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Taxonomy of the endoconidial black yeast genera *Phaeotheca* and *Hyphospora*

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Abstract: The genus *Phaeotheca* contains melanized endoconidial fungi which may have unicellular, meristematic or hyphal thalli. The morphologically similar genus *Hyphospora*, anamorph of *Comminutispora*, is mainly distinguishable by cultural characteristics. The species accepted in the two genera are keyed out and their cultural, morphological and physiological characters are provided.

Key words: black yeasts, endoconidial fungi, taxonomy, *Dothideales*, *Phaeotheca, Hyphospora, Comminutispora.*

Introduction

The genus *Phaeotheca* was established by Sigler *et al.* (1981) to accommodate dematiaceous endoconidial fungi. In the type species, *P. fissurella* Sigler *et al.*, hyphae were nearly lacking, the entire thallus consisting of sporangia-like mother cells with one to several endoconidia. Ramaley (1996) described a fungus with endoconidial propagules in the new genus *Hyphospora*, the anamorph of *Comminutispora agavaciensis* Ramaley. *Phaeotheca* was not mentioned in Ramaley's (1996) paper. At first sight *Hyphospora* seems to differ significantly by being strongly hyphal and initially hyaline, becoming melanized only in a later stage of development. However, since hyphae can be present or absent in *Phaeotheca*, clear-cut morphological criteria for separation of the two genera are absent. On the basis of 5.8S rDNA sequence comparison, de Hoog *et al.* (1999) established that the genera are closely related, but at some distance away from each other within the ascomycete order *Dothideales*. *Hyphospora agavaciensis*, *Phaeotheca fissurella* and *P. triangularis* de Hoog & Beguin are reconsidered below in the light of these new data. Diagnostic keys to three accepted species are provided, using morphological and physiological properties.

Methods

Strains studied are preserved in the culture collections of CBS and MZKI (Microbiological Culture Collection of National Institute of Chemistry, Ljubljana, Slovenia). Microscopic morphology was studied on MEA, OA and PDA (malt extract, oatmeal and potato-dextrose agars); MEA was tested without and with 6% additional NaCl. Maximum diameters listed are those colony sizes that do not change any more, even after prolonged incubation. Physiological profiles were determined in liquid medium as described in detail by Untereiner *et al.* (1999). All tests were performed two times in duplicate.

Phaeotheca Sigler, Tsuneda & Carmichael

Phaeotheca Sigler, Tsuneda & Carmichael, Mycotaxon 12: 450. 1981.

Colonies restricted, initially pink to olivaceous brown, becoming olive-brown to black, slimy or dry, smooth or cerebriform. Hyphae absent or present. Unicellular thalli dark- and thick-walled, producing pale to dark brown endoconidia by irregular rupture of the mother cell wall. Hyphal thalli initially (sub)hyaline, soon becoming dark- and thick-walled, producing similar endoconidia.
Type species: *Phaeotheca fissurella* Sigler *et al.*

Discussion. – Sterflinger *et al.* (1999), using 18S rDNA sequencing, demonstrated that *Phaeotheca* and *Hyphospora* both belong to the order *Dothideales*. Rela-

tively close kinship was revealed with *Aureobasidium pullulans* (De Bary) G. Arnaud and *Hortaea werneckii* (Horta) Nishimura & Miyaji. The former species is a common inhabitant of slightly osmotic substrates such as phyllosphere, whereas the latter is halophilic (Zalar *et al.*, 1999). Mainly on the basis of the 5.8S rDNA gene, de Hoog *et al.* (1999) noted that *Phaeotheca* and *Hyphospora* are members of a clade containing several halophilic and epilithic taxa. It was therefore suggested that a predilection for substrates with limited water activity might be an evolutionary tendency in the genus *Phaeotheca*. However, *Phaeotheca fissurella* originates from living plants. Only *P. triangularis* de Hoog & Beguin was proven to be halophilic (Zalar *et al.*, 1999).

Another general ecological feature in the endoconidial *Dothideales* and their relatives are growth responses to different temperatures. *Hortaea werneckii* is unable to grow at temperatures above 35°C (de Hoog & Gerrits van den Ende, 1992), but nevertheless it is particularly abundant at relatively high temperatures in saltpans during the warmer season (Gunde-Cimerman *et al.*, 1999). In contrast, *P. fissurella* is psychrophilic, with an optimum at 18°C. It was isolated in northern Canada.

