CHAPTER 4: TAXONOMY OF ARBUSCULAR MYCORRHIZAL FUNGI

Spores of twelve AMF species were recovered from the soil from the agroforestry and maize monocrop systems. Spores of seven species were determined as *Acaulospora rehmii* Sieverding & Toro, *Glomus* aff. *aggregatum, Glomus etunicatum, Glomus* aff. *geosporum*, *Gigaspora margarita, Scutellospora cerradensis* and *Scutellospora dipurpurascens*. Specimen represented as *Archaeospora* sp. was determined to genus level as it failed to establish in pot culture in this study, hence there was insufficient material to accurately diagnose the species. *G. margarita* and *G. geosporum* also failed to establish in pot culture, however, all morphological features of spores from the field enabled complete and accurate reference with the INVAM specimen and the type specimen description in Schenck and Perez (1990). Specimen represented as *Acaulospora* sp. 1, *Acaulospora* sp. 2, *Glomus* sp. and *Gigaspora* sp. although successfully established in pot culture, had some morphological characters that strongly overlapped with their allies, hence were also represented to genus level. Nine species were successfully pot cultured on *Senna siamea* (Lam.) Irwin & Barneby (syn. *Cassia siamea*); *Senna spectabilis* (DC) Irwin & Barneby (syn. *Cassia spectabilis*), *Sorghum bicolour* (L.) Moenche; *Zea mays* L., *Sesbania sesban*; *S. macrantha* and *Gliricidia sepium*.

4.1 ACAULOSPORA sp. 1. (Plate 4.1 A)

4.1.1 Spore characteristics

Spores found singly in soil; hyaline, white and cream (0-0-5-0 to 0-0-15-0) when young and yellowish cream (0-0-20-0 to 0-0-40-0) at maturity (INVAM Colour Chart & Colour

Chart for Fungi, Edinburgh Botanic Garden 1969); globose to subglobose; 78-114 to 99-105 ×114-120 µm.

Spore wall consists of four layers (**L1-4**) in two groups (groups A and B). Group A with an outer granular uneven sloughing layer (**L1**) 1.5–2.5 µm thick; usually adherent to the middle layer; middle layer (**L2**) laminate; 3.0–6.0 µm thick; white to cream (0-0-5-0 to 0-0-15-0) in immature spores and yellowish cream to yellow (0-0-20-0 to 0-0-40-0) in mature spores. Group B with a bi-layer, outer sub-layer (**L3**) hyaline, thin, 0.5-1 µm thick, visible in mild and vigorously crushed spores, completely separating from L2. Inner sub-layer (**L4**) <1.0 µm thick, appearing wrinkled in crushed spores. Spore has a circular cicatrix 6-12 µm indicating the region of contact between spore and saccule neck during spore development. Some spores appear smooth with no indication of an attachment to a sporiferous saccule. L2, darkening to yellow (0-0-60-0) in Melzer's reagent other walls not reacting. The surface of spores glitters and the spore is fragile and breaks with light pressure on crushing (Plate 4.1A).

4.1.2 Discussion

Spores of this *Acaulospora* sp.1 were distinguished by the small size, hyaline, white to cream colour, smooth glittery surface and fragile spores. Hyphal attachments were absent and spores rarely had sporiferous saccules. A circatrix was present in some spores.

The species from Malawi is compared with published reference literature (Schenck & Perez 1990) and INVAM specimen description. The spore colour, shape and surface appearance of the Malawi species are similar to *Acaulospora delicata* Walker, Pfeiffer & Bloss. The Malawi species record is however smaller than *A. delicata*. Spore size of the Malawi species range from 78-114 to 99-105 ×114-120 µm compared with 80-125(-150) × 80-110(-140) µm diam. of *A. delicata* from type specimen description but closer to size 80–120 µm of *A. delicata* specimen from INVAM. There are possibilities of errors in the interpretation of range of spore size because of non-statistical comparisons, hence it was suggested that it should not be weighted highly in species determination (Morton 1988). I therefore feel this size difference is not significant. The number of spore wall layers of the species from Malawi, are similar to the descriptions of *A. delicata* specimens from INVAM and the type description, however, the wall thickness differ slightly. The Malawi specimen differs slightly by a thicker outer layer (**L1**) (1.5–2.5 µm compared with 1 µm of type specimen description and 0.4-0.8 µm of the specimen from INVAM) and a thicker middle layer (**L2**) (3.0–6.0 µm compared with 2.5-3.5 µm of the type specimen description and 1.8-3.2 µm of the INVAM specimen). The inner wall group, L3 (0.5-1 µm compared with ±0.5 µm in the type specimen description and 0.5-0.8 µm in the INVAM specimen) and L4 (<1.0 µm compared with ±0.75-1.0 µm in the type specimen description and 0.6-0.8 µm in the type specimen description) are within the range of *A. delicata* description. The type and number of layers in spore wall is a much more stable character than sizes of the layers. The outer sub-layer of the inner wall group (L3) of the Malawi species never had ornamentation visible under light microscopy. This is contrary to observations made in *A. delicata*. The

beaded layer in the INVAM specimen tended to dislodge with applied pressure or became invisible after the specimen was mounted in PVLG for several months or longer. The orange red colour observed in the inner membranous layers of the type specimen description of *A. delicata* was never observed in the inner wall group of the Malawi species. The middle laminate layer (L2) however darkened to yellow as in L2 of *A. delicata* specimens. L2 of the INVAM specimen stained light pink to a slightly darker pink. The range of size of the circatrix (6-12 μm) is close to the size of the circatrix for the INVAM specimen (6.5-9.6 μm). At maturity, the spores of specimen from INVAM detached from the saccule and were sessile. The spore completely separated from the parent saccule neck and the spore wall had no break or opening region of attachment to the saccule neck. The circatrix in the species from Malawi was also not commonly found on spores.

Spore size and the thickness of layers are continuous variables that may be affected by environmental conditions. I do not therefore consider these differences taxonomically significant to classify *Acaulospora* sp. 1 differently from *A. delicata*. Staining of the inner membranous layer is considered taxonomically important (Morton 1988). However, reaction to Melzer's reagent was also not consistent in *A. delicata* type specimen and INVAM specimen. I therefore consider this species taxonomically close to *A. deilcata* but further comparisons need to be undertaken with *A. delicata* from pot cultures from ex-type material.

Only one record of *A. delicata* has been reported on the African continent. Ba *et al.* (1996) reported *A. delicata* in association with *Acacia holosericea* A. Cunn. ex G. Don. and

Acacia mangium Willd. in Sudan. If the species is confirmed to be *A. delicata*, it will be a new record for Malawi.

