Crypt. Bot. 1, 204–207 (1989)

Cryptogamic Botany

Acaulospora cavernata (Endogonales) – a New Species from Poland with Pitted Spores

Janusz Błaszkowski

Department of Plant Pathology, Academy of Agriculture, Szczecin, Poland

SUMMARY

Acaulospora cavernata, sp. nov. was found only at one site in Poland as associated with roots of *Thuja occidentalis* growing at the Hel Peninsula. *A. cavernata* forms sessile spores on the neck of a sporiferous saccule. The spores are yolk yellow to light brown, (115–) 140 (–170) µm in diam and have a 5-walled wall structure arranged in three groups. Group A consists of a coloured, unit outer wall, ornamented with evenly distributed, round or slightly ovate depressions, and of a hyaline, unit inner wall. A single hyaline, membranous wall forms the wall group B. Group C is composed of a hyaline, coriaceous wall and an amorphous innermost wall, which stains red in Melzer's reagent. This species seems to be a very rarely occurring species of the Endogonaceae in Poland.

Introduction

In 1987 a soil sample was taken from under *Thuja occidentalis* L., which contained spores similar to those of *Acaulospora foveata* Trappe et Janos [2]. Unfortunately, they were incomplete, lacking sporiferous saccules. So, it was impossible to clearly place the spores into an appropriate genus. In 1988 an additional soil sample from the same site was taken, in which numerous complete specimens representing the genus *Acaulospora* Gerd. et Trappe emend. Berch were found. Detailed morphological studies showed, however, that the spores differ in many respects both from those of *A. foveata* originally described by Janos and Trappe [2] and those found by Schenck et al. [8], as well as from spores of all other species of this genus. They are proposed in this paper to represent a new species, *A. cavernata*.

Material and Methods

The soil samples were taken from a depth of 5–30 cm and then refrigerated until processing. Spores were isolated by wet sieving and decanting [1]. About 50 freshly isolated spores as well as 100 g of soil from the samples were used to prepare pot cultures with *Lolium perenne* L., which were harvested after eight months and examined for the presence of new spores and mycorrhizae. Roots were stained according to Phillips and Hayman [7]. About 100 field- and pot-collected spores were mounted in polyvinyl alcohol/glycerol/lacticacid (PVLG) [4] to make microscope observations on their morphology. The terminology used in this paper in descriptions of walls and murographs is based on the propositions by Walker [10, 11] and Morton [5], and observations on spore colour were made with the aid of a stereomicroscope from fresh specimens immersed in water. Colours were determined according to the Methuen Handbook of Colour [3]. The holotype and other specimens are deposited in the Department of Plant Pathology (DPP), Academy of Agriculture, Szczecin. All specimens are preserved in 5% formalin.

Results

Acaulospora cavernata Błaszkowski sp. nov. (Figs 1–7) Etym. *cavernata* (Latin), referring to the pitted outermost spore wall.

Sporae singulae in solo efformatae, lateraliter gestae ad collum sacculi sporangiferi. Sacculus sporangifer hyalinus vel pallide aurantiacus, globosus vel subglobosus, 130–150 µm diam; collum 100–120 µm longum, attenuatum ab 30 µm diam ad sacculi sporangiferi ad 20 µm diam ad sporam. Sporae luteae vel pallide brunneae, globosae vel subglobosae, (115–) 140 (1–170) µm diam,

0935-2147/89/0001-0204 $ 3,50/0

sessile ad collum sacculi sporangiferi. Tunicae sporae e stratis quinque in turmis tribus. Turma externa e stratis duobus (strati 1, 2); uno, luteo vel pallide brunneo, rigido, (5.1–) 5.7 (–6.6) µm crasso, cum cavernis ordinatis, 2–5 µm diam vel ovatis, 2–2.9 × 4.2–5 µm et 1.7–2.5 µm profundis; duobus hyalino, rigido, (1.2–) 2.0 (–3.4) µm crasso. Turma media e strato uno (stratum 3), hyalino, membranaceo, (0.7–) 1.0 (–1.5) µm crasso. Turma interna e stratis duobus (strati 4, 5); quarto hyalino, coriaceo, (1.5–) 1.7 (–2.9) µm crasso; quinque hyalino, amorpho, (0.8–) 1.0 (–1.3) µm crasso. Formans vesicular-arbusuclares mycorrhizae.