Phaeotheca is characterized by melanized, one-celled conidia formed endogenously from meristematically enlarging cellular clumps. *Hyphospora* differs morphologically only by initially having a pale pigmented thallus. *Botryomyces* (de Hoog & Rubio, 1982) produces similar clumps of cells but lacks endoconidia; it is a member of *Pleosporales* (Sterflinger *et al.*, 1999), close to *Alternaria* (de Hoog *et al.*, 1997).

In the present paper, two *Phaeotheca* and one *Hyphospora* species are accepted. DesRochers & Ouelette (1993) introduced a further *Phaeotheca* species, *P. dimorphospora*. No material was sent upon request. It was described as having dry, filamentous colonies exuding a yellow pigment into the agar.

Morphological key to the species

1a. Colonies on MEA initially pink, becoming black after three weeks; endoconidia hyaline, 1.5-3.8 × 1.5-3.0 µm ..... ***H. agavaciensis***
1b. Colonies on MEA immediately dark; endoconidia coloured, over 3.5 µm long and 3 µm wide → **2**
2a. Colonies on MEA wrinkled, cerebriform and dry; endoconidia dark brown, 7.0-9.5 × 4.0-5.5 µm ..... ***P. fissurella***
2b. Colonies on MEA flat, slimy and shiny; endoconidia olivaceous-green, 3.5-7.0 × 3.0-5.5 µm ..... ***P. triangularis***

Physiological key to the species

1a. L-Sorbose, L-rhamnose and methyl-α-D-glucoside assimilated ..... ***P. triangularis***
1b. L-Sorbose, L-rhamnose and methyl-α-D-glucoside not assimilated → **2**
2a. D-Glucosamine, ribitol and *meso*-erythritol assimilated ..... ***H. agavaciensis***
2b. D-Glucosamine, ribitol and *meso*-erythritol not assimilated ..... ***P. fissurella***

Phaeotheca fissurella Sigler, Tsuneda & Carmichael – Plate 1a-c, Fig. 1

Phaeotheca fissurella Sigler, Tsuneda & Carmichael, Mycotaxon 12: 450. 1981.

CULTURAL CHARACTERISTICS. – Colonies on MEA reddish-brown, becoming dark brown, initially slimy with yeast-like appearance, later becoming dry, granular and cerebriform, heaped; margin irregular. Daily growth rate 0.5 mm, maximum diam (2 months) 10 mm.

Colonies on OA black, granular and moist, later dry and cerebriform, slightly elevated above the substratum; margin irregular. Daily growth rate less than 0.5 mm, maximum diam (2 months) 5 mm.

Colonies on PDA brownish-black, initially with yeast-like appearance, becoming dry, cauliflower-like, about 3 mm elevated above the substratum, exuding an olive-brown pigment into the agar; margin irregular, sometimes with sectors of yeast-like appearance. Daily growth rate 0.5 mm, maximum diam (2 months) 11 mm.

MICROSCOPY. – Description based on cultures grown on MEA at 18°C.

After inoculation cells swelling, becoming spherical, up to 10 µm in diam, thick-walled, olivaceous black. After a few days some cells becoming divided by cross septa in several directions. Cell complexes falling apart into separate daughter cells, interpreted as endoconidia because they often are partly covered by mother cell wall remains; thickening of the cell wall being noted which results in one endoconidium after dissolution or rupturing of the cell wall. Endoconidia dark brown, thick-walled, frequently flattened at one side, 7–9.5 × 4–5.5 µm. Number of daughter cells per mother cell variable between one and three. Hyphae occasionally present, submerged, olivaceous black, thick- and rough-walled, 5-15 µm wide. Hyphae initially transversely septate, later septate in all directions, irregularly branched. Hyphae disintegrating after disruption of the cell wall, releasing daughter cells which contain one

