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GLOMUS KERGUELENSE SP. NOV., A NEW GLOMALES SPECIES FROM SUB-ANTARCTIC

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ABSTRACT

Glomus kerguelense sp. nov. was isolated from a gravelly mineral soil at Kerguelen Islands, in association with the rhizosphere of introduced *Agrostis stolonifera*, Poaceae. This golden yellow spored species is distinguished from other Glomeraceae species by the granular ornamentation of the interior surface of the inner spore wall layer, and by a spongy zone gradually developed within the middle spore wall layer. The isolate differentiated vesicles and arbuscles inside *Allium porrum* roots and sporulated profusely in pot cultures. *Glomus kerguelense* is, with *G. antarcticum*, the second Glomales species described originally from the Antarctic region.

Glomus kerguelense sp. nov. a été isolé d'un sol minéral graveleux des Îles Kerguelen, dans la rhizosphère d'une Poacées introduite, *Agrostis stolonifera.* L'espèce aux spores jaune doré se distingue d'autres espèces de Gloméracées par une ornementation granulaire à la surface intérieure de la paroi interne et par une zone spongieuse graduellement différenciée dans la paroi médiane. L'isolat cultivé en pots différencie avec *Allium porrum* des vésicules et des arbuscules intraracinaires ainsi que des spores en abondance. *Glomus kerguelense* constitue, avec *G. antarcticum*, la seconde espèce de Glomérales décrite des régions antarctiques.

KEYWORDS : Arbuscular mycorrhizal fungus, Glomales.

INTRODUCTION

Recent field expeditions to Kerguelen Islands supported by the French Polar Institute (IPEV) were performed to study the impact of climatic changes and human activities on the biodiversity of French sub-Antarctic Islands (Frenot et al 2001). Published studies and reports on arbuscular mycorrhizal (AM) symbioses under Antarctic and sub-Antarctic climates have dealt almost exclusively with plant mycorrhizal status (Christie & Nicholson 1983; Smith & Newton 1996; Laursen et al 1996; Strullu et al 1999). Up to now, only one Glomales species, *Glomus antarcticum* Cabello has been originally described from the Antarctic continent, associated with *Deschampsia antarctica* Dev. (Cabello et al 1994). Similarly, only 5 arbuscular mycorrhizal fungal (AMF) species were found sporulating in indigenous Arctic soils within the rhizosphere of *Festuca* species (Dalpé & Aiken 1998), and all other studies have reported plant arbuscular symbiotic associations (Stutz 1972; Kohn & Stasovski 1990; Bledsoe et al 1990; Gardes & Dahlberg 1996).

In the present survey on Kerguelen Islands, an undescribed taxon was found associated with *Agrostis stolonifera* L., an introduced Poaceae with a worldwide distribution. Herein we describe *G. kerguelense* sp. nov., based on juvenile and mature spore morphologies and the ontogenetic sequence of spore wall layer development, supported by ultrastructural observations. Spores are characterized by their inside wall layer ornamentation and a middle spongy wall layer. Mycorrhizal behavior of the new species under greenhouse growing conditions is characterized by an abundant leek root colonization and a high sporulation rate.

MATERIAL AND METHODS

Soil harvesting and inoculum production

Rhizospheric soil samples (20 mL) containing roots sections of *Agrostis stolonifera* were collected from a gravelly mineral soil at Port-aux-Français, Kerguelen Island, Southern Indian Ocean. Soil samples were mixed with calcined clay, Oil-Dri US-special Ty/IIIR particle size average 5 mm (Oil-Dri Company, Chicago, USA) and used as mycorrhizal inoculum. Leek plants (*Allium porrum* L. cv "Alex") were grown on calcined clay watered weekly with a nutrient solution (Plenchette, 1982) and placed in a greenhouse with a controlled environment (20°C/24°C; natural light supplemented high pressure sodium lamps to maintain a day/night period of 14/10h during autumn, winter and early spring. Three growth cycles of 6 months were required to purify mycorrhizal inoculum during which colonized roots were used to reinoculate leek plants.

