachment to sporogenous cell. In the specimen from Malawi the laminate layer (L3) is also surrounded by an outer bi-layer (L1 & L2) and a single unit layer (L4). The INVAM laminate layer (L3) is surrounded by an outer bi-layer (L1 and L2) and a thin hyaline layer (L4) that is easily mistaken for a separate flexible inner wall. The type specimen description does not mention L4. This unit layer (L4) could easily be mistaken for part of the laminate layer (L3). This could possibly have led to observation of four layers by Spain & Miranda (1996). L5 in the specimen from Malawi was observed as a wrinkled layer. The single layer of wall group C (L5) in the specimen from Malawi is comparable with the germinal membranous layers in the type specimen description and the INVAM specimen description with the possibility of the wrinkles obscuring the sub-layers observed in the type and the INVAM specimen description. In outer sub-layer (L3) of wall group B for the specimen from Malawi, two slightly separated laminae were observed and staining with Melzer's reagent showed non-staining of these laminae in L3 and heavily staining L4. In the type specimen description, the stained specimen was observed to have five layers. Reaction with Melzer's reagent in the type specimen was observed on the laminate layer (L3) (reddish yellow-reddish brown) and the outer of inner layer 1 (reacting weakly to pink tinge), inner layer 2 (pink), two layers of inner wall two (a light pink outer layer over a reddish purple inner layer). In the species from Malawi, reaction to Melzer's reagent was observed on rigid layer (L2) to reddish orange and L4 & L5 to reddish purple. Except for the differences in perception of layers by Spain and Miranda (1996), spore

reaction to Melzer's reagent and number of spore layers of the type specimen and the INVAM specimen agrees well with stained specimen from Malawi. The inner membranous layer stained pink to purplish red in the specimen from Malawi, INVAM and type specimen. There was no consistency in reactions to Melzer's reagent in the remaining layers. According to Morton (1988) staining of all layers except the innermost membranous layer is unstable and hence of no taxonomic importance, hence the differences in staining of the outer layer may be considered of no taxonomic importance. The difference in staining of outer layer is minimal and not substantial enough to reconsider classification.

The confusion in the perception of particularly the inner layers by Spain & Miranda (1996) was acknowledged in the INVAM specimen description. They confused the numerous folds of the highly plastic L2 of the germinal layer 2 as being a separate layer. The perception of the innermost L5 as one wrinkled layer could be explained by observations made in the INVAM specimen description. They observed both germinal walls (gw1 and gw2) not to separate easily even in vigorously crushed spores and thus resembling one very thick hyaline wall, much like found in *Scutellospora erythropha* (Koske & Walker) Walker & Sanders. The more pronounced warty ornamentations in the younger spores of specimen from Malawi were not mentioned in the type and INVAM specimen description.

The location of the shield was between germinal inner wall one (gw1) and two (gw2) in the type specimen description and on the germinal layer 2 in the INVAM specimen compared with the inner bilayer (L4 & L5) in the specimen from Malawi. The colour of the germination shield is consistently light brown to dark brown.

The yellowish-brown to brown sporogenous cell of the type specimen description and INVAM specimen resembles the cinnamon to pale brown colour of the specimen from Malawi. However, the emergence of hyphae from several pegs in the type specimen description was not observed in the specimens from Malawi. There was no evidence of two spores of variable sizes developing at the apex of sporogenous cell as was observed by Spain *et al.* (1989). These are however not significant diagnostic features. The yellowish brown coiled hyphae of the type specimen description corresponds with the rusty tawny to date brown colour of the specimens from Malawi. The size of sporogenous cells (15-24  $\mu\text{m}$ ) of the specimen from Malawi is smaller compared to the size (20-33  $\mu\text{m}$ ) of cells of the type specimen description and 32-45  $\mu\text{m}$  of cells of the INVAM specimen description. I consider the sporogenous cells, location of germination shield and the auxiliary cells taxonomically similar. I therefore consider this species to be *S. cerradensis*.