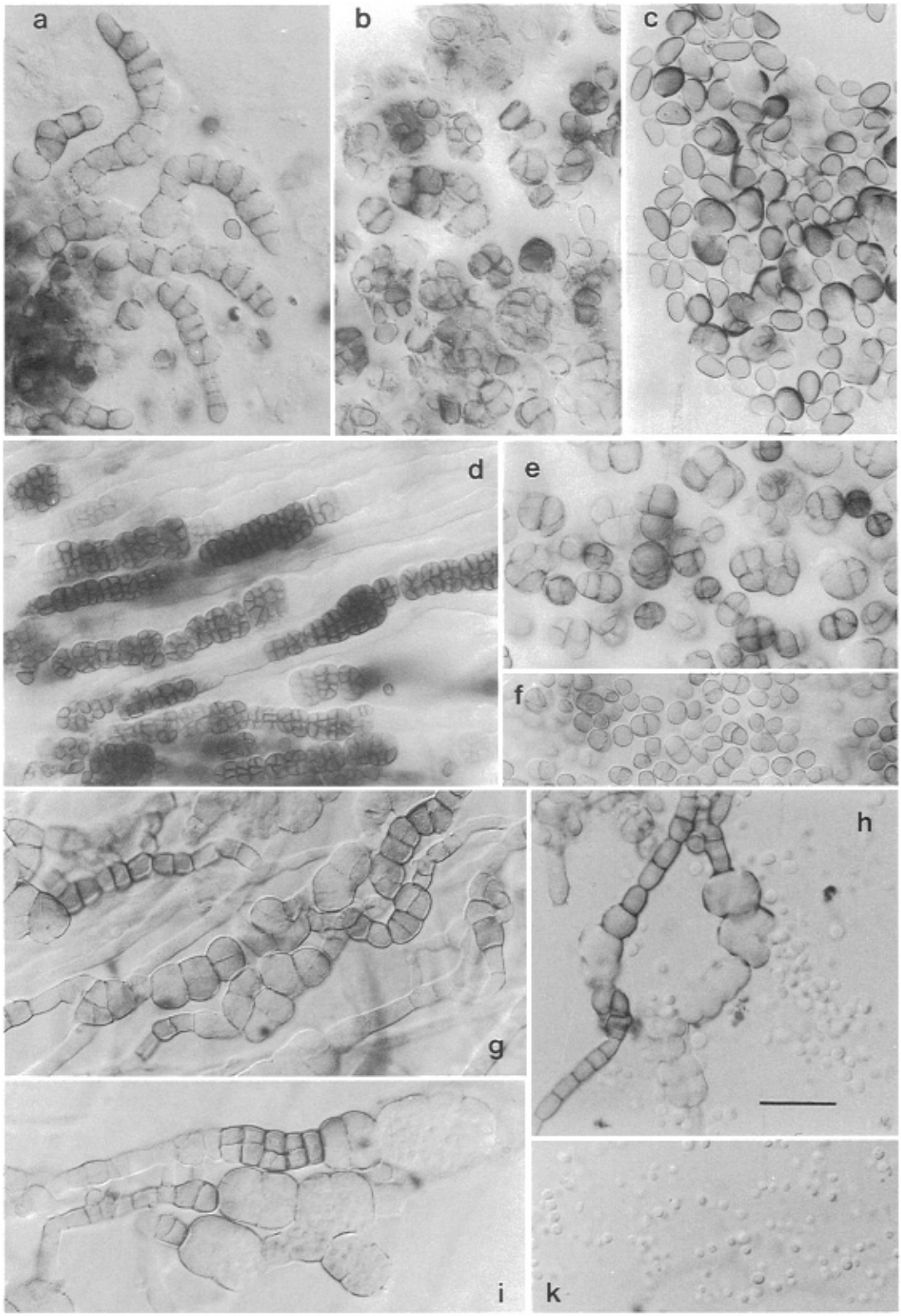

Plate 1. a–c. *Phaeotheca fissurella*, CBS 520.89. a. hyphae on OA growing in the medium, after 7 days of growth; b. cellular clumps containing and releasing endoconidia, on OA after 7 days of growth at 18°C. c. endoconidia on MEA after 7 days of growth. d–f. *Phaeotheca triangularis*. d. MZKI B-733; e-f. CBS 471.90. d. hyphae on MEA growing in the medium, after 14 days of growth; e. mother cells containing endoconidia on MEA, after 7 days of growth; f. endoconidia on MEA after 7 days of growth. g, h, i, k. *Hyphospora agavaciensis*, CBS 619.95. g, j. hyphae growing in the medium, on MEA, after 7 days of growth; h. hyphae releasing endoconidia by rupture of the cell wall, on MEA after 7 days of growth; k. endoconidia after liberation, on MEA after 7 days of growth. Scale bar indicates 20 μm for all figures.

to few endoconidia. Liberated cells may shed off their walls iteratively.