Spores formed singly in the soil, laterally on the neck of a sporiferous saccule (Fig. 1). Sporiferous saccule hyaline to pale orange (5A3) [3], globose to subglobose, 130–150 µm in diam; neck 100–120 µm long, tapering from 30 µm in diam at the saccule to 20 µm in diam at the point of spore attachment. Saccule wall of a hyaline, smooth, 1–1.5 µm thick layer. Saccule collapsing at maturity and usually detached among mature spores. Spores yolk yellow (4B8) to light brown (6D8), globose to subglobose, (11–) 140 (–170) µm in diam, sessile on the neck of a sporiferous saccule. Spore wall of five walls (walls 1–5) in three groups (groups A, B, C) (Fig. 7). Group A of a yolk yellow (4B8) to light brown (6D8), unit, (5·1–) 5.7 (–6.6) µm thick outer wall (wall 1), evenly pitted with round, 2–5 µm in diam, rarely ovate, 2–2.9 × 4.2–5 µm and 1.7–2.5 µm deep depressions, separated by ridges 1–4 µm wide (Figs 2, 3), and of a hyaline, unit, (1.2–) 2.0 (–3.4) µm thick inner wall (wall 2) (Figs 3–5), easily separating from the wall 1. Group B of a hyaline, membranous, (0.7–) 1.0 (–1.5) µm thick wall (wall 3) (Figs 3–5). Group C of a hyaline, coriaceous, (1.5–) 1.7 (–2.9) µm thick wall (wall 4) adherring to a hyaline, amorphous (0.8–) 1.0 (–1.3) µm thick innermost wall (wall 5) (Figs 3–6). Spore contents of hyaline lipid globules. Only the wall 5 stains ruby red (12D8) in Melzer's reagent (Fig. 6).

Specimens examined: All collected from under *T. occidentalis* by J. Błaszkowski, Poland, Hel, 20 Aug. 1987, 945–956, 1022–1024; 28 Sept. 1988, DPP 1285, holotype, 1259–1267, 1277–1284, 1286–1298, 1386–1387, isotypes.

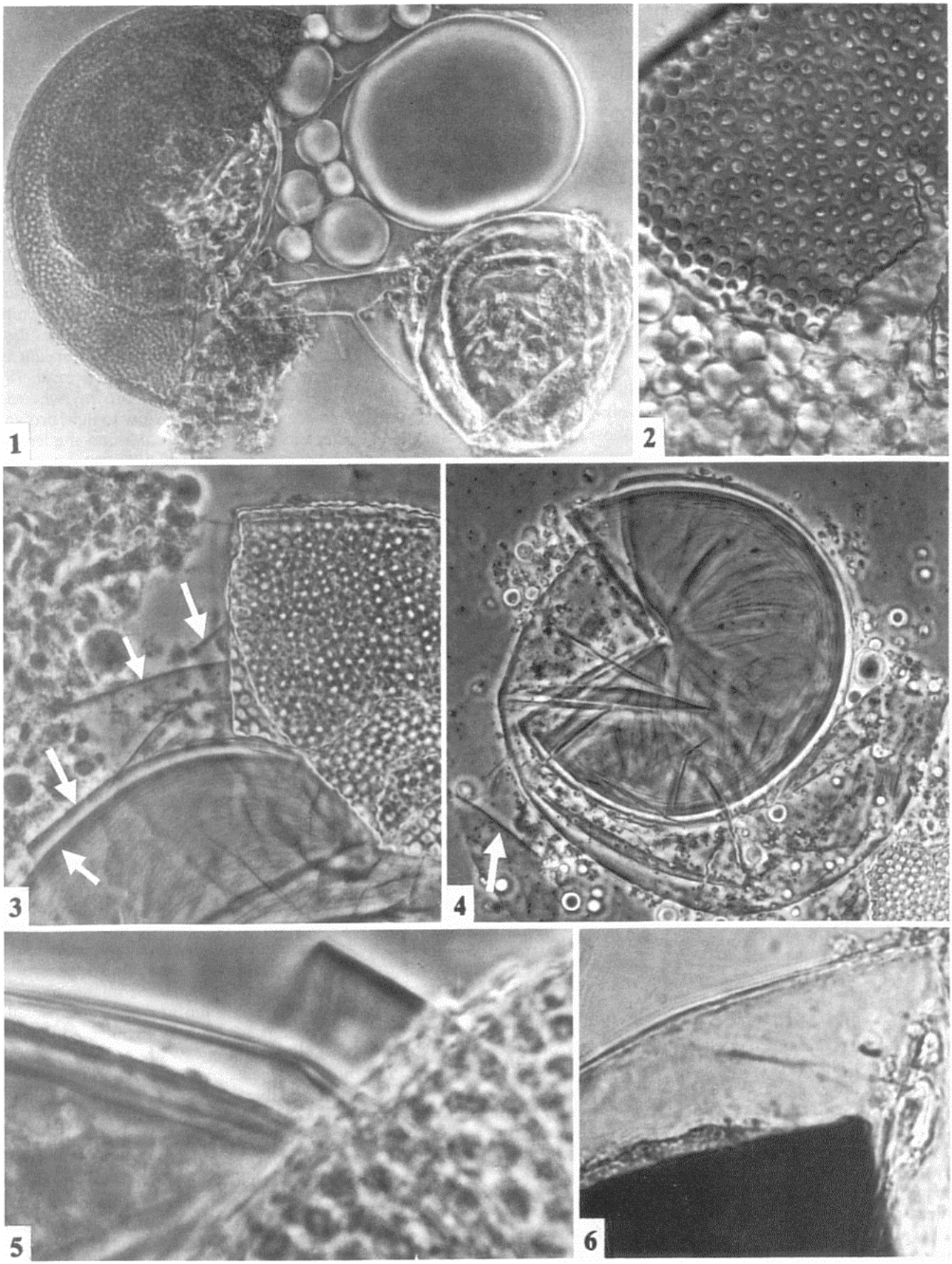
Distribution and habitat: To date, *A. cavernata* was isolated only from one site in Poland situated about 200 m from the Baltic Sea. It was found in the rhizosphere soil of *T. occidentalis* growing in a private garden of a small town, Hel. *T. occidentalis* was the only plant species present at the site. The spore densities of this species were 23 and 234 per 100 g dry soil in 1987 and 1988 respectively. The chemical properties of the soil sample from this site examined in 1988 were: pH 4.7; 48, 15, 10, 24, and 20 mg kg^{-1} NO_3, P, K, Mg, and Cl respectively. This species seems to be an extremely rare vesicular-arbuscular mycorrhizal fungus occurring in Poland, having been found in only two soil samples taken from the same site, but it was absent in over 200 other soil samples collected both from under cultivated and natural plants. 58 of them were also collected from other sites of the Hel Peninsula.

