
Some new recorded species of arbuscular mycorrhizal fungi associated with sugarcane crop in Iran

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Ten species of AMF belonging to four genera including *Entrophospora colombiana*, *Glomus manihotis*, *G. eburneum*, *G. aggregatum*, *G. caledonium*, *G. coronatum*, *G. microcarpum*, *G. diaphanum*, *Paraglomus occultum* and *Pacispora scintilance* were identified. Among them five species of *Entrophospora colombiana*, *Glomus eburneum*, *G. manihotis*, *G. diaphanum*, *G. microcarpum* and *P. occultum* are reported for the first time in Iran; *Glomus aggregatum*, *G. caledonium*, *Glomus coronatum* and *P. scintilance* are new recorded for sugarcane mycoflora of Iran.

Key words: *Glomus*, *Entrophospora*, *Pacispora*, *Paraglomus*, identification, sugarcane, Iran

Introduction

Arbuscular Mycorrhizal Fungi (AMF) are a group of obligate plant symbionts belonging to Phylum Glomeromycota (Schussler *et al.*, 2001). They are a ubiquitous component of most ecosystems throughout the world and play an important role in soil processes (Smith and Read, 1997). This association improves the capacity and longevity of root uptake, boosts up the absorption of low access nutritional elements especially phosphorus (Werner, 1992) and reduces the susceptibility of plants to soil-borne pathogens (Norris *et al.*, 1994). These fungi also reduce plant stresses caused by root-infecting micro-organisms, saline soils, soil moisture stress (Staddon and Fitter, 2001). The fungi belong to the most commonly occurring soil microorganisms of the world are associated with at least 80% of plants of the Earth and among the most important of them are Gramineous plants (Gianinazzi and Gianinazzi-Pearson, 1986). Sugarcane grown for more than forty years in Khuzestan province with approximately sixty thousand hectares under its cultivation. Sugarcane is a member of Gramineous

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plants, among the plantations that has a long lasting relationship with the province soils, but few research works have been identified AMF associated with the plant and their beneficial efficacies. This research was conducted to find out the diversity of Arbuscular Mycorrhizal Fungi associated with sugarcane roots in Khuzestan province of Iran.

Materials and methods

Soil samples were collected from sixty fields of main sugarcane agro-industry companies including Haft-Tape, Karoon, Mian-Ab, Imam-Khomeini and Amir-kabir during November and December of 2004 coincides with the ends of the growing season and the beginning of the harvest. Samples were collected from rhizosphere of four extensively grown sugarcane varieties CP69, CP48, CP57 and NCO-310 in the Khuzestan province. To establish trap cultures 100 grams of soil and root of each sample was mixed with sterile sand, potted with corn seeds and kept for 4 and half month in greenhouse. In order to get access to high and pure population of spores trap culture was repeated again by selecting some of morph types of each sample, using smaller pots and 12 days old corn seedlings as host plant. To confirm symbiotic association in sugarcane and corn, roots were boiled in 10% KOH, stained with 0.05% aniline blue solution in lacto phenol and were examined by optical microscope. Isolation of spores were carried out by centrifugation in 55% sucrose solution and then extracted spores were transferred to 1:1 mixture of PVLG (Koske, 1985) and Melzer's reagent (Hall and Abbott, 1984) and microscopic slides were prepared. Identification of species were carried out base on morphological and morphometrical characteristics of spores, and subtending hyphae using stereomicroscope (Olympus ZSH10) and brigth field microscope (Olympus BH-2).

Results and discussion

In this research, ten species of arbuscular mycorrhizal fungi belonging to three orders Glomerales, Diversisporales and Paraglomerales were identified. In addition to the diversity and population abundance of the isolated species with four commonly cultivating sugarcane varieties i.e (CP-69, CP-48, CP-57 and NCO-310) were investigated. The results are as below:

The most diversity of association was seen with CP-48 and among the identified species only *G. caledonium* was not isolated from the former variety, subsequently, CP-69 had the most symbiotic association with all the identified species but *G. microcarpum*, *K. colombiana* and *P. scintilans*. Comparing others, CP-57 was the third where it was associated with only nine species of isolated fungi except, *G. intraradices*, *G. caledonium*, *P. scintilans* and *K.*

colombiana. The last variety with the least diversity of association was the NCO-310 which had only symbiosis association with five identified species i.e *G. manihotis*, *G. mosseae*, *G. eburneum*, *G. coronatum* and *G. intraradices*.