4.2 ACAULOSPORA REHMII Sieverding & Toro, Angewandte Botanik 61: 217 – 223, 1987. (Plate 4.1 B-D).

4.2.1 Spore characteristics

Spores found singly in soil; borne intercalary on a sporiferous saccule (Plate 4.1 B); cream (0-0-5-0 to 0-0-20-0) and cinnamon (0-10-2-0-0 to 0-10-60-0) in young spores to rusty tawny (0-20-20-0 to 20-40-100-10), date brown (20-60-40-0 to 20-80-100-10), mouse gray and smoke gray (40-60-50-10 to 40-60-10010) in older spores (INVAM Colour Chart & Colour Chart for Fungi, Edinburgh Botanic Garden 1969); globose to subglobose; 74-90 × 153-177 µm.

Spore wall characteristics of four layers (**L1-4**) in three wall groups (A, B & C). Group A consists of outer ornamented layer (**L1**), cream (0-0-5-0 to 0-0-20-0), cinnamon (0-10-2-0-0 to 0-10-60-0), date brown (20-60-40-0 to 20-80-100-10) and mouse gray to smoke gray (40-60-50-10 to 40-60-100-10). L1 forms depressions 1–1.5 µm deep 0.5-1 µm wide, creating a network of ridges organized in a labyrinth pattern. The labyrinth pattern is distinctly variable with spore age with intensity increasing with age. The ornamentation on young spores is fine to thickly spiny and highly crowded (Plate 4.1 C) and sinusoidal in mature spores (Plate 4.1 D). Group B single, hyaline, rigid layer (**L2**), 3–4 µm, usually adhering to L1. Group C a bi-layer, outer sub-layer (**L3**) with warts, 0.5-1.5 µm; inner sub-

layer (**L4**) hyaline, thin and wrinkling membranous layer. The inner sub-layer (**L4**) turns slowly to light pink in Melzer's reagent. Other layers do not react.

Sporiferous saccule with white contents; globose; 135 × 135 µm; wall of single hyaline smooth layer; distance from saccule to spore 69–75 µm. Spore consists of a circular cicatrix 15-21 µm.

4.2.2 Discussion

Spores of *A. rehmii* were separated from other species by the rusty brown colour in mature spores to cream and light brown in young spores. The spores are slightly large in size and surface is darl and granular in appearance. Most spores have sporiferous saccule or the remnant of a thick hyaline pedicel or a circatrix.

The specimen from Malawi resembles the type description and specimen from INVAM in size, shape and number of layers. Slight variations are observed in colour. The range of colours in the type description specimen is light yellow to brown with older spores often appearing brown to black and INVAM species is yellow-brown to orange-brown. The Malawi specimen differs in colour, being cream and cinnamon in young spores to rusty tawny, date brown, mouse gray and smoke gray in older spores. This colour differences in my opinion does not constitute sufficient reason for reclassifying this specimen. There are slight variations in L2, with adherence observed with only L1 in the species from Malawi and not to L1 and L3 as in the type specimen description. In the specimen from INVAM, L1 was described as a hyaline degrading layer that sloughs early in spore wall

differentiation so that it was usually absent in mature spores. The sloughing layer was not detected in the specimens from Malawi. The ornamented L2 of the INVAM species is therefore described as the ornamented L1 in the specimen from Malawi. As in the specimen from Malawi which has the ornamented L1 adherering to only L2, the ornamented L2 of the INVAM specimen adhered to L3. In the INVAM specimen, L3 consisted of three zones with the inner zone with sub-layers that separated collectively or only the innermost sub-layer separated and appeared as a distinct structure that resembled a flexible inner wall with two germinal wall layers (gw1 and gw2). The gw2 had granular excresences (or beads) that tended to become dislodged and float away with applied pressure. In the specimen from Malawi, the single hyaline rigid L2 was separated from the inner flexible group C bi-layer. This bi-layer comprised of two flexible sub-layers, L3 and L4. The inner sub-layer (L4) of the specimen from Malawi did not appear beaded. The beaded appearance was probably present but since no consideration was made on the degree of pressure applied to spores, it might have been dislodged. This difference is, however, minimal and not substantial enough to reconsider classification as a new species. A major observation of spores of *A. rehmii* from this site was the distinct variations in surface ornamentations at different stages of spore development. The range of ornamentation encompassed the types in *Acaulospora denticulata* Sieverding & Toro, *Acaulospora foveata* Trappe & Janos, *Acaulospora gerdemanii* Schenck & Nicolson and *Acaulospora lacunosa* Morton. Although this difference appears very distinct, the overall similarities in size and wall characteristics warrant identification of our specimen as *A. rehmii*. Except for *A. lacunosa* and *A. denticulata*, the remaining species were described before the concept of wall characteristics as diagnostic features was well understood and

overstressed (Walker 1983). I support the postulate role in wall layers having a significant role in species identification. The variation in different stages of development will be confirmed further by a series of single spore cultures of *A. rehmii*. Until the variation in surface ornamentation in the different stages of spore development is confirmed, it will be maintained as a possibility.

In the description of the type specimen of *A. rehmii*, similarities were noted with *Acaulospora gerdemanii* (Sieverding & Toro 1987) now referred to as *Archaeospora gerdemanii* (Rose, Daniels & Trappe) Morton & Redecker. Preliminary work on phylogenetic relationships amongst the AMF taxa placed *A. denticulata* and *A. rehmii* at the same level (Morton 1990). Similarities were also noted in in spore colour between *A. foveata* and *A. lacunosa* with distinctions made in only spore size, size of pits and reaction with Melzer's reagent (Schenck & Perez 1990). I am in support of maintaining distinctions for these species.

The species *A. rehmii* was first recorded in Colombia from a crop field in association with cassava, sorghum and *Crotolaria* species (Sieverding & Toro, 1987). The species was also found in pot culture with *Peuraria phaseoloides* (Roxb.) Benth. This species record from Malawi is a first record for the African continent. The species was recovered from agroforestry sites with *Gliricidia sepium*/maize intercrop and *Sesbania sesban*/maize intercrop, *S. macrantha*/maize intercrop and maize monocrop systems.

4.3 ACAULOSPORA sp.2 (Plate 4.1 E-F)

4.3.1 Spore characteristics

Spores found singly in soil borne intercalary on a sporiferous saccule (Plate 4.1 E); hyaline, white, ivory, cream (0-0-20-0 to 0-10-20-0) to yellowish cream (0-10-40-0) at maturity; globose to subglobose 60-105 × 78-135 µm.

Spore wall characteristics of four layers (**L1-4**) in three wall groups (groups A, B and C) (Plate 4.1 F). Group A of single outer layer (**L1**); hyaline, white, ivory, cream (0-0-20-0 to 0-10-20-0) to yellowish cream (0-10-40-0); 3-5 µm; ornamented; ornamentation with circular to elliptical pits 1.0-1.5 µm apart, 1-1.5 µm deep and 1.5-2.0 µm wide. Group B of single hyaline thin layer (**L2**); 1-1.5 µm. Group C of bilayer with two adherent sub-layers; outer sub-layer (**L3**) beaded and inner sub-layer (**L4**) membranous. L4 slowly turning pink with Melzer's reagent (Plate 4.1 F). Sporiferous saccule subglobose; 108-114 µm; distance from saccule to spore is 27-75 µm; with white contents; circatrix circular; 7.5-12 µm.