DEVIATIONS ON OTHER MEDIA. – OA: dark brown, irregular hyphae more abundant; mother cells clustered in irregularly-shaped clumps, constricted at the septa (Fig. 1b) up to 20 μm in length; number of endoconidia per mother cell is usually more than three.

PDA: thalli somewhat darker; clustered mother cells as on OA; endoconidia darker, with thicker walls, somewhat rough-walled.

PHYSIOLOGY. – Assimilative properties are listed in Table 1. Fermentation absent. Cardinal temperatures: slow growth at 7°C, optimal growth at 18°C, no growth at 25°C. The fungus is not able to grow on MEA with 6% additional salt.

MATERIAL EXAMINED. – CBS 520.89 (= UAMH 4285), type strain, isolated from canker of *Pinus contorta* caused by *Cronartium coleosporioides*, Saskatchewan, Alberta, Canada.

Discussion

The species differs from the remaining *Phaeotheca* species by larger endoconidia and near absence of hyphae. Details on the proces of endoconidiogenesis in *P. fissurella* were provided by Tsuneda & Murakami (1985). Physiologically *P. fissurella* is recognizable by assimilation of salicin, D-glucuronate, D-galacturonate, DL-lactate, and showing no growth with L-arabinose, D-arabinose and *meso*-erythritol. In addition, it is unable to grow in the presence of salt. The species is known only from a single strain. The species was isolated, together with *Hormonema dematioides* Lagerb. & Melin, from the surface of a fungal canker on *Pinus contorta* (Sigler *et al.*, 1985); it is difficult to speculate on its micro-niche.

Phaeotheca triangularis de Hoog & Beguin – Plate 1d-f, Fig. 2f-k

Phaeotheca triangularis de Hoog & Beguin, Antonie van Leeuwenhoek 71: 290. 1997.

CULTURAL CHARACTERISTICS. – Colonies on MEA brownish to jet-black, slimy, shiny, moist, sometimes cerebriform at the centre, elevated up to 3 mm above the substratum; margin sharp; sometimes hyphae growing into the medium are present. Daily growth rate 0.5 mm, maximum diam (2 months) 18 mm.

Colonies on OA olivaceous to jet-black, sometimes chocolate-brown, moist, glistening with oily shimmer, growing very superficially; margin sharp; usually marginal hyphae growing into the medium present. Daily growth rate 0.5 mm, maximum diam (2 months) 10 mm.

Colonies on PDA olive-brown, dark reddish-brown to brownish-black, shiny and slimy, glistening, sometimes cerebriform, elevated up to 3 mm at the centre; margin sharp or irregular where colonies are cerebriform. Daily growth rate 0.5 mm, maximum diam (2 months) 15 mm.

MICROSCOPY. – Description based on cultures grown on MEA at 22°C.

Cells subhyaline, swelling after inoculation, becoming dumbbell-shaped, soon developing transverse septation, finally septate in all directions, enlarging to cauliflower-like clumps about 20 μm in diam, sometimes reaching up to 150 μm. Some cells becoming olivaceous brown, rupturing and liberating endoconidia.

Hyphae sometimes present at the edge of the colony, at first hyaline, 3 μm wide at the tip, soon becoming transversly septate, 6 μm wide, widening up to 20 μm, with development and maturation of endoconidia, finally becoming dark brown and muriform. Hyphae containing numerous endoconidia which are released by rupture of the cell wall.

Endoconidia olivaceous green, smooth- and rather thick-walled, broadly ellipsoidal, 3.5-7.0 × 3.0-5.5 μm directly after liberation, often unilaterally flattened up to triangular, soon swelling to spherical before becoming septate. Number of endoconidia per mother cell ranging from one to five.