Spore isolation and microscopic observations

Spores were extracted from the pot culture by wet sieving and decanting techniques (Gerdemann & Nicolson, 1963) prior to vacuum filtering over filter paper *(Whatman #2)*. Spores were then manually isolated under the dissecting

microscope and permanently mounted in: 1) PVLG (Polyvinyl, lactic acid, glycerol) media (Omar et al 1979), 2) PVLG : Melzer's reagent (1:1, v/v), and in 3) PVLG : Cotton Blue (0.155 in lactic acid) (1:1,v/v). For better observation of inner wall ornamentation, spores were submitted to a 30 sec. ultrasonic treatment in order to remove remaining soil debris attached to the evanescent outer wall layer. Light microscopy was done under regular light and DIC (differential interference contrast) and color observations referred to the International Culture Collection of Arbuscular and vesicular-arbuscular mycorrhizal Fungi (INVAM) color chart codes. For transmission electron microscopy, (TEM), spores were fixed at 4°C for 18 h in 2.8% glutaraldehyde, 1.5% p-formaldehyde in 0.2M phosphate buffer, washed and centrifuged in 0.1M phosphate buffer followed by double distilled water, postfixed in 2% aqueous osmic acid before dehydration, stained in uranyl acetate, and embedded in LRWhite (London Resin Company Ltd.). Ultrathin sections were mounted on grids and observed under a Zeiss EM902 electron microscope at 80 kv. For scanning electron microscopy (SEM), spores were fixed similarly, coated with gold, and observed under a Zeiss DSM940A digital SEM.

Estimation of root colonization

Root sections were cleaned in water to remove soil debris, cleared in 10% KOH at 90°C for 30 min, rinsed in water, stained in fuchsin acid (0.5%) - lactoglycerol (H2O:lactic acid: glycerol, 1:1:1,v/v/v) at 90°C for 15 min, rinsed in water and preserved in lactoglycerol till use . Root colonization levels were estimated using light microscope, 40X objective, using the grid-line intersect methodology (Giovannetti & Mosse, 1980). Root sections were mounted in PVLG for morphological observation under light microscopy.

LATIN DIAGNOSIS

Glomus kerguelense Dalpé & Strullu *sp. nov.* FIGS. 1-2

Sporocarpia ignota. Juveniles sporae, globosae vel subglobosae, 75-162 µm diam., hyalinae ad perpallidae flavidae; maturae sporae luteae, 153-216 µm diam., laeves, faciei externe visae apparenter verrucatae propter interni parietis ornamentatum, singulatim vel aggregatim in solo et abundanter intra radices efformatae; crescentes apici hyphae sustinentis solitariae. Juvenilium tunicae sporarum stratis tribus 3.5-6.2 µm crassae; tunica externa hyalina, 0.6-1.8 µm crassa, laevis, evanescens, mucilagina; stratum secundum, hyalinum ad perpallidum flavidum, 2.4-3.6 µm crassum, laeve, tenuiter laminatum; stratum tertium, hyalinum ad perpallidum flavidum, 0.5-0.8 µm crassum, tenuiter laminatum, praeditum minutas verrucas, 0.2-0.3 µm latas, irregulariter 1.2-1.8 µm distantes. Maturarum tunicae sporarum, stratibus tribus, 6.5-10.8 µm crassae; tunica externa eadem ac juvenilium tunicae sporarum; stratum secundum, luteum, 4.3-6.8 µm crassum, laminatum, formans mature spongiosam

mediam zonam 1.5-1.9 µm crassam ; stratum tertium, aureum, laminatum, 1.6-2.2 µm crassum, verrucatum. Hyphae sustinentes, hyalinae ad aureae, cylindricae vel subinfundibuliformes, 8.5-14 µm crassae, aliquando tumidae, 16-21 µm crassae. Porus sporae curvo septo clausus. Mycorrhizae cum vesiculis et arbusculis formans.

