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ACAULOSPORA DELICATA SP. NOV. — AN ENDOMYCORRHIZAL FUNGUS FROM ARIZONA*

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SUMMARY

A newly discovered endomycorrhizal fungus, *Acaulospora delicata,* which forms typical vesicular-arbuscular mycorrhizas with Sudan grass and sorghum, is described and illustrated.

INTRODUCTION

Sand from a greenhouse bed, used for the propagation of cuttings of various ornamental plants, was wet-sieved and the resultant sievings centrifuged in sugar solution (Walker, Mize & McNabb 1982) in order to extract endogonaceous spores. After spores had been extracted, they were suspended in water and observed under a dissecting microscope. Among the spore types present was one we did not recognise. Spores of this type were separated and a representative sample studied on slide mounts under a compound microscope. All these spores proved to be morphologically similar and unlike any described species. We then selected more of these spores and added them to pots of sterilised sand sown with seeds of *Sorghum vulgare* Pers. (sorghum), *S. sudanense* (Piper) Staph. (Sudan grass) and *Lolium perenne* L. (perennial rye grass) in attempts to produce pure pot cultures. These cultures were maintained in a growth chamber with a diurnal temperature range of 15° C to 27° C and illuminated for a 16 hour photoperiod with a mixture of fluorescent and incandescent light.

The plants and their potting medium were examined after 90 days for the presence of mycorrhizas and spores. Perennial ryegrass proved to be ineffective as a host. Vesicular-arbuscular mycorrhizas were observed in the *Sorghum* spp. after clearing and staining the roots (Phillips & Hayman 1970), and abundant spores, all of similar morphology were extracted. The spores were formed laterally on the neck of a sporiferous saccule (Walker, Reed & Sanders 1984), a characteristic of the genus

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Acaulospora Gerd. & Trappe, but were different from those of any other described species in the genus (Gerdemann & Trappe 1974, Trappe 1982, Trappe & Schenck 1982, Schenck *et al* 1984).

The description of wall structures and the murographs (Fig. 1) follow the standardized terminology of Walker (1983). The species description is made from specimens mounted on microscope slides in distilled water or in polyvinyl alcohol lactophenol (PVL), to which stains and reagents had been added as required.

ACAULOSPORA DELICATA Walker, Pfeiffer & Bloss sp. nov. (Fig. 1-3)

Sporae singillatim in terra vel intra radicellas senescentes enatae, lateraliter gestae in sacculo sporifero hyalino 60-95 x 70-125 μm, hyalinae vel flavo-eburneae, ob sporae contentis scintillantes, globosae, subglobosae vel raro ovoideae vel obovoideae, 80-125(-150) x 80-110 (-140) μm. Sporarum tunicae 3-4 in turmis duabus: Turma A cum tunicae hyalinae extimae evanescenti ca. 1 μm crassae (Tunica 1), ad Tunicam 2 adhaerenti; Tunica 2, 2.5-3.5 μm crassa, laminata. Tunicae Turma B cum tunicarum una vel duabus (Tunicae 3 et 4), unaquaeque tenuis, hyalina, membranaceaque. Tunica 3 minute granulosa. Tunicae membranaceae in solutioni Melzeri cinnabarinae.

SPORES borne singly in the soil laterally on the neck of a sporiferous saccule; hyaline to pale yellowish-cream, sparkling from the nature of the spore contents; globose to subglobose (rarely ovoid to obovoid), 80-125(-150) x 80-110(-140) μm. Occasionally spores occurring in the cortical cells of senescent roots (Fig. 3C).

SPORIFEROUS SACCULE (Fig. 2A) usually broader than long, 60-95 x 70-125 μm, hyaline, consisting of the swollen tip of a thin-walled coenocytic hypha, 10-12.5 μm diam, with a single wall 1-2 μm thick. Soil particles and debris often adhering to the exterior of collapsed saccules.

SPORE WALL STRUCTURE of four walls (Walls 1-4) in two groups (Group A and Group B). (See Murograph, Fig. 1A).