4.3.2 Discussion

Spores of this *Acaulospora* sp. 2 appeared under the stereomicroscope as small white to cream spores distinguished from *Acaulospora* sp. 1 by the darl appearance and a fine granular surface. Spores with white sporiferous saccule or a hyaline collapsed sporiferous saccule were frequent (Plate 4.1 E).

This species from Malawi resembles *Acaulospora scrobiculata* Trappe and *Acaulospora undulata* Sieverding. The spore size of the species from Malawi (60-105 × 78-135 µm) is smaller than spores of type description of *A. scrobiculata* (100–240 × 100–220 µm) and INVAM specimen of *A. scrobiculata* (80-160 µm) but larger than spores of *A. undulata* (84 × 74–60 × 54 µm). The hyaline to white and cream to yellowish cream colour of the *Acaulospora* species from Malawi is within the range of colours of the type specimen description of *A. scrobiculata* (hyaline to light greenish yellow), INVAM specimen of *A. scrobiculata* (subhyaline, pale yellow to straw) and *A. undulata* (hyaline to subhyaline). The surface wall ornamentation of the species from Malawi showed discrete circular to elliptical pits 1.0-1.5 µm apart, 1-1.5 µm deep and 1.5-2.0 µm wide compared the type specimen description of *A. scrobiculata* with circular to elliptical occasionally linear to Y-shaped depressions, 1-1.5 × 1-3 µm separated by ridges 2-4 µm and INVAM specimen of *A. scrobiculata* with ovoid concave depressions 0.6-2.0 µm across and 0.5-1.4 µm deep with some merging together to form channels 5-12 µm long. *A. undulata* has similar ovulate or slightly irregular shaped pits. However, the space between the pits for *A. undulata* (4-9 ×4-9 µm) is wider than the species from Malawi (1.5-2.0 µm). The spore wall ornamentation of the species from Malawi is distinctly different from the description of type specimen and INVAM specimen of *A. scrobiculata*. The spore wall ornamentation of species from Malawi appears distinct from *A. scrobiculata*.

The spore walls of the species from Malawi and *A. scrobiculata* have four layers compared with three layers in *A. undulata*. The species from Malawi has three wall groups with

Group A of single ornamented outer layer, group B of single hyaline thin layer and group C of bi-layer with two adherent sub-layers of outer sub-layer beaded and inner sub-layer membranous and turning pink with Melzer's reagent. The type specimen description of *A. scrobiculata* has an outer ornamented layer, an adhering but separable, smooth, hyaline layer (L2), an adhering but separable smooth hyaline layer (L3) and a separated sometimes minutely roughened hyaline inner layer (L4) that turns deep red with Melzer's reagent. The *A. scrobiculata* specimen from INVAM has a hyaline layer (L1) degrading and sloughing early in spore wall differentiation and often absent, an ornamented L2 and L3 that is sometimes considered to be a sub-layer (lamina) of spore wall and two flexible germinal layers which may separate when each spore is broken. The outer layer of *A. undulata* has wall group A of bi-layer with outer sub-layer evanescent and appressed to ornamented L2, group B single layer of a hyaline, often minutely roughened unit layer (L3) often turning yellowish orange in Melzer's reagent. Based on the inner flexible layer (wall group) and the reaction to Melzer's reagent, the species from Malawi does not match the descriptions of *A. undulata* but has number of layers and reaction to Melzer's reagent matching that of *A. scrobiculata*. This species has characters that overlap with *A. scrobiculata* and *A. undulata*. The spore wall surface ornamentation and number of layers in a spore wall are significant diagnostic characters. I therefore consider *A. scrobiculata* and *A. undulata* different and also different from the *Acualospora* sp. 2 from Malawi. *Acaulospora paulineae* Blaszkowski, a spore similar to *A. undulata* could not be compared with the species from Malawi as there was neither published information nor reference specimen materials for this species. There is need to study the morphology of ex-type cultures of *A.*

scrobiculata, *A. undulata* and *A. paulineae* species and also use molecular techniques to verify the species.

A. scrobiculata has a wide distribution. It has been recorded in South America, USA, Japan and Australia (Schenck & Perez 1990). There are three records of this species from Africa. *A. scrobiculata* was isolated from Cameroon (Mason *et al.* 1992) and Ivory Coast (Wilson *et al.* 1992b) from *Terminalia ivorensis* A. Chev. plantation. In Kenya, it was found in association with *Leucaena leucocephala* and *Calliandra calothyrsus* Meissner (Shepherd *et al.* 1996). *A. undulata* has so far been extracted from the highlands of Zaire (Sieverding, 1988) while there is no record of *A. paulineae* from Africa.

4.4 ARCHAEOSPORA sp. (Plate 4.1 G-H)

4.4.1 Spore characteristics

Spore found singly in soil; white to pale cream (0-0-5-0 to 0-0-15-0); globose to sub-globose; 187-242 × 209-275 µm.

Spore wall characteristics of five layers (**L1-5**) in four groups (A, B, C, & D) (Plate 4.1 H). Group A with bi-layer; outer sub-layer (**L1**) with fissures, hyaline, rigid layer upto <1.5 µm. Inner bi-layer (**L2**) pale cream, laminated, brittle layer, 7.8-8.7 µm. Group B of single, thickly beaded layer (**L3**) separating from L2; 3-6 µm. Group C of single hyaline, unit layer (**L4**), 3-4.5 µm. Group D of single, membranous; hyaline layer (**L5**), 1 µm; circatrix circular, 6-7.5 µm (Plate 4.1 G).

4.4.2 Discussion

The species looks like the acaulosporoid phase of a species in the genus *Archaeospora* Morton and Redecker possibly *Ar. leptoticha*. Spores of the species *Archaeospora* can easily be separated from other species by the white to pale cream colour and darl rough surface. The spores are much larger in size than the ornamented *Acaulospora* sp. 2 and some spores had remnants of sporiferous attachment that appeared as a circatrix.