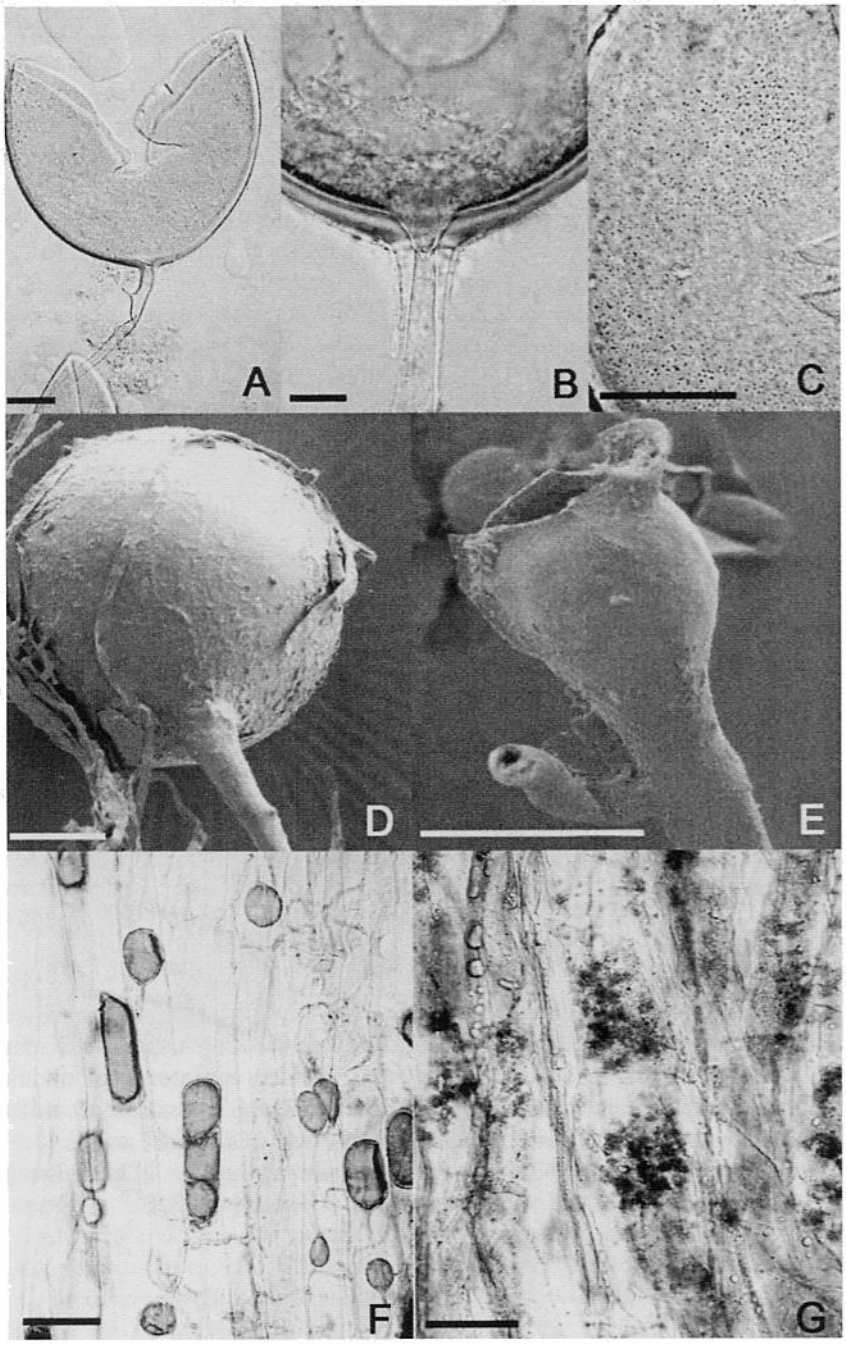
Sporocarps : unknown.

Spores (Fig 1A-D) globose to subglobose formed singly or in clusters of 3 to 5 spores in soil, and also inside roots between cortical cells; differentiated at the apex of a single cylindrical coenocytic subtending hypha. Juvenile spores yellow (0/10/100/0), 75 - 162 µm in diameter, mature spores yellow (0/40/100/0), 153 - 216 µm in diameter (mean diam. 186.3 µm), smooth surfaced; in water mounting media, most juvenile and some mature spores exhibit a granular appearance due to the inside inner wall rugosity.