Mycorrhizal association: *A. cavernata* was associated with vesicular-arbuscular mycorrhizal roots of *T. occidentalis* growing at the site from which the original soil samples were collected, although many other spores of the genera *Acaulospora, Glomus* Tul. et Tu., and *Scutellospora* Walker et Sanders were present in these samples as well. This species formed vesicular-arbuscular mycorrhizae in pot cultures with *L. perenne*.

Discussion

A. cavernata most resembles *A. foveata* by possessing the almost identically pitted outermost spore wall. However, *A. cavernata* produces smaller spores (115–170 µm in diam *vs.* 135–480 µm in diam) with smaller pits (2–5 µm in diam *vs.* 4–16 µm in diam) [2, 8]. Besides, Janos and Trappe [2] characterized spores of *A. foveata* as yellowish brown to brownish black, Schenck et al. [8] stated them to be dark brown to red brown, whereas spores of *A. cavernata* are only yolk yellow to light brown. Also, the diameter of sporiferous saccules and the lenght of hyphal necks are significantly different among specimens of A. cavernata (130–150 µm in diam and 100–120 µm long) and A. foveata (170–310 µm in diam and 38–70 µm long). However, the wall structure and properties of spore walls of both species are parameters of the highest taxonomic weight, univocally separating them into two distinct taxa. Janos and Trappe [2] characterized *A. foveata* as forming 2-walled spores, of which the outer one is 11–15 µm thick and coloured, and the inner wall is 3 µm thick and hyaline. Schenck et al. [8] found *A. foveata* with three walls, of a total thickness of 6.3–17.5 µm, of which the second wall is butterscotch to brown, and the innermost wall is hyaline. According to Walker (pers. comm.), the wall of *A. foveata* spores consists of a unit, ornamented outermost wall in the group A and of a membranous and an amorphous wall in the group B. In contrast, spores of *A. cavernata* have five walls, and their outermost wall is almost two times thinner than that characterized by Janos and Trappe [2] or overlaps only the bottom limit of the total wall thickness of specimens found by Schenk et al. [8].

Also, *A. scrobiculata* Trappe [9], *A. dilatata* Morton [5], and *A. lacunosa* Morton [5] form spores with a pitted outermost wall. Although the diameter of *A. scrobiculata* spores partly overlaps that of *A. cavernata* spores, the former has an outermost wall pitted with smaller depressions (1–3 µm in diam *vs.* 2–5 µm in diam), and its wall structure is characterized as 4-walled, of which, addittionally, the second and penultimate walls are significantly thinner than those of *A. cavernata*. *A. dilatata* generally produces somewhat smaller spores (73–130 µm in diam). Its outermost wall (a single wall in the group A) is laminate (*vs.* unit in *A. cavernata*) and ornamented with minute pits (*vs.* relatively large pits in *A. cavernata*); the wall group B consists of two adhering rigid walls, each 0.1–1 µm thick, and the wall group C is composed of a membranous, "beaded", 0.5–1 µm thick wall and of an amorphous wall of highly variable thickness in lactophenol-based mountants.