The abundance of population of arbuscular fungi associated with the sugarcane varieties was studied. By comparing others the most population frequency of isolated species was seen with CP-69 and after that the population abundance of identified species associated with CP-48, CP-57 and NCO-310 had the most abundance in soil sample of the mentioned varieties respectively. The descriptions of isolated species are as follows:-

1. *Kuklospora colombiana* (Spain and Schenck) Sieverding and Ohel (2006) J. Appl. Bot. Food Qual 80: 69-81

Spores produced singly in soil or in roots, developed from inside the neck of the sporiferous sacule .spores are globose (57.8) 75.1 (-91) μm in diameter, hyaline to pale purple with granular or reticulate contents. Spore wall consist of tree layers (L_1 , L_2 , L_3). L_1 : a hyaline, evanescent layer which is continuous with the wall of the sporiferous sacule neck. (1.1-) 1.2 (-2.1) μm thick, attached to second layer (L_2). This layer is only seen on young spores. Second layer (L_2): a light yellow laminated layer (1.6-) 1.4 (-2.1) μm thick. (L_3): a hyaline layer, <1.1-1.1 μm thick, separating from second layer on broken spores (Fig. 1a-c).

Germinal walls consist of two inner layers (gw_1 and gw_2), purple in color, composite walls thickness, 1.1-<1.5 μm (Fig. 1 and 2). Soporiferous sacule white to hyaline, 47-65 μm in diameter at first globose, shrinking after discharging its content into spore, tending to detach from spore, accumulating of organic materials frequently is seen on its wall. Soporiferous sacule wall consist of one layer 1.1-3.2 μm thick. Interconnecting hypha between spore and sporiferous sacule 31.5-42 μm long, 3.2-5.3 μm wide at the point of attachment to sporiferous sacule and 5.3 μm wide at the point of attachment to spore. Produced opposite to interconnecting hypha, subtending hypha 4.2-5.3 μm wide (Fig. 1). Shape, color, dimensions and spore wall structure and characteristic of sporiferous sacule are comparable with protologue, however the most and the least range of spore and sporiferous sacule dimensions are approximately a little less than it. Also color of spores is a little lighter than protologue (Spain and Schenck, 1984). Shape, spore composition and sporiferous sacule characteristic are comparable with Spain and Schenck description (Spain and Schenck, 1984). However maximum and minimum spore and sporiferous sacule dimensions are less than protologue to some extent. Also Spore color is somehow lighter than Spain and Schenck description. *Entrophospora colombiana* has been reported from Colombia (Spain and Schenck, 1984). This is the first record of this species in Iran.

2. *Glomus eburneum* Kennedy *et al.* (1999) Mycologia 91(6): 1083 -1093

Spores formed singly in soil; globose to subglobose (42-) 72 (-115.5) μm diam; sometimes ellipsoid to tear drop in shape, (42-) 86.4 (-104) \times (54-) 68.7 (-118) μm diam; bright white when newly formed, becoming pale ivory in old spores, less frequently hyaline. Spore wall consist of two adherent hyaline layers. The outer layer (1) is 1-1.2 μm thick, semiflexible and usually remain intact long after spore has matured. This layer especially on the surface of ivory color spores is well visible, making a fingerprint pattern (Fig. 2a-c). The inner layer (2) is also semiflexible (forming folds in broken spores) and finely laminate, (1.1-) 2.7 (-4.2) μm thick.

Subtending hypha at the point of attachment to spore, cylindrical to a little flared, (2.1-) 4 (-7.4) μm wide. Pore: closed by a curved septum, 1-2 μm thick, forms from continuation of the laminate layer of spore wall positioned 1-10 μm in the hyphal lumen. The subtending hyphal wall consists of a continuation of both layers of spore wall to the septum, after which it consists of only the outer lyer of spore and is 1 μm thick.