Juvenile spore wall (Fig 2 A,D,E) composed of three layers, 3.5 - 6.2 µm total thickness ; layer 1: hyaline, 0.6 - 1.8 µm thick, evanescent, mucilaginous, surface smooth to roughened irregular, sometimes disappearing with spore maturation. Layer 2: hyaline to pale yellow, 2.4–3.6 µm thick, finely laminated under light microscope, surface smooth, wall showing a fibrillar texture of different opacity under TEM, corresponding to the sub-layers developed during subsequent maturation. Layer 3: hyaline to pale yellow, 0.5 - 0.8 µm thick, finely laminated, inside surface ornamented with irregularly spaced wart, 0.2-0.3 µm wide distanced by 1.2 - 1.8 µm, giving by transparency a fine granular texture to the spores under the light microscope. Under TEM, the inner surface ornamentation appearing as a succession of irregular cavities, 1.3-1.5 µm wide. All layers stained with Cotton Blue, facilitating the observation of inside ornamentations; no reaction observed with Melzer's reagent.

Mature spore wall (Fig 2 B,C,F,G) composed of three layers, 6,5 - 10.8 µm total thickness; layer 1: identical to the one of juvenile spores; layer 2: yellow, 4.3 - 6.8 µm thick, laminated, maturing by differentiating a spongy middle zone, 1.5 - 1.9 µm thick sandwiched between an outer (1.2 - 1.6 µm thick) and

Fig. 1 A-G *Glomus kerguelense* A. Juvenile crushed spore with subtending hyphae; B. Septum at subtending hyphal attachment of a mature spore; C. Spore granular appearance from inside wall; D. Spore and subtending hyphal attachment under SEM; E. Subtending hyphal attachment with lateral peg under SEM; F. Vesicles colonizing leek (*Allium porrum*) roots; G. Arbuscules inside cortical cells of leek colonized roots. (Scale bars = 20 µm)


A
B
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an inner (2.0 - 2.7 µm thick) section; the spongy area made of holes forming reticulations that appeared under light microscopy as a line of wrinkles between two laminated sections and under electron microscopy as a lacunar tissue in between two fibrillous zones; layer 3: golden yellow, 1.6 - 2.2 µm thick, laminated, inner surface covered with fine granulosity disappearing gradually with maturity. Under TEM, this third layer is already detectable in juvenile spores but laminations are not yet strongly contrasted . At spore maturity, all layers react to Cotton Blue and none to Melzer's reagent.

Subtending hyphae (Fig 1 B.D.E), cylindrical to slightly flared with juvenile spores, 8.5 - 14µm wide, sometimes funnel-shaped with mature spores, 16 - 21 µm at the largest diameter. During the spore maturation process, the pore is closed at an early stage by a curved septum formed by the inner lamination of layer 3.

Wall of subtending hyphae, hyaline to golden yellow, 2.8 - 5.8 µm thick at pore level, thinning to 1.0 - 1.4 µm thick 24 - 32 µm distant from the spore.

Mycorrhizae (Fig 1 F,G) arbuscular mycorrhizae with intra- and extraradical spore differentiation, vesicles mainly ovoid to ellipsoid, 12 - 21 X 24 - 42 (64) µm, and arbuscules arising from a central trunk that ramifies 3 to 4 times before branching intensively producing cauliflower shaped structures.