WALL GROUP A consisting of a thin, hyaline, outer evanescent wall (Wall 1) approximately 1 μm thick before disintegration, closely attached to Wall 2 which is a relatively thick (2.5-3.5 μm) laminated wall with up to 6 subequal laminations that often are very difficult to observe. Soil particles and debris often adherent to the evanescent outer wall (Wall 1) (Fig. 2C).

WALL GROUP B of two thin, hyaline, membranous walls (Walls 3 & 4), ± 0.5 μm and 0.75-1 μm thick respectively. Wall 3 covered by minute granular excrescences that become increasingly crowded with age and that are difficult to see under the light microscope, but which can be observed readily with the aid of a scanning electron microscope (Fig. 3A, 3B). Membranous walls rapidly turning orange-red in Melzer's reagent. Wall 1 not reacting in this reagent; Wall 2 deepening in colour to become a slightly darker yellow.

ETYMOLOGY: Latin — *delicata,* referring to the rather fragile nature of the spores due to their thin walls.

MYCORRHIZAL ASSOCIATIONS: The species forms typical vesicular-arbuscular mycorrhizas with *Sorghum sudanense* and *S. vulgare* (Fig. 3D).

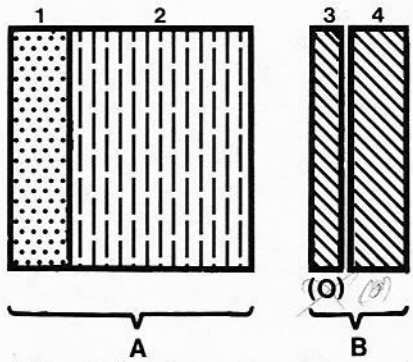

Fig. 1A: *Acaulospora delicata*

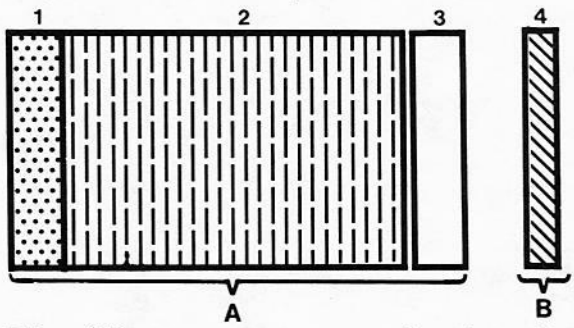

Fig. 1B: *Acaulospora nicolsonii*

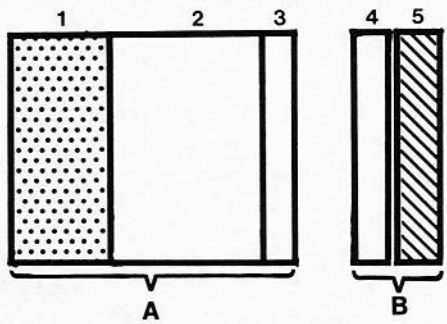

Fig. 1C: *Acaulospora longula*

Fig. 1 Murographs (after Walker 1983) of *Acaulospora delicata* (Fig. 1A) and two superficially similar species, *A. nicolsonii* (Fig. 1B) and *A. longula* (Fig. 1C). Evanescent walls are shaded with dots, laminated walls with broken lines, membranous walls with diagonal lines, and unit walls are left unshaded. Wall 3 is ornamented, but the ornamentation is often difficult, and sometimes impossible, to observe with a light microscope.

COLLECTIONS EXAMINED

Holotype: ARIZONA - Pima County, Tucson; from a pot culture with S. *sudanense* (OSC, isotype ARIZ, K). The origin of the sand in the green house at the University of Arizona (Building 42-2R) from which the original spores were extracted is unknown.

