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TWO NEW *GLOMUS* SPECIES FROM ARABLE LAND

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SUMMARY

The vesicular-arbuscular mycorrhizal fungi *Glomus fistulosum* and *Glomus fragilistratum* are described. The former is characterized by a fistular laminated wall, the latter by a unit wall which breaks into flakes and strips when spores are broken.

INTRODUCTION

Investigations into the ecology and agronomic importance of vesicular-arbuscular mycorrhizal (VAM) fungi in cultivated soils have been carried out in Denmark for several years (cf. *e.g.* Jensen & Jakobsen, 1980; Jakobsen & Nielsen, 1983; Jakobsen, 1986). A number of VAM fungi were collected. *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe predominated in five soils and *G. caledonium* (Nicol. & Gerd.) Trappe & Gerd. in one. Further, one sixth of the spores isolated from one soil were identified as *Gigaspora calospora* (Nicol. & Gerd.) Gerd. & Trappe (syn. of *Scutellospora calospora* (Nicol. & Gerd.) Walker & Sanders). Some isolates (Jensen & Jakobsen, 1980) were regarded as new species, and two of these are described in this paper.

MATERIALS AND METHODS

Isolates of VAM fungi were collected in 1978 at Askov, southern Jutland, at Hillerslev, northern Jutland, and at Jullerup, Funen, Denmark. The fungi, taken from soil suspensions, initially were propagated on white clover (*Trifolium repens* L.). Similar-looking spores then were selected and reinoculated on maize (*Zea mays* L.). Homogeneous cultures were obtained by five repeats of this procedure.

The spores were examined in several different mountants: PVLG using the recipe of Omar, Bolland & Heather (1979), and prepared according to the method of Koske & Tessier (1983); Shear's mounting fluid (2% CH_3COOK in 0.2M

pH 8 McIlvaine's buffer: glycerol: ethanol (95%) in a 5:2:3 proportion; Punithalingam, 1971); 3% KOH for clearing (cf. *e.g.* Baral, 1987); 1M sucrose (osmoticum; cf. *e.g.* Villanueva, 1966), or Melzer's reagent.

Identification of the isolates were based mainly on the INVAM species guide (Schenck & Pérez, 1988) and by consideration of details discussed by Morton (1988). For each species, spore size was based on more than 200 measurements made with an ocular screw micrometer.

RESULTS

Glomus fistulosum Skou & Jakobsen sp. nov.

Etymology: The Latin epithet '*fistulosum*' means fistular and refers to the pronounced fistular laminated wall of the spores.

Descriptio: *Sporocarpia ignota. Sporae in terra singulatim efformatae, luteae, globosae, 78 - 137 - 200 µm magnae, inter quas 11 pro 100 late ellipsoideae vel pyriformes, 67 - 121 - 166 x 94 - 138 - 178 µm magnae. Sporae complexis tunicis, 5.5 - 8.9 - 13 µm crassae, quinque stratis (1-5) instructae, in duo turmis (A-B) formatae. Stratum externum evanescens et stratum unitum (sequens), haec duo strata adherentia, tenuibus, non plus quam 1 a 2 µm, et hyalina. Stratum tertium laminatum, luteum, variabiliter crassum, fistulas habens, quo aditus tenuiter declivis ad aperturam angustam, quae plerumque 0.5 µm. Strata membranacea duabus in turma B, hyalina, non plus quam 1 a 2 µm crassa. Hyphae affixae, hyalinae, non occlusae, non septatae, 6-10 µm crassae ad basim sporae pariete 2.7 µm plerumque. Mycorrhizas vesicular-arbusculares formans.*

Habitat in terra ad Askov et Hillerslev, Jutlandia, Dania.

Holotypus increvit ad Zea mays L. anno 1989, in Museo et Herbario Hauniensi (C), Dania depositus. Isotypus aeque ac cultura ad INVAM Universitas Florida, Gainesville, U.S.A. depositus.

Description: Sporocarps unknown. Spores formed singly in soil, pale yellow and yellow in reflected and transmitted light, respectively, globose, 78 - 137 - 200 µm diam. with 86% between 120 and 160 µm; 11% broadly ellipsoid or pyriform, 67 - 121 - 166 x 94 - 138 - 178 µm diam. The spore wall consists of five walls in two groups, with composite wall 5.5 - 8.9 - 13 µm thick (Fig. 1).