The type specimen was recorded from Brazil from a dark red latisol, mostly clayey and poor in phosphorus availability (3.5  $\mu\text{g/g}$  soil) and pH (water 1:2.5) 4.8-5.0. The conditions of soil from the collection site of Malawi specimen was that of a ferric lixisol with 52% sand and 32% clay, pH (water 1:2.5) 6.0 and phosphorus 0.51  $\mu\text{g/g}$  soil. The species from Malawi were also extracted from soils without inorganic fertilizer and soils that received 40 kg P ha<sup>-1</sup> fertilizer since 1992, implying tolerance in this species to high phosphorus as well as the predilection for low phosphorus soils. The type specimen site of collection progressed from predominantly native grass to pasture to soybeans and sites that had been left for a fallow period of five years followed by planting of different legumes for green manure. The type species was successfully cultured on *Tephrosia candida* (Roxb.) DC. In

my case, the natural vegetation of southern Malawi is predominately ceasalpinoid species. My species was recovered from the agroforestry systems with *Gliricidia sepium*/maize, *Sesbania sesban*/maize and *S. macrantha*/maize intercrops and maize monocrop. This too implies a lack of sensitivity to plant type and this soil condition. The species was successfully cultured on *G. sepium*, *S. sesban*, *S. macrantha*, *S. siamea*, *S. spectabilis* and *Sorghum bicolor*.

4.12 SCUTELLOSPORA aff. DIPURPURASCENS Morton & Koske., Mycologia 80 (4): 520-524, 1988. (Plate 4.4 F-I)

#### 4.12.1 Spore characteristics

Spores found singly in soil, terminally on sporogenous cell, cream (0-0-5-0 to 0-0-15-0) to yellowish cream (0-0-20-0 and 0-0-40-0), globose 165-300  $\mu\text{m}$ .

Spore wall characteristics of four layers (L1-4) in two groups (group A & B) (Plate 4.4 G & H). Group A, a bi-layer with outer sub-layer (L1) smooth, pale cream, rigid, breaks only under high pressure, <1.5 $\mu\text{m}$ , adhering to L2. Inner sub-layer (L2), laminate, 6-9  $\mu\text{m}$ , cream (0-0-5-0 to 0-0-15-0) to yellowish cream (0-0-20-0 and 0-0-40-0), separates from an inner layer (L3). Group B, bi-layer of inner hyaline layer with two equal sub-layers (L3 & L4), two sub-layers separating at a point enclosing the germination shield, <1.0  $\mu\text{m}$  each (Plate 4.4 G). Reaction to Melzer's reagent, L2 turning orange brown and inner membranous bi-layer (L3 & L4) turning purplish red (Plate 4.4 H).

Sporogenous cell borne vertically, sub-hyaline, 36-54  $\mu\text{m}$ , cell wall of two layers 1-1.5  $\mu\text{m}$ , outer layer continuous with the outer rigid spore layer (L1) and inner hyaline thin layer of cell continuous with middle spore layer (L2), peg 4.4-9  $\mu\text{m}$  long, arising from sporogenous cell and projecting towards spore; subtending hyphae septate, 15-24  $\mu\text{m}$  wide near sporogenous cell reducing to 6-9  $\mu\text{m}$  at point of first septum, hyphal wall 1-1.5  $\mu\text{m}$ .

Germination shield, pale cinnamon (0-30-50-10 to 0-30-70-10) (Plate 4.4 F), ovoid 105  $\times$  117  $\mu\text{m}$ , location of the germination shield underneath the sporogenous cell, below the laminate layer (L2), between the two inner sub-layers (L3 & L4), 5-10 germination tubes emerging from the shield.

Auxiliary cells in aggregates of 4-10 cells per coiled hyphae, hyaline to sub-hyaline; clavate; top margin of cell undulated with 4-5 lobes, 1.5-4.5  $\mu\text{m}$  high, 3-9  $\mu\text{m}$  wide, 3-6  $\mu\text{m}$  apart, cells borne on a coiled sub-hyaline to pale cinnamon (0-30-50-10 to 0-30-70-10) hyphae, 3-12  $\mu\text{m}$  wide, with hyphal wall 0.5-1.5  $\mu\text{m}$  wide (Plate 4.4 I).

#### 4.12.2 Discussion

The spores of this *Scutellospora* species were distinguished from spores of other species by the large size, sporogenous cell and the yellowish cream colour. The spore is robust, the surface smooth, shiny and consistently globose.