Unlike *Acaulospora* species, the species has no evidence of a flexible layer and it has a spore wall consisting of at least two permanent layers; an outer layer varying in thickness and a thicker laminate layer. The species differs in the thick inner beaded L3 that has not been described in *Acaulospora* species with ornamented surface. The five layers in this species were also observed in *Acaulospora longula* Spain & Schenck (Schenck & Perez 1990). The layers in *A. longula* are in three wall groups with L1 described as a mucilaginous ephemeral outer layer compared with the hyaline, rigid, fissured L1 in the *Archaeospora* species from Malawi. L2 and 3 are inseparable in *A. longula* while in the specimen from Malawi L3 is a separate wall group. L4 and 5 of the *Archaeospora* sp. from this site are also separated unlike in *A. longula* and no reaction with Melzer's reagent was observed in L5 as was the case with *A. longula*. The size of *A. longula* (55-)90(-100) to 110-115 × 66-98 µm is significantlty smaller than the size 187-242 × 209-275 µm of the *Archaeospora* sp. from Malawi. The differences between *A. longula* and the *Archaeospora* sp. are substantial to maintain the two as separate species. The fissures observed on L1 of the *Archaeospora* sp. were observed in *Acaulospora nicolsonii* Walker, Reed & Sanders. Two wall groups with four layers were described in *A. nicolsonii* compared with four wall

groups and five layers in the specimen from Malawi. Further differences exist between the two species (Schenck & Perez 1990). The description of the outer brittle L1 of *A. nicolsonii* that is first smooth but later roughened as it breaks up and sloughs leaving granular fragments attached to L2, resembles the characteristics of L1 of the *Archaeospora* sp. from Malawi. Both species display a distinct laminated L2. However, L3 of the *Archaeospora* sp. is thickly beaded, a characteristic not described in *A. nicolsonii*. The brittle unit L3 in *A. nicolsonii* that often separates when the spore is crushed was similar to unit L4 of *Archaeospora* sp. from Malawi. The inner membranous L5 in *Archaeospora* sp. from Malawi was similar to the innermost membranous L4 in *A. nicolsonii*, except for the lack of reaction with Melzer's reagent. The size, 99-198 × 109-218 µm of *A. nicolsonii* is within the range of the size 187-242 × 209-275 µm, of spores of the *Archaeospora* sp. The circatrix was distinct in the *Archaeospora* sp. Although the species from Malawi appears similar to *A. nicolsonii* in most aspects, the lack of the distinct beaded L3 is significant enough to maintain the two as separate species.

The *Archaeospora* sp. from Malawi could not be established in pot culture in this study, hence the materials were insufficient to fully describe the species. The specific epithet could not be confirmed as *Archaeospora leptoticha* as I had neither published nor pot culture reference materials for comparisons.

Plate 4.1 A

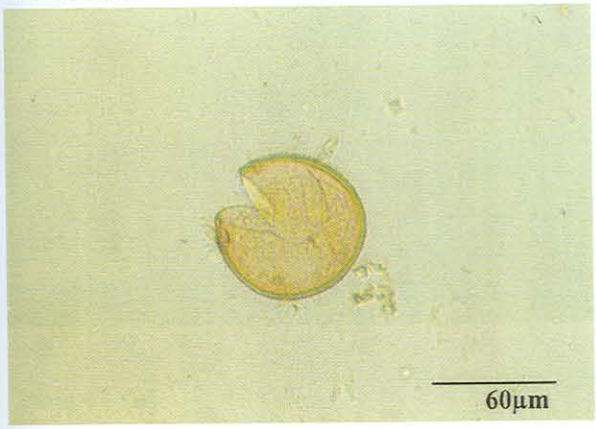

Plate 4.1 B

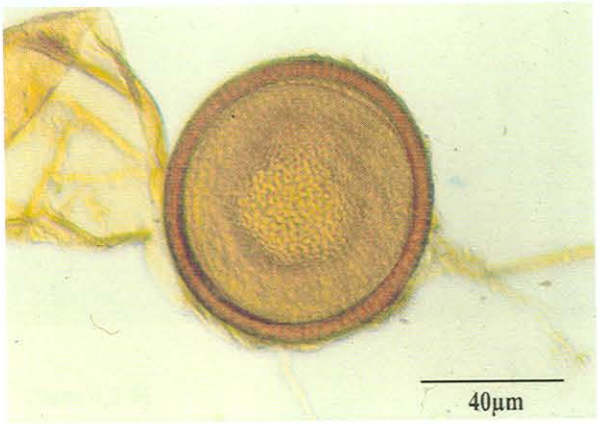

Plate 4.1 C

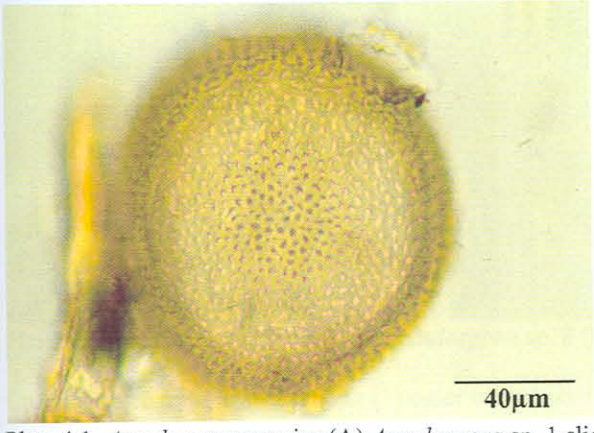

Plate 4.1 D

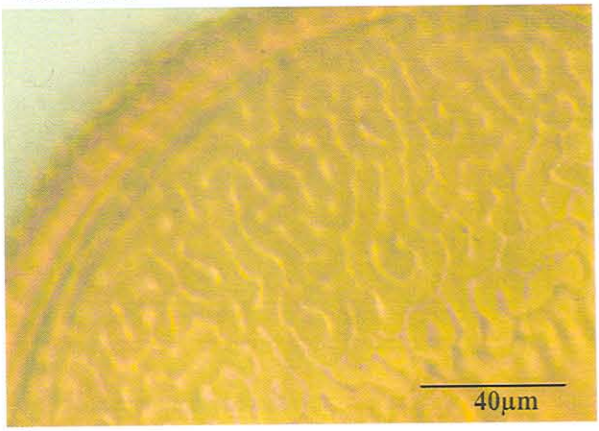

Plate 4.1. *Acaulospora* species (A) *Acaulospora* sp. 1 slightly crushed in Melzer's reagent. (B-D) *Acaulospora rehmii* (B) Spore with collapsed soporiferous saccule (C) Intermediate stage of spore with denticulate ornamentation (D) Mature spore with sinusoidal ornamentation.