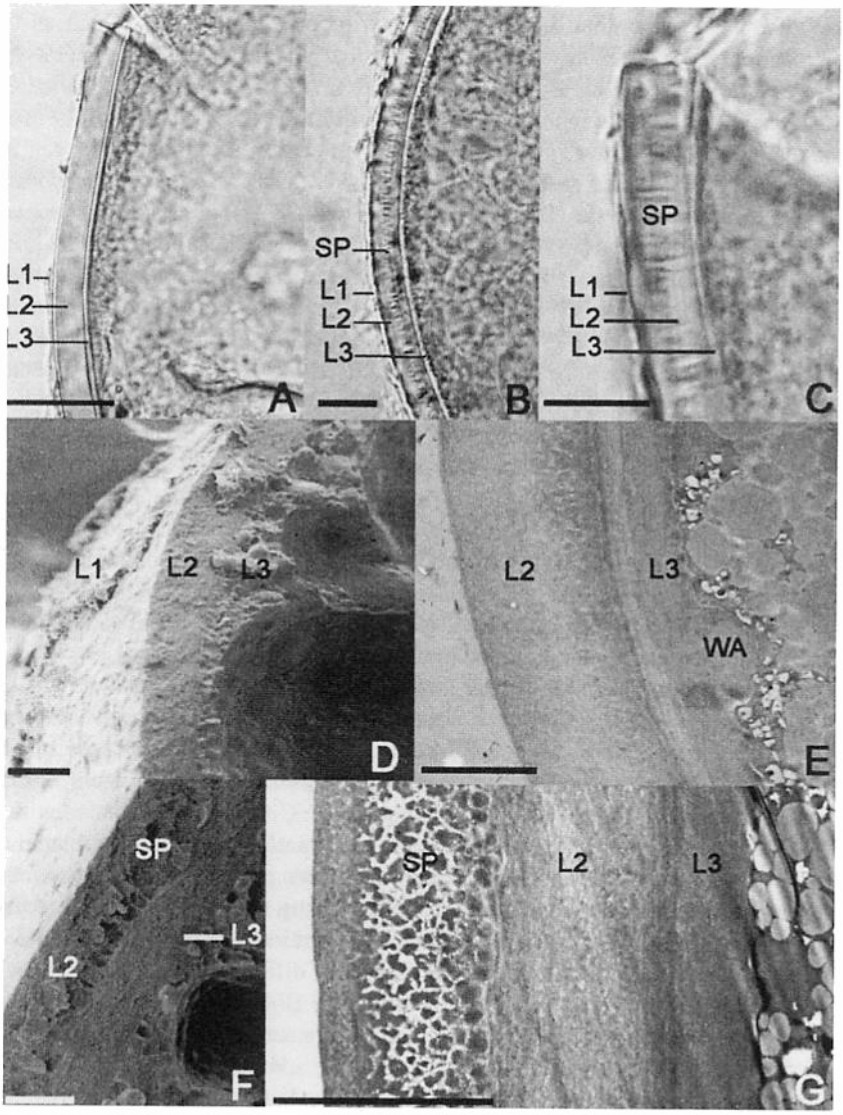
Mycorrhizal association: In the field, isolated from a gravelly mineral soil, from the rhizosphere of *Agrostis stolonifera.* In the greenhouse, pot cultured with *Allium porrum* L.

Distribution: *Glomus kerguelense* is only known from a single site at Port-aux-Français, Scientific Base, Kerguelen Islands, 49 21'S 70 13'E, gravelly mineral soil from a cool oceanic climate with low temperature fluctuating from 2°C in winter to 8 °C in summer.

Etymology: referring to the harvesting locality, Kerguelen Islands, named in honor of the French navigator, Yves-Joseph de Kerguelen de Trémarec, who discovered the Islands in 1772.

Collection examined: FRANCE . Kerguelen Islands Overseas Territory: Port-aux-Français, Feb.1 1998, J-C. Gloaguen. HOLOTYPE DAOM 229603 comprising juvenile and matures spores mounted in PVLG.