DISCUSSION

Spores of *Acaulospora delicata* bear a superficial resemblance to those of *A. trappei* Ames & Linderman, *A. nicolsonii* Walker, Reed & Sanders, and *A. longula* Spain & Schenck, but there are differences in the size range and colour of the spores, and in the spore wall structure of the species. The size range of spores of *A. trappei* and *A. delicata* over-lap slightly 42-99 x 42-70 μm in the former compared with 80-150 x 80-140 μm in the latter) but, in general, spores of *A. trappei* are much smaller than those of *A. delicata. A. trappei* is described as having only a single wall, whereas *A. delicata* has a complex wall structure of three or four walls in two groups. There is considerable overlap in the size range of mature spores of *A. nicolsonii,* which are 99-198 X 109-218 μm, and those of *A. delicata,* though most spores of the former are larger than most of the latter. However, the differences in wall structure between these two species is clear, and is illustrated in the murographs (Fig. 1). Wall 1 of both species is evanescent, hyaline, and approximately 1 μm thick, but although Wall 2 is laminated and may be pale yellow in both species, in *A. nicolsonii* the laminae are thicker and much easier to see than in *A. delicata* and the surface of the former fractures to form fissures at maturity, whilst in the latter it remains smooth. Wall 2 is also much thicker in *A. nicolsonii* (3-10 μm) than in *A. delicata* (2.5-3.5 μm). Wall 3 in *A. delicata* is an ornamented, membranous wall in Wall Group B; whereas in *A. nicolsonii* it is a thin, brittle, unit wall in Group A. Occasionally, the inner lamina of Wall 2 in *A. delicata* becomes partially detached and could be mistaken for a separate unit wall, but examination of a number of specimens should resolve the real nature of this artefact. Wall Group B differs between the species. Mature spores of *A. delicata* have two membranous walls one of which becomes increasingly ornamented with age (Fig. 1A); whereas *A. nicolsonii* has only one membranous wall in this group which is

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FIG. 2 Light photomicrographs of spores of *Acaulospora delicata.*

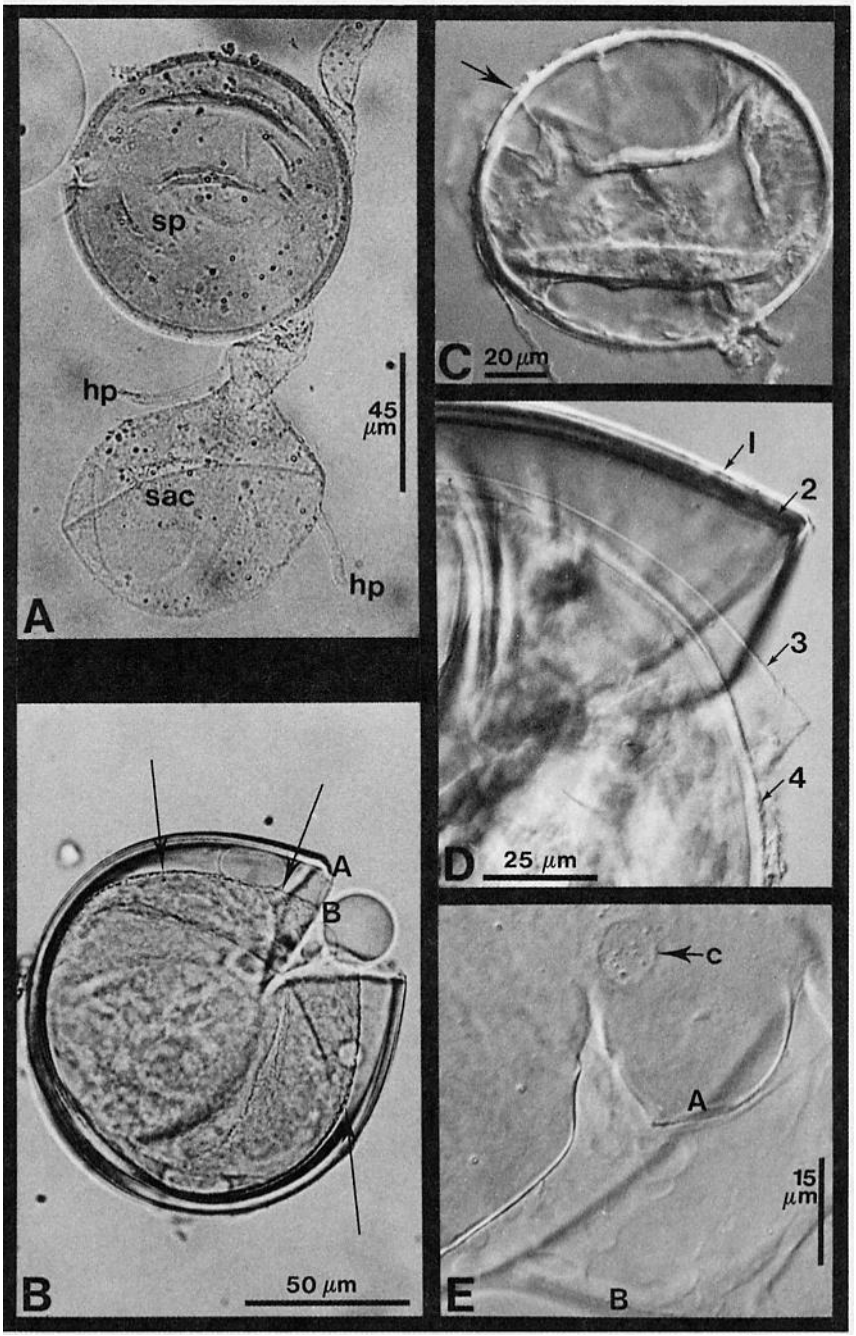
A. Sporiferous saccule (sac) with a fully-formed spore (sp) still attached. The saccule has two of the hyphal protuberances (hp) typical of the genus *Acaulospora.*