Plate 4.1 E

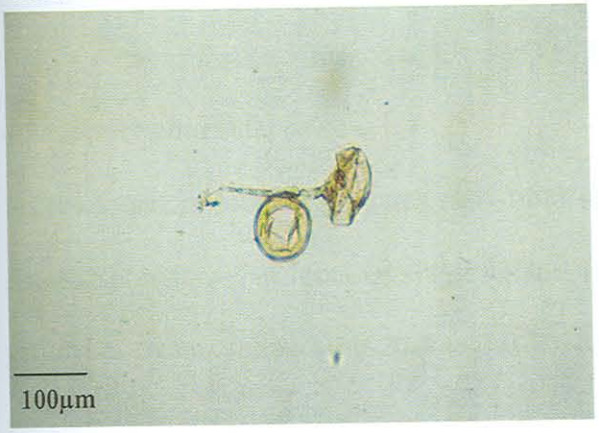

Plate 4.1 F

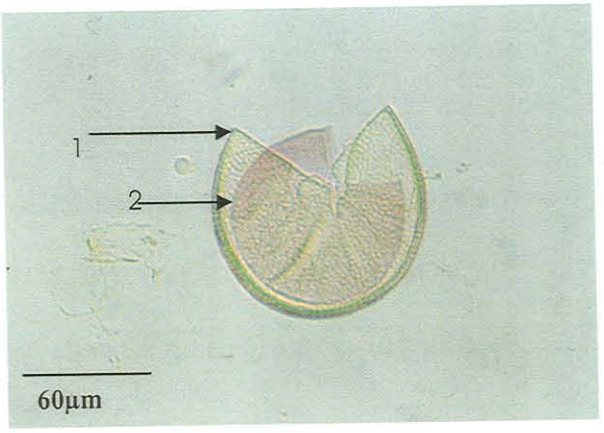

Plate 4.1 G

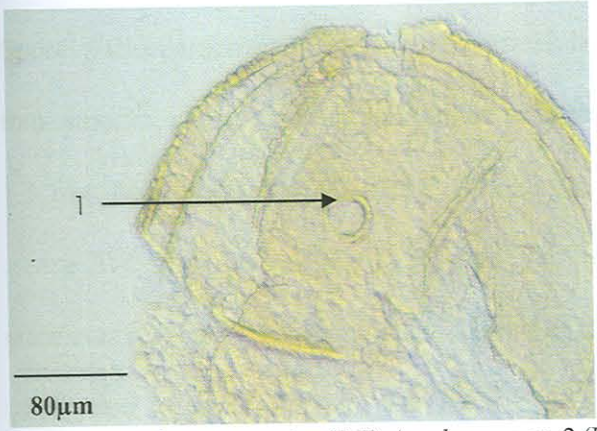

Plate 4.1 H

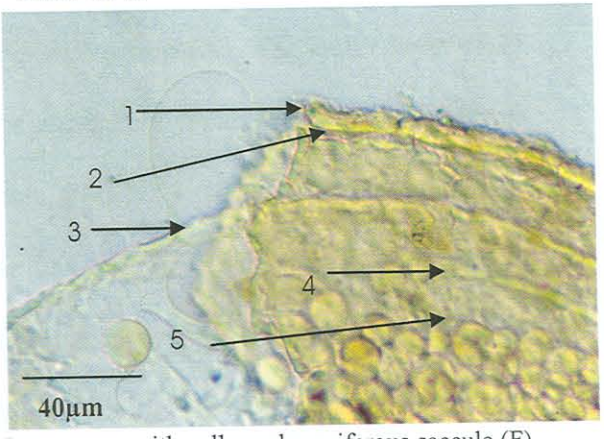

Plate 4.1 *Acaulospora* species (E-F) *Acaulospora* sp. 2 (E) Intact spore with collapsed sporiferous saccule (F) Crushed spore in Melzer's reagent (1) outer ornamented layer (2) inner membranous layer staining pink with Melzer's reagent.

Plate 4.1 *Achaeospora* species (G-H) *Achaeospora* sp. (G) Circatrix (H) Crushed spore with (1) outer layers with fissures (2) thick laminated layer 2 (3) beaded L 3 (4) unit L 4 and (5) flexible L 5 and a.

4.5 GLOMUS aff. AGGREGATUM Schenck & Smith emend. Koske, Mycologia 77: 619, 1985 = *Glomus fasciculatum* complex. Koske, R.E. (Plate 4.2A)

4.5.1 Spore characteristics

Spores found in loose aggregates, 300-1500 µm wide, lacking a peridium, in irregularly shaped sporocarps, in roots or single in soil (Plate 4.2A); cream (0-0-50-0 to 0-0-15-0); yellowish cream, yellow (0-0-20-0 to 0-0-70-10) to yellowish green (10-0-70-10); globose, subglobose to irregular; 25-75 × 33-120 µm.

Spore wall characteristics of three layers (**L1-3**) in one group (A). The outer layer (**L1**) thin, smooth, rigid, hyaline, sometimes with debris and rarely separating from layer (**L2**), 1.5 µm. Middle layer (**L2**), single, laminate, cream (0-0-50-0 to 0-0-15-0) to yellowish cream (0-0-20-0 to 0-0-40-0), 1.5-2.5 µm. Inner layer (**L3**), hyaline, thin, adhering to middle layer, rarely distinguished from the middle layer except at the point of hyphal connection to spore, <1 µm, the inner layer appears wrinkled in some spores. All three layers do not stain with Melzer's reagent.

Subtending hyphae straight; irregular and flared, cream tint (pale cream); width variable (3.0-14 µm); point of attachment with spore 3-12 µm wide. Hyphal wall of two layers, slightly thicker at the point of attachment; hyphal wall continuous with spore wall, pore open, 3.0-12 µm in young spores or closed by a thin septum that forms part of the inner spore wall.

4.5.2 Discussion

The spores of this species are easily distinguished from others by the small size, cream to yellowish cream colour with a green tint and the smooth shiny surface. Unlike the spores of *Acaulospora* sp. 1, the spores are robust and do not break easily and may occur singly, with or without subtending hyphae or in groups interconnected by hyphae.

The spores from this site resemble description of *G. aggregatum* in most aspects and also bear resemblance to *Glomus fasciculatum*. The spores of both species are found in loose aggregates, single in soil or in sporocarps. Considering that the spores occur in loose aggregates, the size of the aggregate is variable. The sizes 25-75 × 33-120 µm of species from Malawi is within the range of sizes (30-)40-120(210) × (20-)40-85(-120) × (20-)40-85(-120) µm of the type description of *G. aggregatum*. The size range is a highly variable character and of no taxonomic significance. The species from Malawi displayed the very pale yellow and the greenish tint which is also described in the *G. aggregatum* type specimen description but did not display the yellow-brown to orange-brown colour observed in both *G. aggregatum* and *G. fasciculatum*. The composite wall of spores of the species from Malawi is thicker (4.5-9 µm) than *G. aggregatum* description (2-6)-10 µm) but less than composite wall of *G. fasciculatum* (5-12 µm). The species from Malawi has three layers with two of the layers continuous with the two layers of the subtending hyphae and the inner layer most visible as a thin septum occluding the pore and also appears as a wrinkled layer in some spores, slightly separating from L1 and L2. This layer was absent in young spores which lacked an occlusions. The pore occlusion also appears as an inner wall thickening in some spores. Spores with and without occluded pore were observed in the

same aggregate of spores. The spore wall of *G. aggregatum* has two layers with the innermost layer extending for a short distance down the subtending hypha. The inner spore layer of spores that have not reached full maturity collapses in mounting reagents such as polyvinyl alcohol (Walker 1983). Spores of *G. fasciculatum* have three layers. The differences in spore wall characteristics and colour in the species from Malawi, the type and INVAM description of *G. aggregatum* and *G. fasciculatum* are not distinct. However, reaction with Melzer's reagent distinguishes the species from Malawi and *G. aggregatum* from *G. fasciculatum*. The species from Malawi and *G. aggregatum* are distinguished from *G. fasciculatum* by the complete absence of reaction of spore wall with Melzer's reagent. An intense dextrinoid reaction of L2 and possibly spreading to L3 is observed in *G. fasciculatum*. However, staining of L2 is considered an unstable diagnostic character and inner membranous layer of taxonomic importance (Morton 1988). I therefore consider the species from Malawi to be affiliated to *G. aggregatum* species.