The specimen resembles *S. dipurpurascens*, *Scutellospora calospora* and *Scutellospora fulgida* Koske & Walker in most aspects with slight variations noted. The spores from Malawi were consistently globose and larger in size (165-300  $\mu\text{m}$ ) compared with subglobose to oblong and sometimes irregular spores of INVAM specimen descriptions of *S. dipurpurascens* (140-240  $\mu\text{m}$ ) and *S. calospora* (120-220  $\mu\text{m}$ ), but was similar in shape of globose to subglobose spores and still larger than *S. fulgida* (120-240  $\mu\text{m}$ ). Four spore layers were recorded from the specimen from Malawi compared with two spore layers and one flexible inner germinal layer in *S. fulgida*, two spore wall layers and two hyaline flexible inner germinal layers in *S. calospora* and *S. dipurpurascens*. The descriptions of the first two layers (L1 & L2) were similar to the two layers that make up the spore wall in the INVAM specimen description of the three species. The inner flexible layers that enclose the germination shield in the species from Malawi are similar to the inner germinal layers in the three species. *S. fulgida* has one germinal layer with two layers that appear so adherent particularly in field collected spores and separates in pot culture spores with the outer layer appearing as folds from parts of the wall. The outer layer of the germinal layer is so thin and difficult to resolve without differential interference optics. *S. dipurpurascens* has two germinal layers with germinal layer 1 (gw1) with one hyaline layer often adherent to the laminate spore wall (L2) and it is difficult to resolve and an inner gw2 with two hyaline layers that are almost always adherent. *S. calospora* has two germinal layers each of two layers with gw1 with two layers that are usually adherent, and are positioned very close to spore wall, and thus are difficult to detect, and gw2 with two layers always adherent. It is difficult to separate these three species from the species from Malawi based on the inner flexible layers unless a higher resolution and differential interference optics is

used. However, reaction of spore layers to Melzer's reagent is able to separate the *Scutellospora* species from Malawi from some of the species. L2 of the species from Malawi turns orange brown and the inner flexible bilayer (L3 & L4) turns purplish red contrary to L2 of *S. fulgida* that is bright yellow to gold colour in Melzer's reagent, the inner flexible layers not reacting. Since the staining of the inner membranous layer is considered taxonomically diagnostic (Morton, 1988), the *Scutellospora* species from Malawi, *S. dipurpurascens* and *S. calospora* are distinct from *S. fulgida*. The reaction to Melzer's reagent for the species from Malawi is similar to *S. calospora* and *S. dipurpurescens*. No reaction to Melzer's reagent was detected in L2 of *S. dipurpurescens* and *S. calospora*, hence differing from the orange brown colour observed in the specimen from Malawi. In *S. dipurpurescens*, gw1 stained light pink in Melzer's reagent and in gw2, the outer layer stained a weak pink and the inner layer stained red-purple to dark red-purple. In *S. calospora*, reaction to Melzer's reagent is detected in only the gw2 with outer layer 1 producing a weak pink reaction in Melzer's reagent and inner layer 2 staining red-purple to dark red-purple in Melzer's reagent. It was difficult to separate the intensity of reaction to Melzer's reagent in the species from Malawi for L3 and L4 since they did not separate. Staining of the outer wall layers was noted to be unstable and hence of no taxonomic importance (Morton 1988), therefore the species from Malawi differs from *S. fulgida* but greatly resembles both *S. calospora* and *S. dipurpurascens*. A comparison of auxiliary cells places the species from Malawi closer to *S. dipurpurascens*. The species from Malawi has aggregates of 4-10 cells per coiled hyphae compared with 1-10 cells in *S. dipurpurascens* and only 1 cell in *S. calospora*.

With the exception of *S. calospora* that was recorded from Libyan soils as *Gigaspora calospora* (Nicolson & Gerd.) Gerd. (El-Giahmi *et al.* 1976), There is no record of *S. fulgida* and *S. dipurpurascens* from the African continent. *S. fulgida* was described from the coastal sand dunes on the eastern North America seaboard in the root zones of *Ammophila breviligulata* Fern., *Solidago sempervirens* L., and *Uniola paniculata* L., but the plants were not proven mycorrhizal (Koske & Walker 1986b). *S. dipurpurascens* was first recorded in several unmanaged pastures and partially reclaimed surface coal mine in West Virginia (Morton & Koske 1988) and *S. calospora* was recorded in Washington in sand dunes, in east of the Cascade range in Oregon and in orchards, open pine forests, and semi-arid mountains (Gerdemann & Trappe 1974b) and the Forestry Commission in Scotland, sand dunes of California and Massachusetts, in lake dunes in New Jersey and beach dunes in Rhodes island (Koske & Walker 1986b). The natural vegetation of southern Malawi is predominately ceasalpinoid species. The *Scutellospora* sp. was recovered from agroforestry systems with *Gliricidia*/maize, *S. sesban*/maize and *S. macrantha*/maize intercrops and maize monocrop. The isolate from Malawi was successfully cultured on *G. sepium*, *S. sesban*, *S. macrantha*, *S. siamea*, *S. spectabilis* and *S. bicolor*. This implies lack of sensitivity to plant types.