WALL GROUP A consists of three walls. Outermost, two thin, hyaline, adhering walls, each not more than 1-2 µm. Wall 1 is evanescent and wall 2 is a rigid unit wall (Fig. 2g). Wall 3 is a yellow, laminated and fistular wall which increases in thickness and number of laminar fissures with spore age (Fig. 2a-i). The openings of the fistules in wall 3 gently slope into an aperture of 0.5 µm on an average (Fig. 2h, i). The fistules appear as points at the focal plane. As the focus is lowered, the fistules incline and appear as diffuse radial lines until they are in horizontal position and clearly visible. This characteristic appear-

ance results from the degrees of inclination of the fistules across the spores as they are seen in the microscope.

The laminated wall 3 breaks rather easily along the laminar fissures. The fractures take on a denticular appearance at the broken edge of this wall, and thus considered ornamented (Fig. 2i).

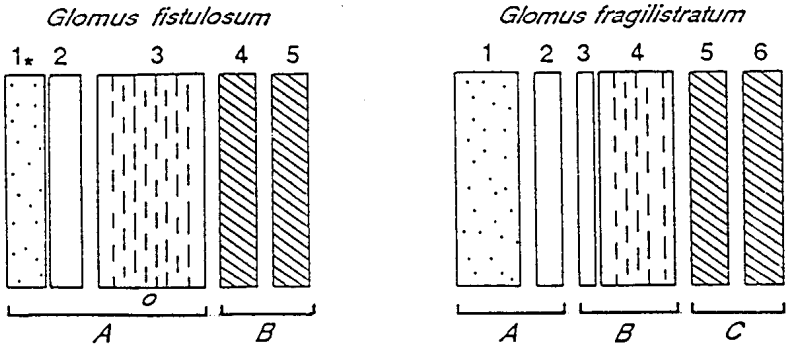


Figure 1. Murographs of the spore wall structure of *G. fistulosum* and *G. fragilistratum*. Muronyms are A(E_*UL_0), B(MM), and A(EU), B(UL), C(MM), respectively.

WALL GROUP B consists of two thin, hyaline membranaceous walls, each not more than 1-2 µm thick. The innermost wall 5 is most easily seen when the cell contents contract on plasmolysis in 1M sucrose (Fig. 2a, e).

A subtending hypha appears to be inserted in the spore walls. It is 6-10 µm wide at the spore base. Hyphal walls are hyaline and with an averaged thickness of 2.7 µm. The pore in the subtending hyphae is open without occlusion or septum (Fig. 2a, b). Spore contents are colourless and appears as variable-sized globules.

G. fistulosum forms vesicular-arbuscular mycorrhizas on several cultivated plants.

Distribution. *Glomus fistulosum* was collected in two habitats by I. Jakobsen in July 1978. Isolate No. 21 was collected in a crop of winter wheat (*Triticum aestivum* L.) on a long-term experimental field with sandy loam at Askov (Askov lermark) in southern Jutland, Denmark. Isolate No. 22 was collected in a crop of spring barley (*Hordeum vulgare* L.) on a loamy soil at Hillerslev in northern Jutland, Denmark (cf. Jensen & Jakobsen, 1980).

Mycorrhizal associations. *G. fistulosum* was collected under conditions that suggest associations with wheat and barley in the field. Further, the fungus (Nos 21 and 22) formed VA mycorrhizas in pot cultures with leek (*Allium porrum* L.), maize (*Zea mays* L.), and white clover (*Trifolium repens* L.).

Types. For purification of *G. fistulosum* (Nos 21 and 22), ten alike spores were separated under the microscope and added to pre-sterilized soil seeded with maize in pot cul-

tures. The holotype (No. 21) was selected in 1989 after five repeats of this procedure and deposited at the Botanical Museum and Herbarium, Copenhagen, Denmark (C). The isotype and a living culture from No. 21 are deposited at the International Culture Collection of VA Mycorrhizal Fungi (INVAM), University of Florida, Gainesville, U.S.A.

Glomus fragilistratum **Skou & Jakobsen sp. nov.**

Etymology: The Latin epithet, '*fragilistratum*' from *fragilis* = fragile, easily shattered, and *stratum* = layer, refers to the third spore wall which is characteristically fragile.