The species from Malawi and the *G. aggregatum* display distinct straight to cylindrical (curved) subtending hyphae with similar range of width, composite wall thickness and number of layers. Only one subtending hyphae was present compared with occasionally two in the type specimen description of *G. aggregatum*. The pore in the species from Malawi is variable with some spores occluded by the inner wall thickening, thin septa and some not occluded. The thin septa in the species from Malawi, is similar to the fragile septa formed by L3 in *G. fasciculatum*. The pore in *G. aggregatum* is plugged by a cytoplasmic plug or a thin curved septum. The species from Malawi resembles *G. aggregatum* in most aspects and I feel these similarities warrant determining the species from Malawi as *G.*

aggregatum. This requires confirmation through comparisons with ex-type pot culture materials of *G. aggregatum*.

G. aggregatum has not been documented from the African continent. *G. aggregatum* was recorded from Canada (Schenck & Perez 1990), the USA as occupants of other dead spores (Koske 1984., Schenck & Perez 1990) and England from sand dunes (Mosse, 1959). The soil texture at the ICRAF Malawi site is sandy loamy. There are several records of *G. fasciculatum* from the African continent. *G. fasciculatum* has been recorded in Nigerian soils (Sani 1976), from the rhizosphere of *Faidherbia albida* (del.) A. Chev.(syn. *Acacia albida* Del.) in Senegal (Dalpe *et al.* 2000), in *Terminalia ivorensis* in Cameroon (Mason *et al.* 1992), in Egyptian soils (Mankarios & Abdel-Fattah 1994) and in Libyaan soils (El-Giahmi *et al.* 1976). Some of these species were recorded long before the species *G. fasciculatum* was revised.

4.6 GLOMUS ETUNICATUM Becker & Gerdemann, Mycotaxon. 6: 29, 1977.

= Glomus etunicatus sp. nov. Becker, W. N. and J. W. Gerdemann, 1974. (Plate 4.2 B-E)

4.6.1 Spore characteristics

Spores found singly in soil; white to cream (0-0-5-0 to 0-0-15-0) in young spores, pale ochraceous to ochraceous (0-10-20-0 to 0-10-40-0 and 0-20-60-0 to 0-20-100-0) and sienna (0-30-60-0 to 0-30-100-0 and 0-40-50-10 to 0-40-100-0) in mature spores; globose to sub-globose 60-100 × 77-153 µm.

Spore wall characteristics of three layers (**L1-3**) in one group. Outer layer (**L1**) rough, uneven, with debris adhering 1.5-3.0 µm. Outer sloughing layer variable with age (Plate 4.2 C & D). The middle layer (**L2**) does not separate from L1; laminate, white to cream (0-0-5-0 to 0-0-15-0) in young spores, pale ochraceous, ochraceous (0-10-20-0 to 0-10-40-0 and 0-20-60-0 to 0-20-100-0) to sienna (0-30-60-0 to 0-30-100-0 and 0-40-50-10 to 0-40-100-0) in mature spores; intensity of laminae varies with age of spore; 3-6 µm. The inner layer (**L3**) hardly distinguishable from L2, thin, hyaline < 0.5 µm. Outer layer (L1) staining patchily reddish purple and reddish orange with Melzer's reagent (Plate 4.2 D). Other layers do not stain (Plate 4.2 E).

Subtending hyphae cylindrical (Plate 4.2 B); one subtending hyphae per spore; pale cream; width at point of attachment 9-15 µm, away 3-6 µm. Wall structure of two layers, 3-6 µm at the point of attachment, <1.0 µm further away. Hyphal wall continuous with spore wall; pore plugged by thick curved septum formed by the inner layer.

4.6.2 Discussion

The spores of *G. etunicatum* are easily separated from other spores by the distinct cream to sienna colour, small size and the smooth glittery surface. Long curved subtending hyphae may be present in some spores.

The species from Malawi was found singly in soil. The colour and shape were similar to the type specimen and INVAM specimen description in all aspects. The size, 60-100 × 77-153 µm, of the species from Malawi was at the smaller end of the size range, 68-144(162)

µm, of the type description and at the smaller end and also larger of the INVAM specimen (60-160 µm). Spores of *G. etunicatum* recorded from banana plantation soils in Uganda were 60-125 µm in diameter (Msiska 2001). The composite wall thickness, 4.5-9.5 µm for the species from Malawi was similar to the wall thickness, 4.5-9 µm for the species from Uganda and slightly thinner than the type description wall thickness of 4-13 µm and the INVAM wall thickness of 5-13.2 µm. Spores of the species from Malawi had one wall group with three layers with the inner layer (L3) indistinguishable from the middle laminate layer (L2). Except for the differences in perception of number of layers in the specimen from Malawi, the specimen from Uganda, the type description and the INVAM specimen description, the rough uneven outer layer and the middle laminate layer are consistent in all the three records. Sturmer and Morton (1997) observed an innermost sub-layer that slightly separated from the spore wall. In the species from Malawi, an innermost L3 was also observed.

The subtending hyphae of spores from Malawi are cylindrical in shape compared with flared in the type description and cylindrical and flared in the INVAM specimen description. The 9-15 µm width of the subtending hyphae at the point of hyphal attachment is less than the 30 µm width of the type specimen description and slightly larger than the 5-10.2 µm in the INVAM specimen. The pore in the species from Malawi is occluded by an inner wall thickening as is the case with the type specimen description. The pore in the specimen from INVAM is occluded by an innermost sub-layer of laminate layer of the spore wall in some spores with the bridging structure resembling a septum. The spore contents in the species from Uganda are separated by a bridge structure resembling a

curved septum while mature spores display spore contents separated from hypha by inner spore wall thickening. The Malawi specimen agrees well with descriptions for the species from Uganda and INVAM. The pactchy reddish purple and reddish orange reaction to Melzer's reagent of outer layer (L1) of species from Malawi is similar to reaction of the INVAM mucilaginous outer layer (L1), having some plasticity with uneven outer surface, staining pink to reddish purple in Melzer's reagent. The outer layer (L1) degrading and sloughing as the spore matures, so that it may be present in patches usually detected in Melzer's reagent or appear as a granular layer. The spore morphology of the record from Malawi is similar with the two records. The differences in spore morphology, in my opinion do not constitute significant reason for re-classifying this species. I therefore consider the species from Malawi as *G. etunicatum* species.