Plate 4.3 A

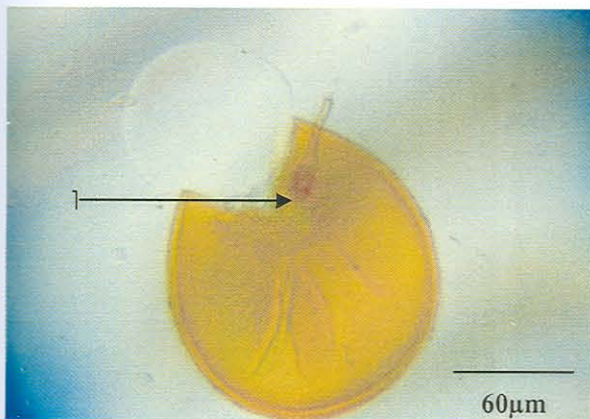


Plate 4.3 B

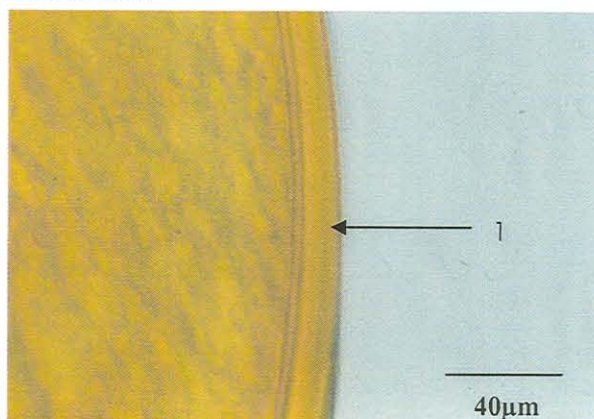


Plate 4.3 C

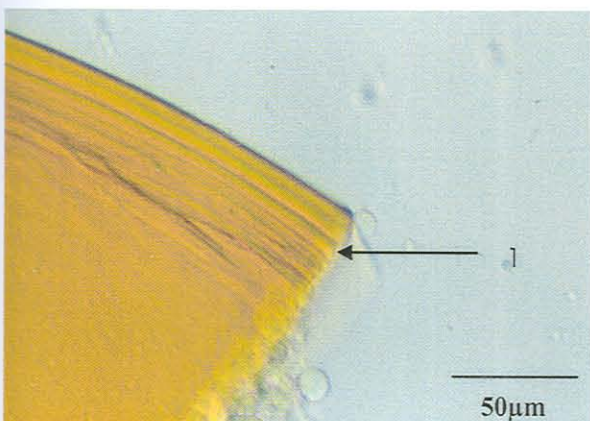


Plate 4.3 D

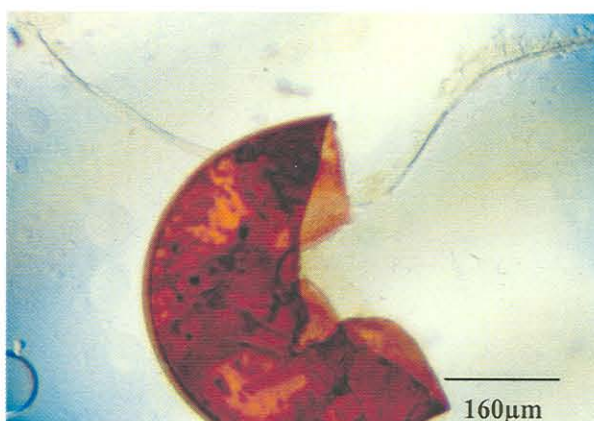


Plate 4.3 Spores of *Gigaspora* sp. (A-D) *Gigaspora* sp (A) (1) Spore with bulbous suspensor cell. (B) (1) Composite wall of young spore without laminations. (C) (1) Laminations in mature spore (D) Patchy staining with Melzer's reagent