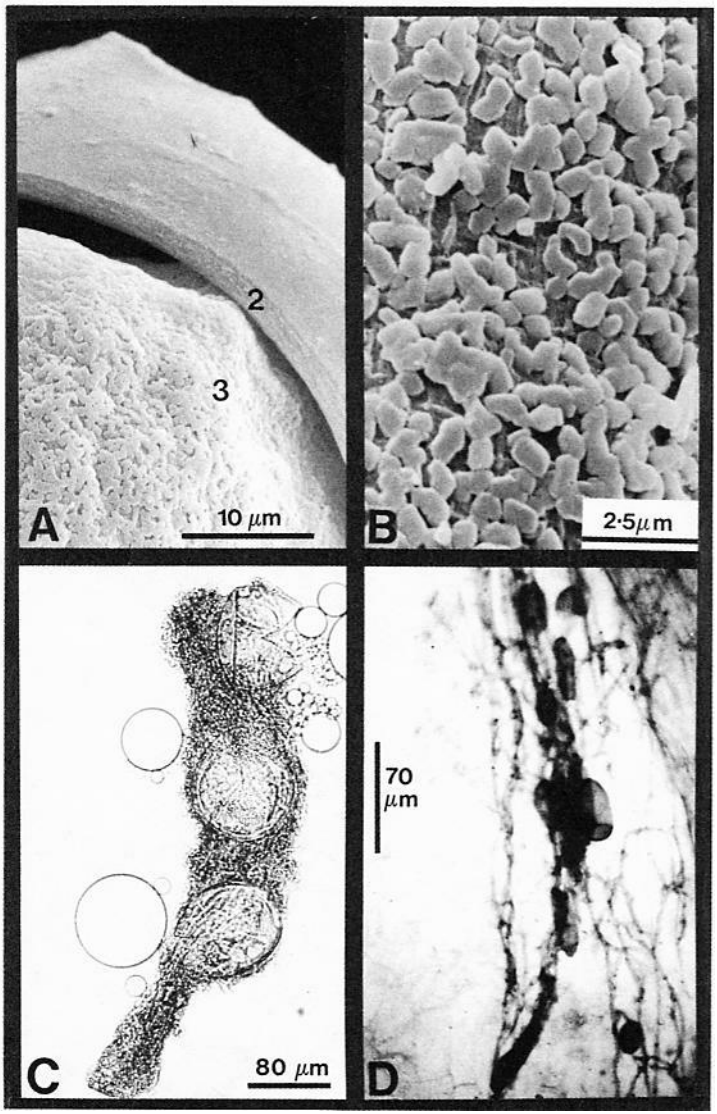
B. A fractured spore, showing the two wall groups (A and B). The ornamentation on wall 3 gives the outer surface of wall group B a finely granular appearance (arrowed).