All the dimensions and other characteristics of spores are the same with protolouge (Kennedy *et al.*, 1999). It has been isolated from Giant Sacaton (*Sporobolus wrightii*), an herbaceous plant, using potted *Sorghum sudanense* as trap culture (Kennedy *et al.*, 1999). According to key references, this is the first report of *Glomus eburneum* in Iran.

3. *Glomus microcarpum* Tulasne and Tulasne (1845) Bot. Ital. 2: 35-63

Spores rarely single in the soil or in a sporocarp with cluster of two to several hundreds spores unenclosed with a peridium, spores light brown to brown, globose to suglobose (5.2-) 10.3 (-14.7) μm diam. Spore walls: Consist of two layers, The outer layer (L_1), a hyaline evanescent layer (< 1.1-) 1.4 (-2.1) μm thick, in young spores it seems like a unit wall making a bright halo around the spore. The inner layer (L_2), a laminate light brown to brown layer, <1.1-1.1 μm thick.

Subtending hyphae funnel shape, hyaline to less frequently light brown with lots of short branches at the tip seemingly radiating to attach with other spores in sporocarp. Subtending hyphae at the point of attachment to spore <1.1 μm wide.

Glomus microcarpum has been isolated from sugarcane rhizospheres soil samples belonging to CP-48 cultivar potted with corn seeds. So far this fungus has been reported from Poland (Blaszkowski, 1993), United States of America (Nicolson and Schenck, 1979), India (Mohankumar *et al.*, 1988), Australia (Hayman and Stovold, 1979), Tasmania (Gerdemann and Trappe, 1974) and Palestine (Blaszkowski *et al.*, 2001).

The closest description to this taxon is *G. tenuis* (Hall, 1977) especially with spore size of 10 – 12 μm diam, but because of the latter produce one layered non sporocarpic spores, it differs from the above explained species. According to key references, this is the first report of *Glomus microcarpum* in Iran.

4. *Glomus manihotis* Howeler *et al* (1984) *Mycologia* 76(4): 685-699

Spores formed singly in soil, light yellow to orange yellow, globose (84-) 227 (-346.5) μm in diam. Spore Wall: Consist of three adherent layers (L_1 , L_2 , L_3), in mature broken spores the third layer might separate from the others. (L_1): A mucilaginous hyaline layer, (1.6-) 3.2 (-5.3) μm thick, disintegrate in mature spores and only part of it remain on the spore surface. On younger spores thicker parts of this layer be seen as disintegrating striations of a laminated layer. (L_2): A persistent laminated layer (4.2-) 3.2 (-19.9) μm thick, despite not fragile it cracks, outer surface of this layer seems uneven because of remnant of first layer. (L_3): Another laminate layer, light yellow to orange yellow (1.1-) 1.7 (-2.7) μm thick (Fig. 4a, b).

Subtending hypha cylindrical to slightly flared at the point of attachment to spore, (15.8-) 24.4 (-33.6) μm wide and (3.2-) 7 (-10.5) μm thick at spore base. Consist of three layers continuous of spore walls, but this continuation short after the base just continue with the third layer of spor wall. Pore usually occluded by a septum that originated from the innermost laminae of third layer (Fig. 4a, b). However others argue that they are two separate taxons (Schenck *et al.*, 1984).

5. *Glomus coronatum* Giovannetti *et al.* (1991) *Canad. J. Bot.* 69: 161-167

Spores generally single in the soil, light orange to orange brown, globose to subglobose (149.5-) 230 (-270) μm wide, usually content of hyaline oil drops. Spore wall: composed of two layers (L_1 and L_2). (L_1): A mucilaginous hyaline layer (1.57-) 2.4 (-3.15) μm thick closely adherent to second layer. (L_2): A laminated smooth, orange brown layer, (4.2-) 5.5 (-7.35) μm thick. Subyending Hypha: composed of two layers continuous with spore wall layers 1 and 2, funnel shaped to completely funnel shaped, upright, sometimes bent, (5.2-) 5.95 (-8.4) μm wide and 3.2-7.5 μm thick at the point of attachment to spore. Pore closed with a curved septum continuous with the innermost sublayers of the laminate spore wall layer two, (1-) 2.2 (-4) μm thick down to (31.5-) 54.6 (-78.75) of subtending hypha.