DEVIATIONS ON OTHER MEDIA. – OA: hyphae usually present at the edge of the colony; white aerial hyphae occasionally appear in a later stage of growth.

PDA: hyphae absent; multicellular cauliflower-like clumps of cells are numerous and may reach up to 500 μm.

PHYSIOLOGY. – Assimilative properties are listed in Table 1. Fermentation absent. Cardinal temperatures: slow growth at 6°C, optimal growth at 20°C, no growth at 30°C. The fungus reaches maximum diameter (30 mm) after 2 months on MEA with 6% additional salt.

MATERIAL EXAMINED. – CBS 471.90, ex-type strain, from humidifier of air-conditioning system, Brussels, Belgium; MZKI B-733, B-945, B-810, B-741 and B-946, all isolated from hypersaline water of saltern, Seca, Slovenia; MZKI B-994, isolated from hypersaline water of saltern, Salinas de la Trinitat, Spain.

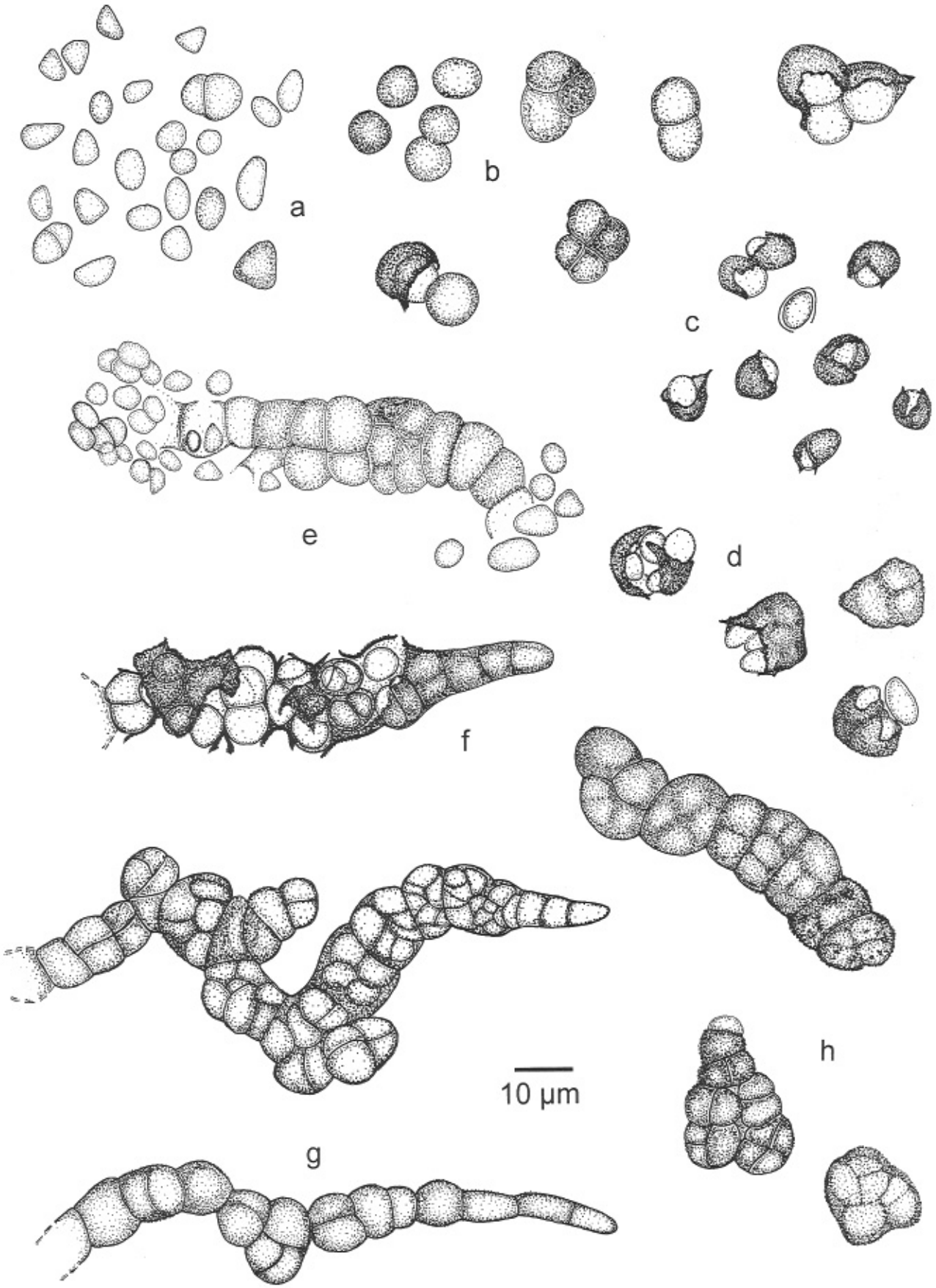

Fig. 1. *Phaeotheca fissurella*, CBS 520.89. a. Young endoconidia; b. swollen endoconidia; c, d. mother cells liberating endoconidia; e, f. hyphae releasing endoconidia; g, h. meristematic development.