There are six records of *G. etunicatum* from the African continent. The species was recorded from Uganda in association with *Musa paradisiaca* L. plants (Msiska 2001), in association with *Terminalia ivorensis* plantations in Cameroon (Mason *et al.* 1992) and Ivory Coast (Wilson *et al.* 1992b), in association with *Acacia nilotica* (L) Del. seedlings in Senegal (Ginwal *et al.* 1997), in the arid regions of Namibia and North America (Stutz *et al.* 2000) and in association with *Vangueria infausta* Berch. Subsp.*infausta* (Rubiaceae) from South Africa (Gaur *et al.* 1999).

4.7 GLOMUS sp. (Plate 4.2 F-J).

4.7.1 Spore characteristics

Spores found singly, in pairs or triplets closely attached, covered with a peridium (Plate 4.2 F); spores also found singly without a peridium (Plate 4.2 G); spores covered by peridia may also have smaller spore emerging from the margin (Plate 4.2 J); peridium cinnamon (0-20-40-0 to 0-20-50-10) to date brown (0-30-50-10 to 0-30-70-10 and 0-40-50-10 to 0-40-70-10); spores without a peridium pale ochraceous to pale citrine and yellowish green (0-10-40-0 to 0-10-70-10); sporocarp globose to subglose 144-282 × 150-432 µm; spores without a peridium, globose to subglobose 120-258 × 135-360 µm.

Spore wall characteristics of three layers (**L1-3**) in one group; tightly adhering layers. Outer layer (**L1**) thin, < 0.5 µm, adhering to layer L2. Middle layer (**L2**), no distinct laminae; pale ochraceous to citrine and yellowish green (0-10-40-0 to 0-10-70-10); 3-6 µm; adhering to L3. Inner layer (**L3**), thin, hyaline, < 1.0 µm. The inner layer of spores without a peridium with projections extending into the spore contents, appearing as dark brown concentric discs in surface view of the spore (Plate 4.2 G). All three layers did not react with Melzer's reagent.

Peridium loose to compact, comprised of septate, sub-hyaline to pale cream, irregular hyphae, width of hyphae is variable, 3-15 µm (Plate 4.2 I). Spore-like structures, 63 × 66 µm, thin walled, 1.5-3.0 µm, sub-hyaline, pale cream to orange tint (0-20-60-0 to 0-0-20-0), attached to the peridium (Plate 4.2 I). The wall of the spore-like structures thickened (Plate 4.2 H).

4.7.2 Discussion

The spores from Malawi were distinguished into spores with and without peridium. The spores with peridium are large, irregular shaped, covered with hyaline mycelia and soil particles adhering on to the mycelia, giving an impression of a soil particle at first glance. The spores with a peridium take the reddish brown colour of the soil and at first glance are easily mistaken for soil particles. Spores without a peridium are large, yellowish cream to citrine in colour and variable in shapes. Spores may or may not have subtending hyphae. When subtending hyphae is present the point of contact of the hyphal attachment and spore appears black.

The spores with a peridium from Malawi resemble *Glomus globiferum*, *Glomus tortuosum* Schenck & Smith and *Glomus mosseae*. Like the species from Malawi, *G. mosseae* and *G. tortuosum* also have spores without hyphal mantle. The description of the hyphal mantle for all the species is similar to the species from Malawi with variation found only in the number of spores per cluster. A cluster of 1-10 spores within hyphal peridia described in the INVAM species description of *G. mosseae* was noted to be unstable and of little taxonomic value. The hyphal mantle was also described as an unstable character in the species description of the INVAM specimen for *G. mosseae*. It was noted that some isolates of *G. mosseae* from the United Kingdom produced abundant sporocarps in the first culture circle, but then reverted to single spores with smooth surfaces in the third culture circle. The spores of *G. mosseae*, without peridia never reverted to sporocarpic nature. The species from Malawi has been in culture since 1995 and has been subsequently recultured until 2003. The species still produces spores with and without hyphal peridia. Contrary to

the spores of *G. mosseae,* which did not revert to sporocarpic form, the culture for the species from Malawi was initiated from spores without hyphal peridia and resulted to a culture of spores with and without hyphal peridia. The spores of *G. tortuosum* are borne single but are occasionally in pairs with young spores without mantle and mature spores with a mantle of sinuous hyphae closely appressed to the spore and flattened. The description of sporocarp for *G. mosseae* by both INVAM and Schenck and Perez (1990) has 1-10 spores, measuring up to 1 mm. This is larger than the 1-3 spored sporocarps of *G. globiferum* (150-260 × 150-270 µm) and the 1-3 spored sporocrp from Malawi (144-282 × 150-432 µm). The size of spores without hyphal peridia for species from Malawi (120-258 × 135-360 µm) is larger than *G. mosseae* (100-260 µm) and *G. tortuosum* (94-180 × 112-230 µm). The size range, unless statistically analysed is a variable character. Spores without hyphal peridia were not described in the type specimen description of *G. globiferum* (Koske & Walker 1986a). There is considerable resemblance of *G. tortuosum*, *G. mosseae* and *G. globiferum* with the species from Malawi. The differences between *G. mosseae* and the *Glomus* sp. from Malawi are so distinct that I feel I should maiantain them as separate species. Except for the lack of spores without peridium in *G. globiferum*, the species from Malawi conformed more to the description of *G. globiferum*.

Thin-walled vesiculates representing the "spore-like" structures were found in the specimen from Malawi. It is postulated that these spore-like structures develop into spores. The vesiculates have so far been described in *G. globiferum* (Koske & Walker 1986a) and *G. tortuosum* (Schenck & Smith 1982). The species from Malawi has both thin-walled and thick-walled (Plate 4.2 H) spore-like structures that fit the description of the vesiculate of

G. globiferum. Wall thickening in vesiculates was only suggested as a possibility by the authors (Koske & Walker 1986a). The smaller spore formed beside a larger spore was not described in *G. globiferum* as is seen in our specimen (Plate 4.2 J).

The spores without a peridium observed in the species from Malawi were not described in *G. globiferum* (Koske & Walker 1986a). The spores without a peridium have concentric disc-like features and colour that is similar to *Glomus clariodeum sensu stricto*. In *Glomus clariodeum* Schenck & Smith, the concentric discs were described as unstable parasitic structures of no taxonomic significance (Walker & Vestberg 1998). The spores of species from Malawi are pale ochraceous, pale citrine to yellowish green while the colour of *G. clariodeum sensu stricto* is hyaline when immature, becoming straw-coloured to ochracceous when mature. The size of the spores of the species from Malawi, 120-258 × 135-360 µm, are slightly larger than the size for *G. clariodeum* (95-)135-178(-220) × (95-) 130–180(-220) µm. The spores of species from this site have one wall group with three layers. Similarly *G. clariodeum* has one wall group with three layers. The spores from Malawi have a thin outer layer adhering to a middle laminate layer with no distinct laminae and a thin hyaline inner layer compared with hyaline outer unit layer and a laminated layer with the innermost laminae sometimes appearing to be a separate unit layer that often forms a septum at the base in *G. clariodeum*. L3 of *G. clariodeum* is membranous, very thin and tightly adherent to L2. The domed, scalloped ingrowths in *G. clariodeum* were observed in the spores of the species from this site. These are now regarded as of no taxonomic importance (Walker & Vestberg 1998). Since it is the concentric discs that linked the species from Malawi with *G. clariodeum,* other features variable, I therefore

consider the spores from Malawi without a hyphal peridium to be different from *G. clariodeum*.