Plate 4.3 E

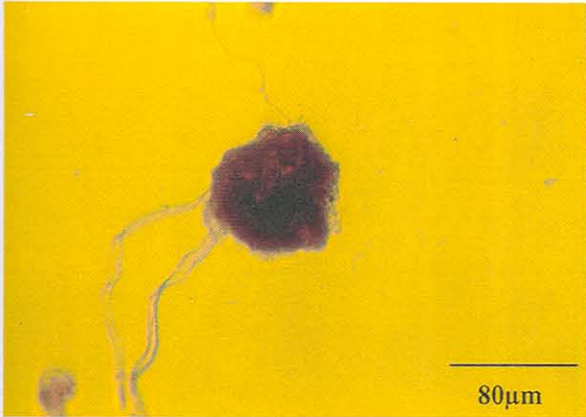


Plate 4.3 F



Plate 4.3 G

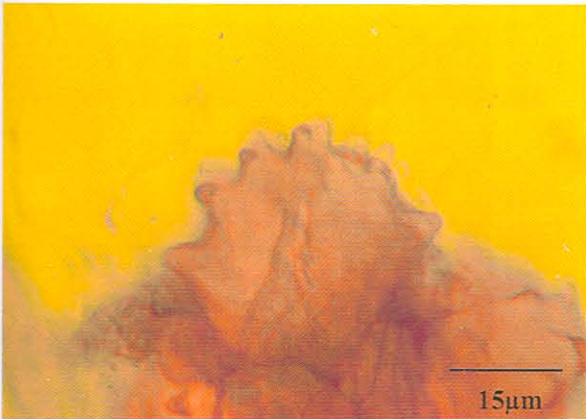


Plate 4.3 (E-G) Auxilliary cells of *Gigaspora* sp (E) clump before auxiliary cell development (F) auxiliary cells exposed (G) spiny auxilliary cell.