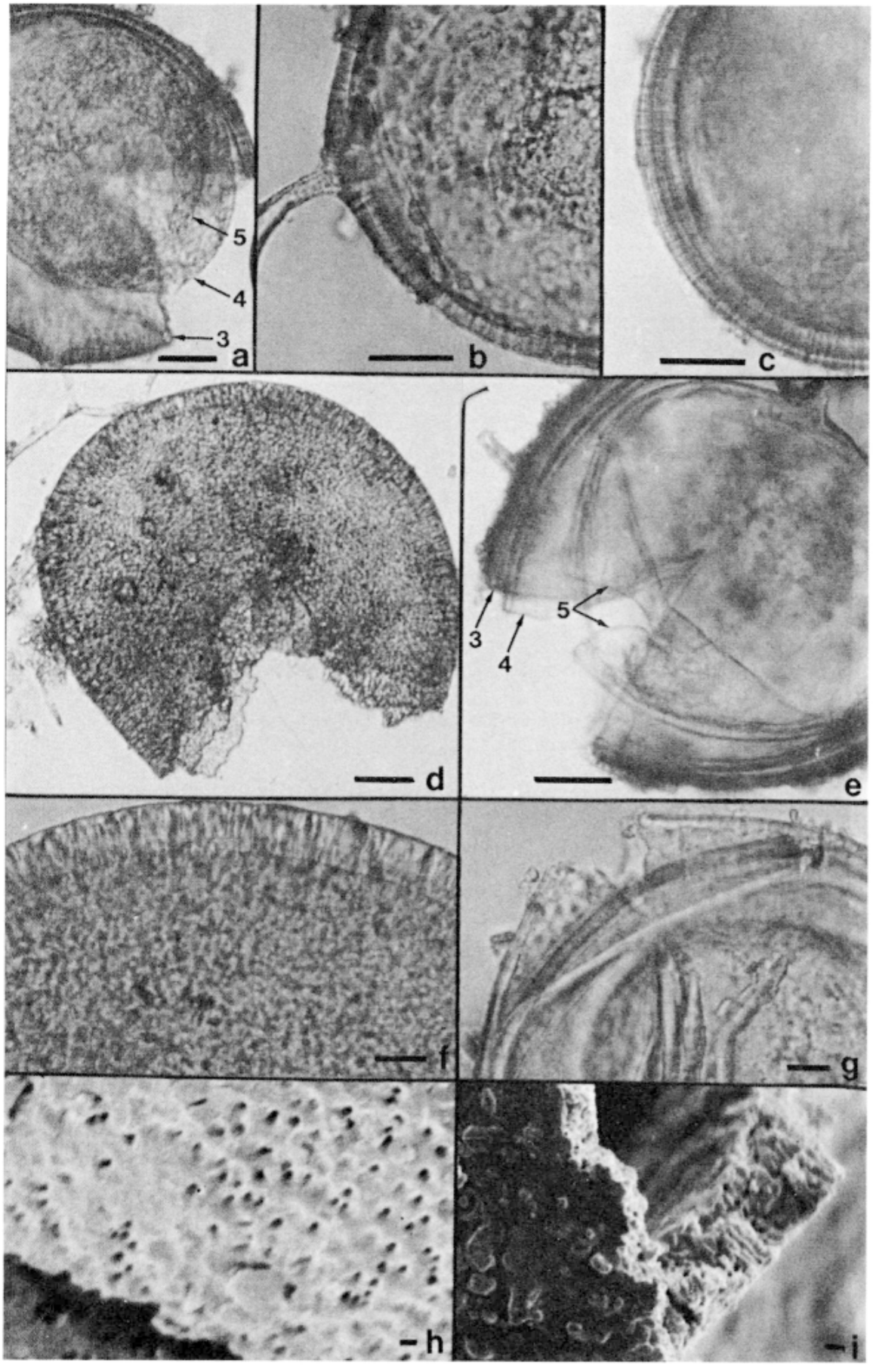
Descriptio: *Sporocarpia ignota. Sporae in terra singulatim efformatae, luteae vel pallide aurantiacae, globosae, 108 - 146 - 191 µm magnae inter quas 18 pro 100 late ellipsoideae vel irregulares, 108 - 142 - 181 x 121 - 169 - 231 µm magnae. Sporae complexis tunicis, 7 - 9 - 12 µm crassae, sex stratis (1-6) instructae, in tres turmis (A-C) formatae. Stratum externum hyalinum, gelatinosum cum granulis, et evanescens, variabiliter crassum attingentia 4.5 µm. Stratum secundum unitum, hyalinum, rigidum, 1.4-3.0 µm. Stratum tertium item unitum, hyalinum, vitri instar, in rimis et vittatis fragilibus, ca. 1 µm crassum, plus minusve ad stratum laminatum adhaerens. Stratum quartum luteum vel pallide aurantiacum, laminatum, variabiliter crassum, plerumque 5.1 µm. Stratum quintum hyalinum, membranaceum, leniter alveolatum, non plus quam 1 µm et stratum sextum hyalinum, membranaceum et granulatum, non plus quam 1 µm. Stratum primum usque ad quartum declive ad hyphas affixas, quae habentes diametrum 9-15 µm ad basim sporarum. Mycorrhizas vesicular-arbusculares formans.*