1
2
3
4
5
6

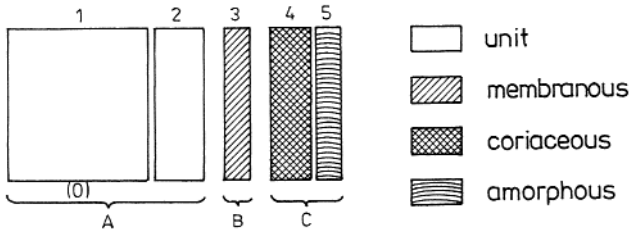


Fig. 7. Murograph of the wall structure of *Acaulospora cavernata,* based on examination of crushed spores in PVLG freshly extracted from soil. Arabic numerals indicate spore walls in order from outer to inner wall; letters indicate wall groups; o = ornamented wall.

Wall structure and wall properties readily distinguish spores of *A. lacunosa* from those of *A. cavernata*. *A. lacunosa* forms reddish yellow spores, whereas those of *A. cavernata* are yolk yellow to light brown. Spores of *A. cavernata* have a muronym [11] A(UoU) B(M) C(CA), and the muronym of *A. lacunosa* spores is A(Lo) B(UU) C(MbA). Whereas the outermost wall of *A. cavernata* is evenly pitted with round or ovate depressions, the ornamentation of *A. lacunosa* spores not only is composed of more irregularly-shaped and distributed depressions, but some have raised cone-shaped edges [5].

The coriaceous wall in the spore wall structure of *A. cavernata* is the next distinctive feature of this species, because this wall type was so far found only among spores of one species of this genus, *A. appendicula* Spain et al. [6], although it is often present among walls of spores of the genus *Scutellospora*. However, *A. appendicula* has larger and lighter spores with a thicker wall, which consists of only four walls. Additionally, its spores are borne on a short pedicel rather than sessile as those of *A. cavernata*.

References

1 Gerdemann, J. W. and Nicolson, T. H. (1963): Spores of mycorrhizal Endogene species extracted from soil by wet sieving and decanting. Trans. Br. Mycol. Soc. *46*, 235–244.
2 Janos, D. P. and Trappe, J. M. (1982): Two new Acualospora species from Tropical America. Mycotaxon *15*, 515–522.
3 Kornerup, A. and Wanscher, J. H. (1983): Methuen Handbook of Colour. Methuen London Ltd, London.
4 Koske, R. E. and Tessier, B. (1983): A convenient, permanent slide mounting medium. Mycol. Soc. America Newsletter *34*, 59.
5 Morton, J. B. (1986): Three new species of Acaulospora (Endogonaceae) from high aluminium, low pH soils in West Virginia. Mycologia *78*, 641–648.
6 Morton, J. B. (1988): Taxonomy of VA mycorrhizal fungi: classification, nomenclature, and identitification. Mycotaxon *32*, 267–324.
7 Phillips, J. M. and Haymann, D. S. (1970): Improved procedcures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. *55*, 158–161.
8 Schenck, N. C., Spain, J. L., Sieverding, E. and Howeler, R. H. (1984): Several and unreported vesicular-arbuscular mycorrhizal fungi (Endogonaceae) from Colombia. Mycologia *76*, 685–699.
9 Trappe, J. M. (1977): Three new Endogonaceae: Glomus constrictus, Sclerocaystis clavispora, and Acaulospora scrobiculata. Mycotaxon *6*, 359–366.
10 Walker, C. (1983): Taxonomic concepts in the Endogonaceae: spore wall characteristics in species descriptions. Mycotaxon *18*, 443–455.
11 Walker, C. (1986): Taxonomic concepts in the Endogonaceae: II. A fifth morphological wall type in endogonaceous spores. Mycotaxon *25*, 95–97.

Key words: Acaulospora, taxonomy, vesicular-arbuscular mycorrhizal fungi, distribution in Poland.

Janusz Błaszkowski, Department of Plant Pathology, Academy of Agriculture, Słowackiego 17, PL-71434 Szczecin, Poland

◀ Fig. 1. A crushed spore with the sporiferous saccule, phase contrast (PC), × 300. – Fig. 2. The evenly pitted outermost wall of a crushed spore is seen, light microscope (LM), × 750. – Fig. 3. The spore wall structure of a spore is visible; the inner walls are arrowed, PC, × 450. – Fig. 4. Inner walls of a crushed spore; the fragment of the cracked wall 2 is arrowed, the stretched membranous wall 3 and the penultimate coriaceous wall slightly separated from the innermost wall can be seen, PC, × 380. – Fig. 5. Another crushed spore showing its wall structure, PC, × 1200. – Fig. 6. The amyloid reaction of the wall 5 in Melzer's reagent is visible, LM, × 1200.