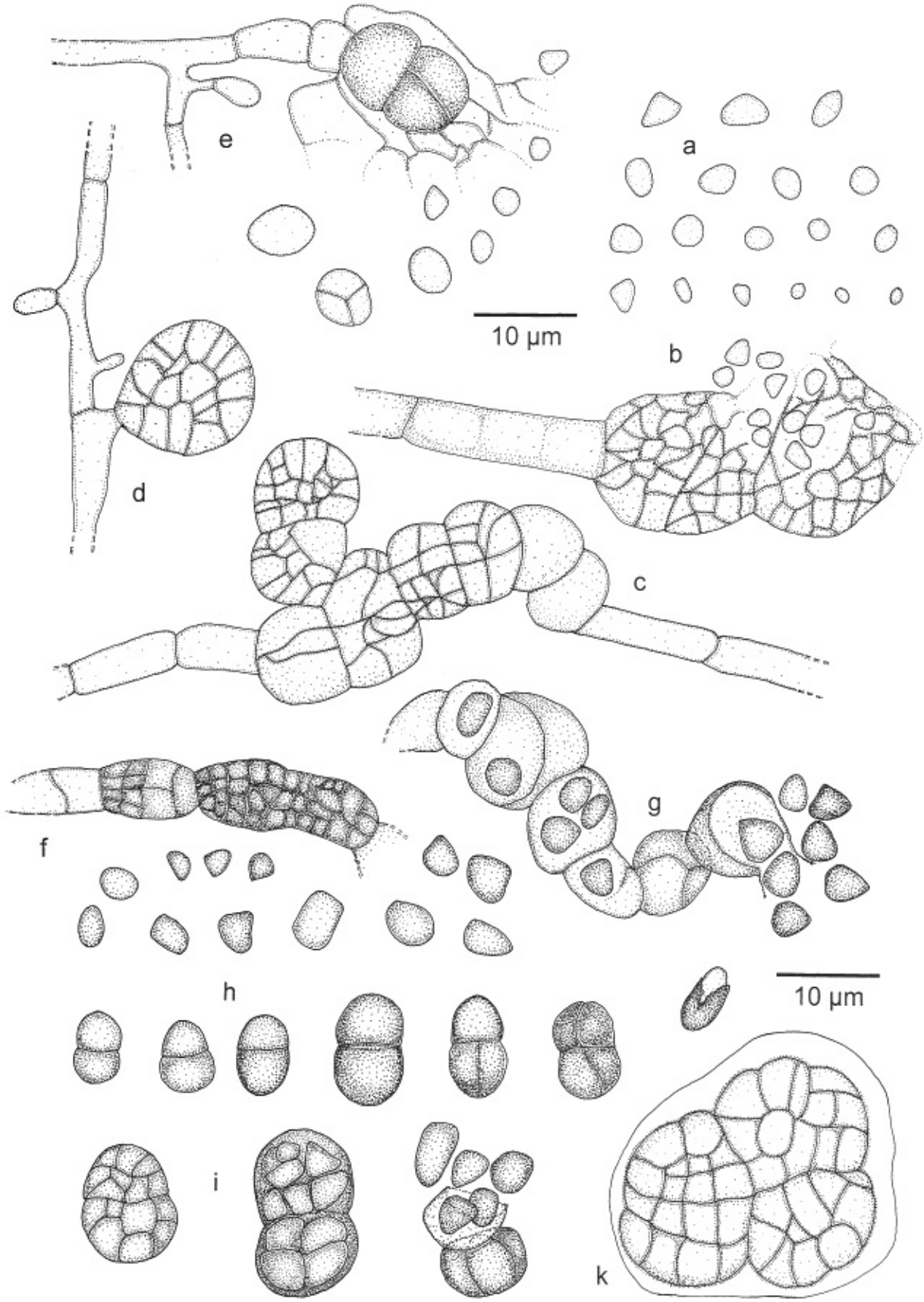

Fig. 2. a-e. *Hyphospora agavaciensis*, CBS 619.95. a. Endoconidia; b-d meristematic hyphae eventually liberating endoconidia; e. meristematic cells developing inside hyphal cell. f-k. *Phaeotheca triangularis*; f-h. MZKI B-810; i,k. CBS 471.90. f. meristematic hypha; g. hyphal cells liberating endoconidia; h. endoconidia; i, k. meristematic development of discrete cells.

Table 1. Physiological profile of described species of *Hyphospora agavaciensis* (CBS 619.95), *Phaeotheca fissurella* (CBS 520.89) and *P. triangularis* (CBS 471.90).

	619.95	520.89	471.90		619.95	520.89	471.90
D-Glucose	+	+	+	D-Mannitol	+	+	+
D-Galactose	+	+	w	Galactitol	–	–	–
L-Sorbose	–	–	+	*myo*-Inositol	–	–	–
D-Glucosamine	+	–	–	Glucono-δ-lactone	–	–	–
D-Ribose	–	w	w	D-Gluconate	w	–	+
D-Xylose	+	+	+	D-Glucuronate	–	+	–
D-Arabinose	+	–	w	D-Galacturonate	–	+	–
D-Arabinose	+	–	w	DL-Lactate	–	+	w
L-Rhamnose	–	–	+	Succinate	–	–	w
Sucrose	–	+	+	Citrate	–	–	–
Maltose	+	+	+	Methanol	–	–	–
α,α-Trehalose	+	+	+	Ethanol	–	–	w
methyl-α-D-Glucoside	–	–	+	Nitrate	+	+	+
Cellobiose	+	+	+	Nitrite	w	–	+
Salicin	–	+	–	Ethylamine	w	+	–
Arbutin	+	–	–/+	L-Lysine	w	+	–
Melibiose	+	+	+	Cadaverine	+	+	w
Lactose	+	+	+	Creatine	w	w	–
Raffinose	+	+	w	Creatinine	w	w	–