Compare to protolouge (Giovannetti *et al.*, 1991), shape, color, dimension and spore layers are the same but maximum and minimum spore sizes are a little more than original description. Dimensions of spores completely differ with and more than Sedaghati (Sedaghati, 2003) and the same with Sadravi's description of the species (Sadravi, 2000). Branched

subtending hypha and oil drops are seen in some spores. As it mentioned before subtending hypha of this species is occluded by a thick curved septum, In some cases the septum is too thin to be seen simply, in this condition the septum is usually located more than 40 μm down to the spore base (Fig. 5d) this is the first report of this species from sugarcane rhizosphere in Iran.

6. *Glomus caledonium* (Nicol. and Gerd.) Gerdemann and Trappe (1974) Mycol. Memoir 5: 1-76

Spores golden yellow to orange yellow, often globose (.8-) 155.2 (-227.5) μm wide. Spore wall: composed of four layers (L_1 , L_2 , L_3 and L_4). (L_1): A hyaline mucilaginous layer, (< 1.0-) 1.3 (-2.6) μm thick (L_2): A hyaline rigid layer, (1.1-) 2.1 (-2.6) μm thick. (L_3): A fragile layer (1.6-) 2.2 (-3.7) μm thick. (L_4): A yellow to pale yellow to somewhat orange yellow laminated layer, (2.6-) 4.3 (-3.7) μm thick (Fig. 6a). Subtending hypha: Cylindrical to often funnel shape or even with constriction at spore base. Golden yellow to orange yellow, composed of four layer continuous of spore wall layers, (9.5-) 16.2 (-25.2) μm wide and (2.1-) 4 (6.3) μm thick at the point of attachment to spore. Pore: closed with curved septum from 0- 15.8 μm down to spore base (Fig. 6b).

Except width of subtending hypha that is slightly more than Sadravi's description (Sadravi, 2000) other dimension of spores in compare with Nicolson and Gerdemann (1968); Gerdemann and Trappe (1974); Sadravi (2000) are the same. Producing spores with four wall layers are also match with Morton (1996) description of the species. So far, *Glomus caledonium* has been reported from Scotland (Nicolson and Gerdemann, 1968), United states (Menge *et al.*, 1977; Gerdemann and Trappe, 1974), New Zealand (Hall., 1977), Australia (Hall and Abbott, 1984), India (Selvaraji and Subramanian, 1987), Poland (Blaszkowski, 1989), China (Mei-Quing and You-Shan, 1991) and Iran (Sadravi, 2000). However, this the first report of this species (*G. caledonium*) from sugarcane rhizosphere in Iran.

7. *Glomus diaphanum* Morton and Walker (1984) Mycotaxon 21: 431-440

Spores single in soil or in loose aggregation, yellow to bright yellow, globose to subglobose (47-) 75 (-130) to elliptical (42-) 75 (-130) \times (-38) 67.7 (-122.2) μm wide. Sporewall: composed of three layers, (L_1): A mucilaginous, hyaline layer <1.0-1.0 μm thick closely adhered to second layer. In mature spores usually disintegrated completely. (L_2): A bright smooth laminated layer (2.1-) 3.7 (-7.3) μm thick, consist of several thin adhered sublayers. (L_3): A flexible to semiflexible bright layer < 1.0- 1.0 μm thick. Protruded into subtending hypha at spore base. Subtending hypha: A hyaline Upright to slightly bent, flared to some extent at spore base (4.2-) 9 (12.6-) μm wide.

Subtending hypha wall hyaline, likely continuous with three spore wall layers but is too thin to be seen in both young and mature spores could be discernible from each other (Morton, 2000), (1.57-) 2.95 (-6.3) μm thick at the point of attachment to spore (Fig. 7a, b).