Habitat in terra ad Jullerup, Fionia, Dania.

Holotypus increvit ad Zea mays L. anno 1989, in Museo et Herbario Hauniensi (C), Dania depositus. Isotypus aeque ac cultura ad INVAM Universitas Florida, Gainesville, U.S.A. depositus.

Description: Sporocarps unknown. Spores formed singly in soil, yellow and bright yellow or pale orange in reflected and transmitted light, respectively, globose, 108 - 146 -

Figure 2. *Glomus fistulosum*. **a**. Broken spore in 1M sucrose. Arrows indicate the laminated wall (3) and the two innermost walls (4 and 5). Note the subtending hypha with the open pore. x 300, bar 25 µm. **b**. Spore showing the open pore to the subtending hypha. The fistules appear as radial stripes on the laminated wall. In PVLG. x 425, bar 25 µm. **c**. The laminated wall with radial lines of the fistules. In PVLG. x 425, bar 25 µm. **d**. Broken spore cleared in 3% KOH. The fistules appear as small points on the inside (note the fractures) as well as on the outside, and as radial lines at the spore periphery. x 300, bar 25 µm. **e**. Broken spore. Arrows point to the laminated wall 3 and the two innermost walls (4 and 5). In PVLG. x 400, bar 25 µm. **f**. Cross section of the fistular, laminated wall in 3% KOH. x 650, bar 10 µm. **g**. Spore in 3% KOH with the outer, hyaline walls (1 and 2) broken. x 550, bar 10 µm. **h**. SEM micrograph of the surface of the laminated wall with fistule openings. x 2500, bar 1 µm. **i**. SEM micrograph of fractures of the laminated wall with the fistules in longitudinal section. x 2500, bar 1 µm.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191 µm diam. with 84.5% between 120 and 170 µm; 18% broadly ellipsoid or irregular, 108 - 142 - 181 x 121 - 169 - 231 µm diam. The spore wall consists of six walls in three groups (A-C), with the composite wall 7 - 9 - 12 µm thick (Fig. 3).

WALL GROUP A consists of two walls. Wall 1 is gelatinous, hyaline and evanescent. It may have a gritty content that is most easily seen in Melzer's reagent. When present, this wall may be up to 4.5 µm thick (Fig. 3a-c). Wall 2 is a rigid, hyaline unit wall, 1.4-3.0 µm thick (Fig. 3e, h, i).