Plate 4.3 H

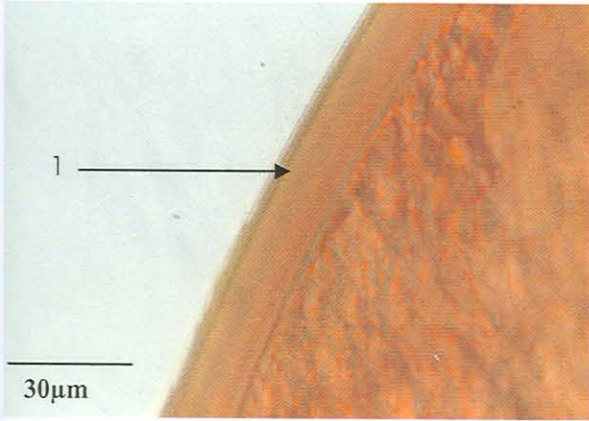


Plate 4.3 I

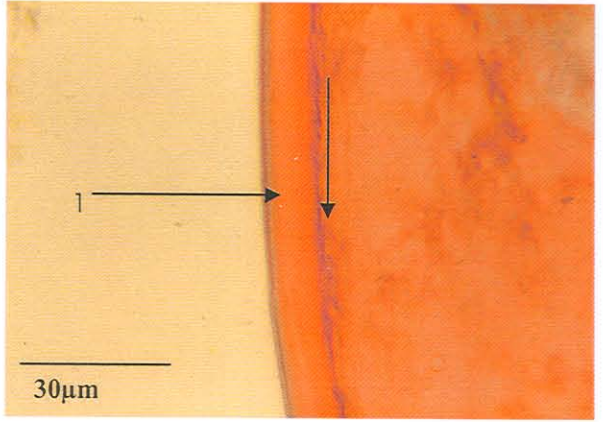


Plate 4.3 J

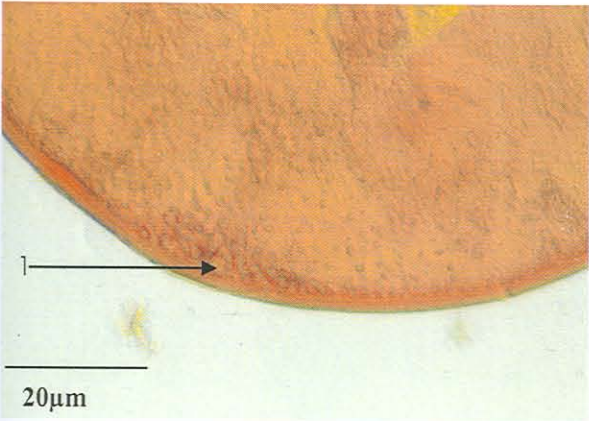


Plate 4.4 A

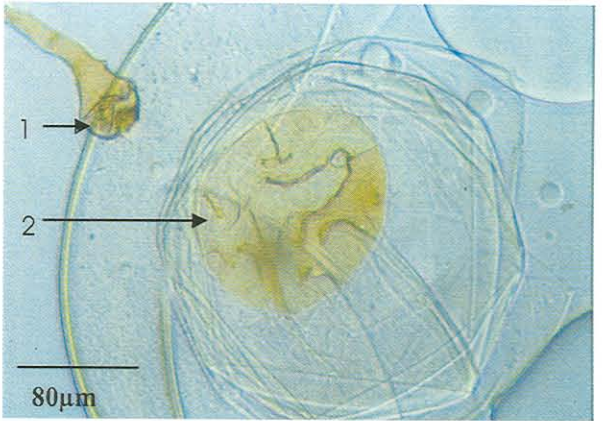


Plate 4.3 Spores of *G. margarita* (H-J) Spore of *Gigaspora margarita* (H) (1) Composite wall in mature spore (I) (1) Composite wall in young spore (J) (1) Germinal point with coalesced spore contents in a localized region.

Plate 4.4 Spores of *S. cerradensis* (A) *Scutellospora cerradensis* (A) Spore with (1) sporogenous cell (2) germination shield.

Plate 4.4 B

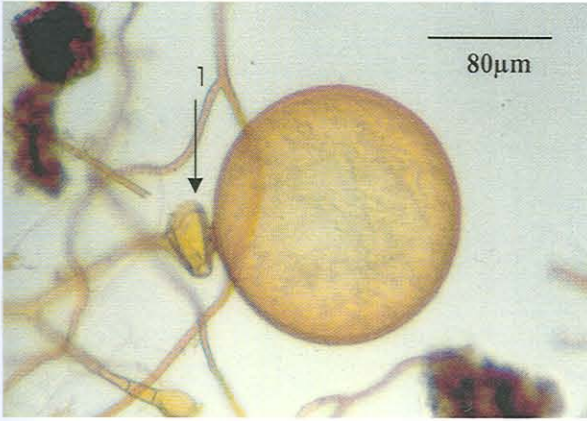


Plate 4.4 C

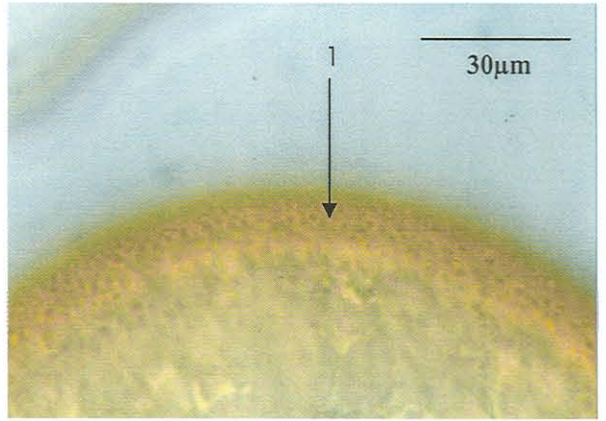


Plate 4.4 D

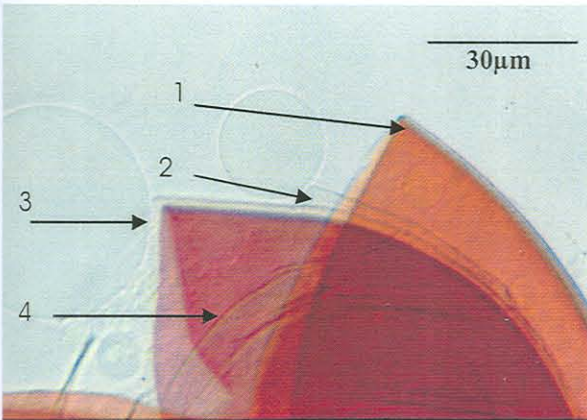


Plate 4.4 E



Plate 4.4 Spores of *S. cerradensis* and auxiliary cells (B-E) *Scutellospora cerradensis* (B) Young spore (1) with large sporogenous cell on hyphae (2) germination shield (C) young spore with outer papillate surface (D) pore reaction with melzter's reagent (1) outer layer with two sub-layers with only inner sub-layer staining (2) L 2 staining lightly (3) inner layer with two sub-layers (L4 & L5) with inner membranous sub-layer staining heavily and (E) knobbed auxiliary cell.