C. A whole spore mounted in polyvinyl alcohol lactophenol to show the wrinkling of the membranous inner wall group and the debris which typically is adherent to the outer wall (arrowed).

D. In this fractured spore, all four walls (numbered as in the description and murograph) can be seen, though the ornamentation on wall 3 is not evident in this specimen.

E. Surface view of a fractured spore, showing the collar (c) formed in the outer wall group at the point of detachment from the sporiferous saccule. The two wall groups are lettered appropriately (A and B).


sp
hp
45 µm
sac
hp
A
C 20 µm
1
2
3
4
D 25 µm
A
B
B
50 µm
c
A
15 µm
E
B


2
3
A
10 μm
B
2·5μm
C
80 μm
70
μm
D

unadorned (Fig. 1B). Young spores of the former species could be confused with those of the latter, as they may seem to have only a single membranous wall with slight or no ornamentation.

Acaulospora longula spores are similar in size and colour to those of *A. delicata,* but the former has five walls and no ornamented wall in the inner wall group whereas the latter has only four walls, and has ornamentations on Wall 3 in Wall Group B (though this ornamentation may be absent in some specimens (Fig. 2D)). In addition, spores of *A. longula* are formed at distance of 100-200 μm from the sporiferous saccule, whereas spores of *A. delicata* are formed close to the base of the saccule (within 100 μm).

All other described members of the genus *Acaulospora* have either ornamented outer walls or have more deeply coloured spores (brown or yellow), and would be unlikely to be confused with *A. delicata.*

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LITERATURE CITED

AMES, R. N. & LINDERMAN, R. G. 1976. *Acaulospora trappei* sp. nov. *Mycotaxon* 3:565-569.

GERDEMANN, J. W. & TRAPPE, J. M. 1974. The Endogonaceae in the Pacific Northwest. *Mycologia Memoir* No. 5, 76 pp.

PHILLIPS, J. M. & HAYMAN, D. S. 1970. Improved procedure for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**:158-160.

FIG. 3 *Acaulospora delicata* spores and root-colonization.

A. Scanning electron micrograph (SEM) showing the laminated nature of wall 2 and the ornamentation on wall 3. Wall 1 has broken down and disappeared.

B. Detail of the ornamentation on wall 3 (SEM).

C. Spores formed in a senescent root from a pot culture with *Sorghum sudanense* (brightfield light microscopy).

D. Vesicles and intercellular hyphae of *A. delicata* in the roots of *S. sudanense* stained in cotton blue (brightfield light microscopy).

SCHENCK, N. C., SPAIN, J. L., SIEVERDING, E. & HOWELER, R. H. 1984. Several new and unreported vesicular-arbuscular mycorrhizal fungi (Endogonaceae) from Colombia. *Mycologia* **76**:685-699.

TRAPPE, J. M. 1982. Synoptic keys to the genera and species of zygomycetous mycorrhizal fungi. *Phytopathology* **72**:1102-1108.

TRAPPE, J. M. & SCHENCK, N. C. 1982. Taxonomy of the fungi forming endomycorrhizas. pp 1-9 In: N. C. Schenck, ed., *Methods and Principles of Mycorrhizal Research,* American Phytopathological Society, St Paul, Minnesota.

WALKER, C. 1983. Taxonomic concepts in the Endogonaceae: spore wall characteristics in species descriptions. *Mycotaxon* **18**:443-455.

WALKER, C., MIZE, C. W. & McNABB, H. S. 1982. Populations of endogonaceous fungi at two locations in central Iowa. *Canadian Journal of Botany* **60**:2518-2529.

WALKER, C., REED, L. E. & SANDERS, F. E. 1984. *Acaulospora nicolsonii,* a new endogonaceous species from Great Britain. *Transactions of the British Mycological Society* **83**: 360-364.