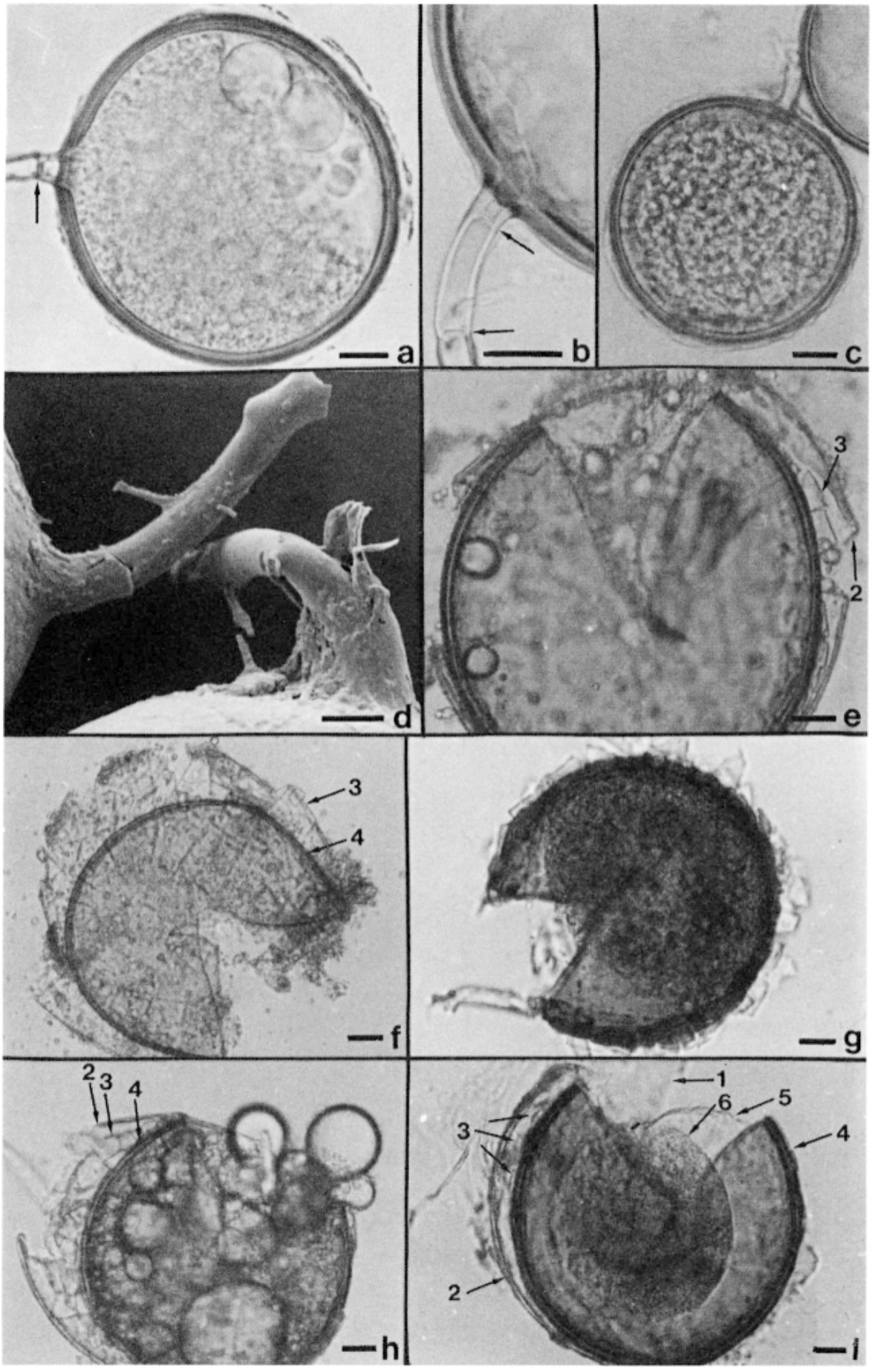
WALL GROUP B consists of two walls (3 and 4). Wall 3 a vitreous hyaline unit wall which rarely exceeds 1 µm on thickness. It breaks into flakes and strips on broken spores. Cracks radiate from the point where the spores are broken with a tapering instrument. This wall sometimes is adherent to the laminated wall (Fig. 3e-i). Wall 4 is a yellow or pale orange laminated wall of varying thickness, 5.1 µm on an average. This wall becomes reddish-brown in Melzer's reagent (Fig. 3a-i).

WALL GROUP C consists of two hyaline membranaceous walls, neither more than 1-2 µm thick. Wall 5 is weakly alveolate and the innermost wall 6 appears granular. The latter wall is most clearly visible when the cell contents contract on plasmolysis (Fig. 3h, i). The spore content consists of differently sized oil globules that become viscid with age (Fig. 3h).

Walls 1-4 extend as walls of the subtending hyphae (Fig. 3d) for a short distance and then tapes off (Fig. 3a, b). Diameter of subtending hyphae at the spore base is 9-15 µm, usually with 1-2 hyphal septa positioned close to the spore. Occasionally, the hypha has an angular bend between the first and the second septum, and narrow branch hyphae may occur at this region (Fig. 3a, b, d).