Fig 2 A-G Spore wall of *Glomus kerguelense* A. Juvenile spore showing three distinct wall layers (L1, L2, L3); B. Mature spore showing a spongious zone (SP) in the middle laminated wall layer; C. Folded appearance of middle laminated wall layer; D. Juvenile spore wall under SEM; E. Juvenile spore under TEM showing the warty inside ornamentations (WA) of the inner wall (L3); F. Mature spore wall under SEM; G. Mature spore wall under TEM showing lacunar appearance of the spongy layer (SP). (Scale bars A,B,C,= 8 µm; D,E,F,G = 2.5 µm)



DISCUSSION

The fine granular appearance of *G. kerguelense* spores, detectable in both juvenile and mature spores, together with the three distinct layers of the spore wall, readily distinguishes the species from most known *Glomus* taxa. The spongy zone differentiated inside the middle laminated wall of mature spores further differentiates the species. The spore ornamentations which occurred at the inner surface of the inside wall layer of *G. kerguelense*, appear to be, with *G. verruculosum* Blaskowski (Blaskowski & Tadych, 1997) unique in *Glomus*.

With other species that differentiated interior ornamented walls such as *G. fistulosum* Skou & Jakobsen (1989), *G. fragilistratum* Skou & Jakobsen (1989), *G. halonatum* Rose & Trappe (1980), and *G. reticulatum* Bhattacharjee & Mukerji (1980), ornamentations always occur at the outer surface of the wall layer, not on the inner surface. In only a few cases, ornamentations can be observed at the inside surface of the wall layer, such as with *G. multiforum* Tadych & Blaszkowski (Blaskowski & Tadych, 1997), and *G. monosporum* Gerdemann & Trappe (1974), but those ornamentations resulted from the print left by the adjacent inner wall. Of the *Glomus* species characterized by a tri-layered wall, such as *G. claroideum* Schenck & Smith (1982), *G. clarum* Nicolson & Schenck (1979), *G. geosporum* (Nicol. & Gerd.) Walker (Walker, 1982) , and *G. lamellosum* Dalpé, Koske & Tews (1992), none has an ornamented inside wall.

When observed under a compound microscope, spores of *G. kerguelense* resemble those of *G. verruculosum* by their size, their inside wall ornamentation, their flared to funnel-shaped hyphal attachment, their septate subtending hyphae, and the absence of spore wall reaction to Melzer's reagent. However, *G. kerguelense* spores differ considerably from *G. verruculosum* in having much paler spore pigmentation, three distinct spore wall layers, a mucilaginous evanescent outer wall compared to the semi-flexible, rigid one of *G. verruculosum*, and the thinner and non-ornamented laminated wall.

The presence of a spongy zone inside the middle laminated wall on *G. kerguelense* spores is a new feature among Glomales. In the present description, it has not been considered as a distinct wall layer because of its sequential differentiation during spore maturation stages and its irregular distribution along the spore wall. In some aspects, it may be associated with a kind of flaw in the spore wall and as such its formation is potentially related to environmental conditions. When examined under a compound microscope, the zone appeared as a series of multiple folds, giving the wall layer a crumpled aspect (Figs 2 B,C). Not detectable in juvenile spores when observed under SEM (Fig. 2D), it appears in mature spores as holes of different sizes separated each other by a fine membrane, similar to a sponge (Fig. 2E). Under TEM, the spongy texture is detectable at the juvenile spore stage (Fig. 2F). It adopts its distinctive reticulated morphology with mature spores (Fig. 2G). Located in juvenile spores within the younger portion of the laminated wall (Fig. 2F), the spongy zone gradually moved toward the outside of the layer with the synthesis of new laminate in this wall layer. Attempts to propagate an isolate on root-organ culture have, up to now, remained unsuccessful but once realized the *in vitro* culture should provide information on the value of this new wall feature for the characterization of the species.

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LITERATURE CITED

Bhattacharjee, M. & Mukerji, K.G. 1980. Studies on Indian Endogonaceae. II. The genus *Glomus*. *Sydowia* **39:** 14-17

Blaszkowski, J. & Tadych, M. 1997. *Glomus multiforum* and *G. verruculosum*, two new species from Poland. *Mycologia* **89:** 804-811.

Bledsoe, C., Klein, P. & Bliss, L.C. 1990. A survey of mycorrhizal plants on Truelove Lowland, Devon Island, N.W.T., Canada. *Can. J. Bot.* **68:** 1848-1856.