The type description of *G. globiferum* has hyphal wall of peridium and spore wall composed of one or two wall groups with 3 or 4 layers respectively. The characteristics of L1 and L2 are similar to spores of the species from Malawi while L3 and 4 are hyaline and membranous. The membranous nature of L3 and L4 was not observed in spores of the species from Malawi. The characteristics of the peridium of the species from Malawi are indistinguishable from the peridium of *Glomus tortuosum* (Schenck & Perez 1990), yet this was the only diagnostic feature separating the two species. The size of the hyphal width of the species from Malawi (3-15 µm) is less than the hyphal width of the *G. globiferum* (5-50 µm) and greater than the hyphal width of *G. tortuosum* (4-10 µm). Unless statistically anaylsed the differences in hyphal width may not not constitute enough reasons to separate the species. The spore-like structures (vesiculates) are described in *G. tortuosum* as swellings on a hyphal attachment. The size of the spore-like structures of the species from Malawi (63 × 66 µm) is within the range of the *G. globiferum* (12-65 × 12-75 µm) although larger than the diameter for the similar structures in *G. tortuosum* (10-20 µm). The size range of the species described from Malawi with peridium (144-282 × 150-420 µm) and excluding the peridium (120-258 × 135-360) encompasses the sizes of *G. tortuosum* (94-180 × 112-230 µm) and the size range of the *G. globiferum* (150-260 × 150-270 µm) excluding the peridium. These two species of *Glomus, G. globiferum* and *G. tortuosum* have strongly overlapping similar characteristics including size, colour and wall layer characteristics. There is a distinct possibility that these two names may be recognized as

synonyms for the same species but it is beyond the scope of this work to undertake such taxonomic revision.

The species from Malawi was successfully cultured in this study and the description done from fresh spore culture. It was proven mycorrhizal with five leguminous agroforestry tree species (*Senna siamea, S. spectabilis, Sesbania sesban, S. macrantha* and *Gliricidia sepium*) and two gramineae species (*Zea mays* and *Sorghum bicolour*). The spore culture was established from spores without peridia, and spores with and without peridia and some with sparse hyphal peridia were recovered from the culture. If the species is confirmed to be *G. globiferum*, this record from southern Malawi will be the first record for the African continent. There is only one record of *G. claroideum* from semi-arid areas of Senegal (Diallo *et al.* 1999) and there is still no record of *G. tortuosum* for Africa. *G. mossae* is widely reported in Africa from Nigerian soils (Sani 1976), in association with *Acacia nilotica*. in Senegal (Ginwal *et al.* 1997), in the arid regions of Namibia (Stutz *et al.* 2000), in Egyptian soils (Mankarios & Abdel-Fattah 1994), arid zones of Morocco (Meddich *et al.* 2000) and Libyan soils (El-Giahmi *et al.* 1976)

4.8 GLOMUS aff. GEOPSPORUM (Nicol. & Gerd.) Walker, Mycotaxon 15: 49-61, 1982. (Plate 4.2 K-L)

4.8.1 Spore characteristics

Spores found singly in soil; bay (40-60-60-0 to 40-60-100-0 and 60-80-100-0 to 60-80-100-10) to rusty tawny and chestnut (20-80-80-0 to 20-80-100-10 and 40-80-80-0 to 40-80-100-10); globose to subglobose 85-115 × 105-205 µm.

Spore wall characteristics of three layers (**L1-3**) in one group (Plate 4.2 L). Outer layer dull, thin, hyaline sloughing layer (**L1**); <1 µm. Middle layer (**L2**) laminate, drab (40-60-60-0 to 40-60-100-0 and 60-80-100-0 to 60-80-100-10) to rusty brown (20-80-80-0 to 20-80-100-10 and 40-80-80-0 to 40-80-100-10); 5-9 µm. Inner layer (**L3**), thin; hyaline; 1-2 µm; with parasitic protrusions extending into the cytoplasm; 9-15µm in length (Plate 4.2 K). All the three layers did not react with Melzer's reagent. Subtending hyphae flared, sub-hyaline, 14.5 µm wide; occlusion of the pore is by a thin septum.

4.8.2 Discussion

The spores of this *Glomus* species were separated from other species by the rusty brown colour and the flared subtending hyphae. It was distinguished from *A. rehmii* by the smooth shiny surface.

The colour, shape and wall characteristics of this *Glomus* species resemble *Glomus geosporum*.The spores of the specimen were described from field materials hence only rusty tawny and chestnut spores were described. The spores of the decribed specimen therefore resemble the dark red brown mature spores of *G. geosporum*. Three layers were described in the specimen from Malawi compared with the two layers plus an inner thin membranous layer, in the type description for *G. geosporum*. The species from Malawi had an outer dull thin sloughing layer, which in the type specimen description has been described as smooth, shiny or with dull appearance or roughened from adherent debris, which outer layer is observed easily in young spores and is sometimes absent in mature

spores. The middle layer was distinctively laminate in both. The specimen described from Malawi appeared to have an inner thin hyaline layer. This was described as a yellow to yellow-brown inner wall (<0.1 μm) that appears membranous and forms a separate septum separating spore contents from the lumen of sub-hyphae.

The flared sub-hyaline subtending hyphae of the species from Malawi slightly differs from the type specimen description of straight to curved, simple to slightly funnel-shaped, yellow to dark yellow subtending hyphae. The pore of the species from Malawi is occluded by a thin septum while the pore of the type specimen forms a septum with the inner membranous wall. The type specimen was observed to have walls that became perforated probably by attacks by microorganisms during aging. In the species from Malawi, parasitic intrusions extending into the cytoplasm were observed. The *Glomus* species was not successfully cultured in this study.

The diagnostic features distinguishing this specimen is limited and are continuous variables that could easily be plastic characters dependent on biotic and abiotic factors. All attempts to culture the *Glomus* species failed thus limiting studies to field samples. The parasitized nature of the spores could explain the inability to establish pot cultures of this species. There are two records of *G. geosporum* from a *Terminalia ivorensis* plantation in Cameroon (Mason *et al.* 1992) and in association with *Acacia holosericea* and *Acacia mangium* in Sudan (Ba *et al.* 1996)