Plate 4.4 F

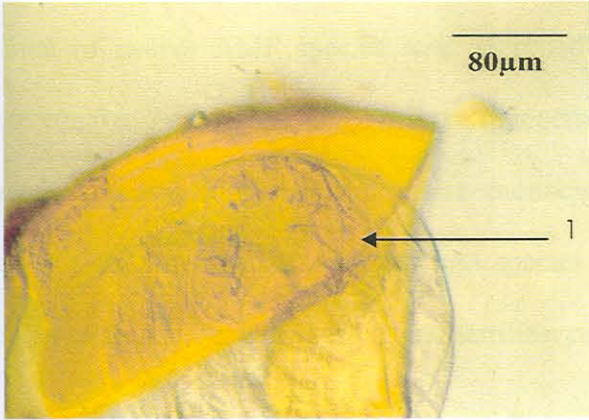


Plate 4.4 G

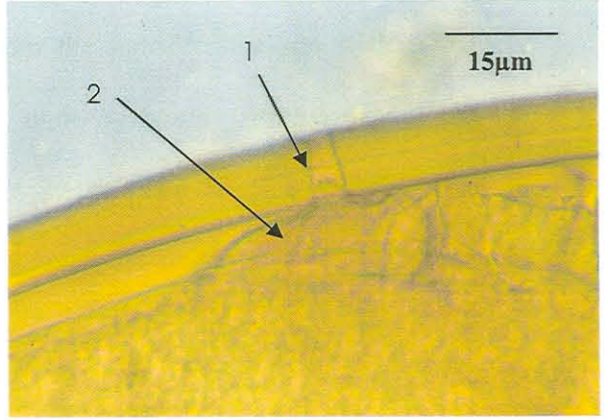


Plate 4.4 H

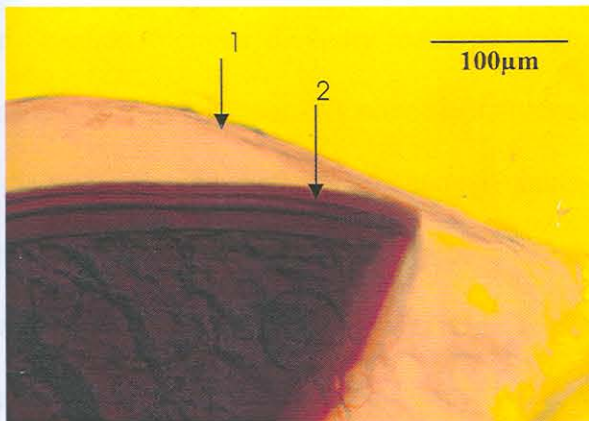


Plate 4.4 I

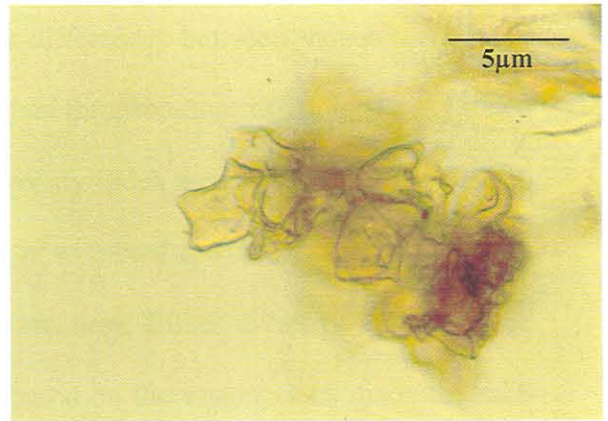


Plate 4.4 Spores of *Scutellospora dipurpurascens*. (F-I) *Scutellospora dipurpurascens*. (F) Spores with (1) pale brown germination shield. (G) Spore with (1) germination tube through wall from the (2) germination shield (H) Crushed spore with (1) outer layer stained lightly with Melzer's reagent and (2) inner layer stained heavily (I) knobbed auxiliary cells.