Pore occluded with the third spore wall layer. Discussion: Except spore color that is slightly stronger (bright yellow to yellow) than protolouge (hyaline to bright), other spore dimensions are the same with Morton and Walker, (1984) descriptions. This species also has been reported from united states (Morton, 1986), Europe (Oehl *et al.*, 2003). *Glomus diaphanum* is reported for the first time from Iran.

8. *Glomus aggregatum* Schenck and Smith emended koske (1985) Mycologia 77: 619-630

Spores single or in loose aggregates in the soil; bright, light yellow to yellow brown, globose to subglobose (31.5-) 72.7 (- 130) μm wide, rarely pyriform to irregular (31.5-) 81.1 (-143) x (52.5-) 116.6 (-160) μm wide. Spore wall: composed of three layers. (L₁): A hyaline, mucilaginous layer, (< 1.0-) 1.2 (-2.1) μm thick, usually become completely disintegrated, if present, extensively demolished. (L₂): A hyaline, semiflexible layer (<1.0-) 1.24 (-2.1) μm thick, gradually disintegrated with spore maturity and only its remnants could be observed on spore. (L₃): A laminated smooth, light yellow to yellow brown layer, (6.3-) 9.4 (-14.7) μm wide at the point of attachment to spore. Subtending hypha wall composed of three layers continuous with spore wall layers. Pore: Usually open, if closed, occlusion occur with the innermost sublayers of third spore wall layer.

Except thickness of third spore wall layer that is slightly more than protolouge, all the other dimensions are completely comparable with Koske, (1985). Also in this research producing sporocarps with numerous spores have not been observed, but aggregation of some spores without covering peridium. Internal proliferation-producing a spore inside the other spore- has been noticed in 11 percent of isolated spores (Fig. 8b and c). *Glomus aggregatum* so far has been reported from Iran (Sedaghati, 2003), Poland (Blaszkowski, 1991) and the United States (Dalpe, 1998). This is the first report of this species in association with sugarcane from Iran (Fig. 8a-c).

9. *Paraglomus occultum* (Walker) Morton and Redecker (2001) Mycologia 93(1): 181-195.

Spores single in the soil, bright to light cream, globose to subglobose (37.8-) 55.6 (-10.4) μm wide. Spore wall: Composed of three adherent layers. (L₁); Less than 1.57 μm thick, generally disintegrated and the remnants make a granular layer on spore, in this case, it seems that spores covered with a

unclean coat of organic materials. (L_2); A laminated layer with very thin sublayers. ($< 1.0- < 1.57$) μm thick. Continue to subtending hypha without any increase in its thickness. (L_3); A permanent layer, $< 1.0- 1.57$ μm thick, usually thickens to 1.57 μm at spore base. This layer also continues into subtending hypha (fig. 9a and b). Subtending hypha: Cylindrical to slightly flared at spore base, ($4.2-$) 6.9 ($- 10.5$) μm wide. Subtending hypha wall bright, made of second and third spore walls, ($<1.0- 2.1$) μm thick each one share equal thickness ($<1.0- 1.05$ μm), gradually the thickness decreases at a distance from spore base and reach to less than 0.3 μm (Fig. 9c).

Pore usually open, sometimes closed due to thickening of both inner wall layers at the point of attachment to spores.

Except minimum spore size and subtending hypha width at spore base which are lesser and more than Morton and Redecker (Morton and Redecker, 2001) description respectively, the other spore dimensions are the same with protolouge. However subtending hypha width at spore base is similar with Schenck and Perez description (Schenck and Perez, 1988). Producing open pore in most cases is comparable with Kenedy *et al.* explanation of the species (Kenedy *et al.*, 1999). *Paraglomus occultum* has been reported from Costa Rica (Morton, 1996), and Egypt (Morton and Redecker, 2001) so far. It is the first report of this species from Iran.