G. fragilistratum forms vesicular-arbuscular mycorrhiza on several cultivated plants.

Figure 3. *Glomus fragilistratum*. **a.** Spore with subtending hypha and remains of the exterior, gelatinous, hyaline wall. Arrow points to a hyphal septum. x 250, bar 25 µm. **b.** Part of spore with subtending hypha. Arrows point to hyphal septa. Note the thick hyphal wall due to the extension of the four outer walls of the spore. x 400, bar 25 µm. **c.** Spore with the exterior, gelatinous, hyaline wall. x 225, bar 25 µm. **d.** SEM micrograph of subtending hyphae. Note the continuation of spore outer walls onto hypha. x 800, bar 10 µm. **e.** Broken spore showing two outer rigid, hyaline unit walls. Wall 3 is broken into radiating sections. x 225, bar 25 µm. **f.** Crushed spore more clearly showing the separation of the third wall in to radial segments. x 175, bar 25 µm. **g.** Hyaline flakes broken off the third wall of a spore in Melzer's reagent. x 225, bar 25 µm. **h.** Broken spore with wall 2-4 and the oily to viscid content visible. x 175, bar 25 µm. **i.** Broken spore with all six wall layers visible. x 175, bar 25 µm. **a-c, e** and **f** in PVLG; **h** and **i** in 1M sucrose.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Distribution. *Glomus fragilistratum* was collected by I. Jakobsen after harvest in July 1978. The fungus (isolate No. 33) was collected in a crop of spring barley (*Hordeum vulgare* L.) on sandy loam at Jullerup (Statens gård), Funen, Denmark (cf. Jensen & Jakobsen, 1980).

Mycorrhizal associations. *G. fragilistratum* was collected under conditions that suggest association with barley in the field. Further, the fungus (No. 33) formed mycorrhizas in pot cultures with leek (*Allium porrum* L.), maize (*Zea mays* L.), and white clover (*Trifolium repens* L.). Inoculation with this fungus improved uptake of phosphorus (P) and growth of barley, maize, and white clover in P-deficient irradiated soils (Jakobsen, unpublished).

Types. After five inoculations on maize in pot cultures, the holotype of *G. fragilistratum* (No. 33) was selected in 1989 and deposited at the Botanical Museum and Herbarium, Copenhagen, Denmark (C).

Isotype and a living culture from No. 33 are deposited at the International Culture Collection of VA Mycorrhizal Fungi (INVAM), University of Florida, Gainesville, U.S.A.

DISCUSSION

We have used the conventional wall-grouping (Walker, 1983) though in reality only the two outermost walls of spores in *G. fistulosum* may be difficult to separate, and as flakes or segments of the vitreous wall 3 may occasionally adhere to the laminated fourth wall of the spores in *G. fragilistratum*.

The spores of *G. fistulosum* and *G. fragilistratum* have more complex wall structures than those of other described *Glomus* species. The five-walled spores of *G. gerdemannii* Rose, Daniels & Trappe is closest in wall complexity, but the sequence of wall types, hyphal attachment, and spore size are different (Rose et al., 1979).

The fistular or pored structure of the laminated wall in spores of *G. fistulosum* is unique. The narrow (about 0.5 µm), fistules pass through the laminar fissures. They are uniformly distributed over the spore wall and are confined to the laminated wall. For this reason, the fistules are not considered artifacts caused by bacterial or fungal activities.

The vitreous unit wall (wall 3) in spores of *G. fragilistratum* is diagnostic for this species and does not compare to any other wall in described *Glomus* species. It is visible, however, only when spores are broken with a pointing instrument. It does not flake as Rose et al. (1979) report for the outermost walls of *G. gerdemannii*.

The outer evanescent, gelatinous wall is clearly thicker on spores of *G. fragilistratum* than on those of *G. fistulosum*. This wall resembles the outer wall of *G. clarum* Nicol. & Schenck (Nicolson & Schenck, 1979), *G. manihotis* Howeler, Sieverding & Schenck (Schenck, Spain, Sieverding

& Howeler, 1984), and probably *G. intraradices* Schenck & Smith (Schenck & Smith, 1982).

The unit wall 2 of both species is hyaline and rigid rather than gelatinous. Therefore, this wall cannot be just a second layer of the outer wall.

The presence of two inner membranaceous walls in spores of *G. fistulosum* and *G. fragilistratum* is unique for *Glomus* species described to date. This wall structure suggests a phylogenetic relationship with members of *Acaulospora* (J.B. Morton, pers. comm.).

Walls 1-4 of *G. fragilistratum* spores extend on or past the closing septum in the subtending hyphae. In that respect, they resemble several other *Glomus* species such as *G. clarum* (Nicolson & Schenck, 1979) and *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe (Nicolson & Gerdemann, 1968).

Globular to pear-shaped, thin-walled, hyaline, vesicle-like cells, 8-15 µm in diameter, occur scattered on the mycelium of *G. fistulosum* between soil particles. Their function, if any, is unknown.

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