Cabello, M., Gaspar, L. & Pollero, R. 1994. *Glomus antarcticum sp. nov.*, a vesicular-arbuscular mycorrhizal fungus from Antarctica. *Mycotaxon* **51:** 123-128.

Christie, P. & Nicholson, T.H. 1983. Are mycorrhizas absent from the Antarctic? *Trans. Br. Mycol. Soc.* **80:** 557-560.

Dalpé, Y. & Aiken, S.G. 1998. Arbuscular mycorrhizal fungi associated with Festuca species in the Canadian high Arctic. *Can. J. Bot.* **76:** 1930-1938.

Dalpé, Y., Koske, R.E. & Tews, L.L. 1992. *Glomus lamellosum* sp. nov.: a new Glomaceae associated with beach grass *Mycotaxon* **43:** 289-293

Frenot, Y., Gloaguen, J.C., Massé, L. & Lebouviera, M. 2001. Human activities, ecosystem disturbance and plant invasions in subantarctic Crozet, Kerguelen and Amsterdam Islands. Biol. Conserv. **101**: 33-50.

Gardes, M. & Dahlberg, A. 1996. Mycorrhizal diversity in arctic and alpine tundra: an open question. *New Phytol.* **133:**147-157.

Gerdemann, J.W. & Nicolson, T.H. 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. *Trans. Br. Myc. Soc.* **46:** 235-244.

Gerdemann, J.W. & Trappe, J.M. 1974. The Endogonaceae in the Pacific Northwest. *Mycol. Memoirs* **5:** 76 pp.

Giovannetti, M. & Mosse, B. 1980. An evaluation of techniques for measuring VAM infection in roots. *New Phytol.* **84:** 489-500.

Kohn, L.M. & Stasovski, E. 1990. The mycorrhizal status of plants at Alexandre Fiord, Ellesmere Island, Canada, a high arctic site. *Mycologia* **82:** 23-35.

Laursen, G. A. , Treu, R., Seppelt, R.D. & Stephenson, S.L. 1996. Mycorrhizal assessment of vascular plants from subantarctic Macquarie Island. *Arctic Alpine Res.* **29:** 483-491.

Nicolson, T.H. & Schenck, N.C. 1979. Endogonaceous mycorrhizal endophytes in Florida. Mycologia 71:178-198

Omar, M.B., Bolland, L. & Heather, W.A. 1979. A permanent mounting medium for fungi. Bull. Br. Myc. Soc. **13:** 31-32.

Plenchette, C. 1982. Recherches sur les endomycorhizes à vésicules et arbuscules. Influence de la plante hôte, du champignon et du phosphore sur l'expression de la symbiose endomycorhizienne, Thèse de Doctorat (Ph. D), Université Laval, Québec, Canada 182 p.

Rose, S.L. & Trappe, J.M. 1980. Three new endomycorrhizal *Glomus* spp. associated with actinorrhizal shrubs. *Mycotaxon* **10:** 413-420

Schenck, N. C. & Smith, G.S. 1982. Additional new and unreported species of mycorrhizal fungi (Endogonaceae) in Florida. *Mycologia* **74:**77-92

Skou, J.P. & Jakobsen, I. 1989. Two new *Glomus* species from arable land. *Mycotaxon* **36:**273-282

Smith, V.R. & Newton, I.P. 1996. Vesicular-arbuscular mycorrhizas at a sub-Antarctic Island. *Soil Biol. Biochem.* **18:** 547-549.

Strullu, D.G., Frenot, Y., Maurice, D., Gloaguen, J.-C. & Plenchette, C. 1999. First study of mycorrhizae in the Kerguelen islands. *CR. Acad. Sci.III-Vie.* **322:** 771-777.

Stutz, R.C. 1972. Survey of mycorrhizal plants. pp.214-216. In Bliss, L.C. (ed.) Devon Island I.B.P. Project: High Arctic Ecosystem, Project Report 1970 and 1971. Edmonton: Department of Botany, Univ. of Alberta.

Walker, C. 1982. Species in the Endogonaceae: A new species (*Glomus occultum*) and a new combination (*Glomus geosporum*). *Mycotaxon* **15:**49-61