10. *Pacispora scintillans* (Rose and Trappe) Oehl and Sieverd

Spores single in the soil, hyaline to bright yellow, globose to subglobose ($143-$) 163.8 (-195) μm wide, rarely elliptical $110.3-169$ x $130-202$ μm wide. Spore wall: Composed of three layers. (L_1); A hyaline to white orange rigid persistence surface warted layer, ($< 1.1-$) 1.7 (-2.1) μm thick closely adherent to second spore wall layer. (L_2); A smooth hyaline laminated layer ($2.1-$) 4.2 (-7.4) μm thick (L_3); A flexible to semiflexible layer less than 1.0 μm thick adherent to second layer (Fig. 10a, b).

A inner wall with three hyaline flexible layer, totally $<1.1- 2.1$ μm thick. Subtending hypha: Hyaline to light yellow, upright cylindrical, usually constricted at the point of attachment to spore, ($6.4-$) 6.9 (-7.4) μm wide and $1.1-2.1$ μm thick at spore base. Pore usually open. Characteristics and dimensions of spore are the same with this species Ohel & Sieverding (Ohel and Sieverding, 2004) and sadravi (Sadravi, 2000). *Pacispora scintillans* has been reported from Poland (Blaszkowski, 1988), Iran (Sadravi, 2000), Spain, Palestine and Turkey (Blaszkowski, 1988). This is the first report of the species in association with sugarcane rhizosphere in Iran.

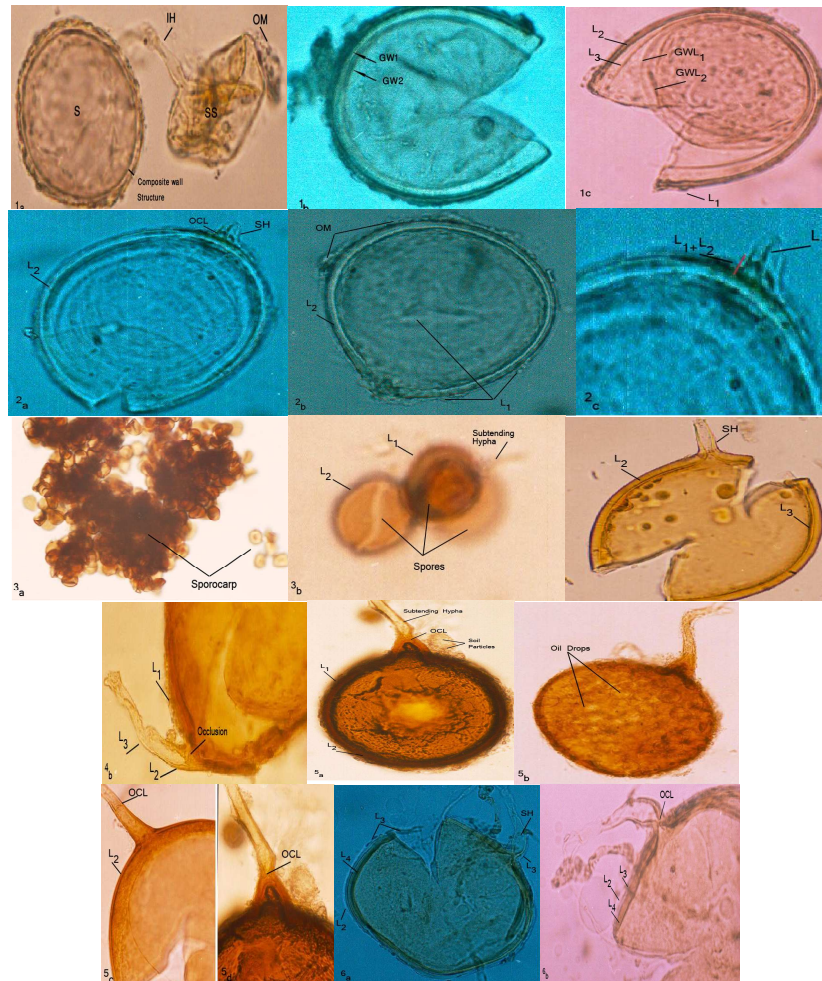


Fig. 1: *Kuklospora colombiana*. **a)** An intact spore, sporiferous sacule and structural details of them. Spore (S); Sporiferous sacule (SS); Interconnecting hypha (IH); Composite spore wall layer and germinal walls; Accumulation of organic materials on sporiferous sacule(OM). **b)** First and second germinal walls on a broken spore. **c)** spore and germinal wall characteristics. Three spore wall layers (L₁, L₂ & L₃) and two inner germinal walls (gw₁& gw₂) on a broken spore. **2:** *Glomus eburneum*. **a)** Mature spores, subtending hypha, Occlusion, first and second spore walls. **b)** First and second spore wall layers (L₁ & L₂) and aggregation of organic materials on spore (OM). **c)** structure and manner of occlusion of subtending hypha. **3:** *Glomus microcarpum*. **a)** sporocarp, mature spores and the way spores attached on each other. **b)** spore and subtending hypha. **4:** *Glomus manihotis*. **a)** Mature spore, Subtending hypha and spore wall layers. **b)** Characteristics of contribution of spore walls in subtending hypha. **5:** *Glomus coronatum*. Characteristic of mature spore; **a)** Spore wall layers (L₁ & L₂), Subtending hypha (SH) and its occlusion by a curved septum. **b)** Oil drops content of spore. **c)** Thin septum located far down the spore base. **d)** Subtending hypha occluded by a thick curved septum **6:** *Glomus caledonium* mature spore. **a)** Second, third and fourth spore wall layers (L₂, L₂, L₄); Composition of Subtending hypha (SH) and involvement of spore wall layers in its construction. **b)** Occlusion (Ocl) of SH with a curved septum near spore base. Second layer separated in a broken spore.

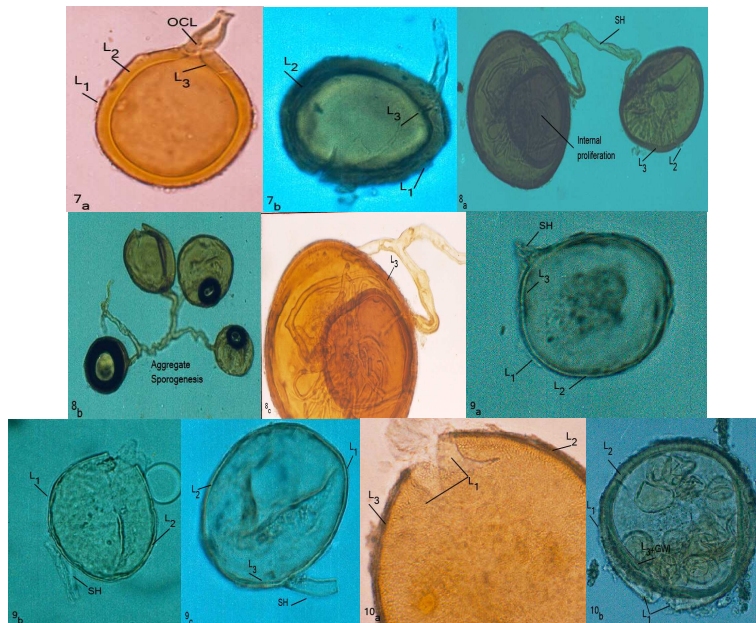


Fig. 2. 7: *Glomus diphanum* mature spore. a) Spore wall layers (L₁, L₂ & L₃) and subtending hypha occlusion. **b)** Mature spore with subtending hypha. **8: *Glomus aggregatum* mature spores. a)** Completely disintegrated first spore wall layer (L₁), Internal proliferation and remnants of second spore wall layer (L₂) on spore. **b)** Loose aggregate of spores **c)** Laminated spore wall (L₃) and internal proliferation. **9: *Paraglomus occultum*. a)** Spore wall layers (L₁, L₂ & L₃) and subtending hypha (SH). **b)** An approximately equal size spore wall layers on a broken spore. **c)** Spore wall layers (L₁, L₂ & L₃) with an open pore subtending hypha. **10: *Pacispora scintilance*. a)** Spore wall layers (L₁, L₂ & L₃), warty surface of first layer on a broken spore. **b)** Germinal walls (Gwl) and spore wall layers.

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