Melezitose	+	+	–	5% NaCl	+	–	+
Inulin	–	–	–	10% NaCl	–	–	+
Soluble starch	+	+	+	20% NaCl	–	–	w
Glycerol	+	+	+	30% NaCl	–	–	–
meso-Erythritol	+	–	+	10% $MgCl_2$	+	+	+
Ribitol	+	–	–	0.01% Cycloheximide	–	–	–
Xylitol	w	–	–	Urease	+	+	+
L-Arabinitol	w	–	–	Fermentation	–	–	–
D-Glucitol	w	–	+				

Discussion. – The species is characterized by melanized endoconidia which are often unilaterally flattened. The type strain, CBS 471.90, was consistently different from the remaining strains by lacking hyphae. Its restricted, granular colonies consisted exclusively of discrete cell clumps on all media used, whereas the strains from salterns frequently were hyphal. Nevertheless, the ITS1-2 rDNA domain of all strains proved to be strictly identical (Zalar *et al.*, 1999). *P. triangularis* can be distinguished from *P. fissurella* and *Hyphospora agavaciensis* by assimilation of L-sorbose, L-rhamnose, methyl-α-D-glucoside, D-glucitol, ethanol, nitrite, and by absence of growth with melezitose (de Hoog *et al.*, 1997). It is markedly halophilic, being able to grow and tolerate up to 20% of NaCl; the species shows better growth with 5% additional salt (Zalar *et al.*, 1999). This explains its occurrence in regularly desiccating air- moisturers (de Hoog *et al.*, 1997) as well as in salt pans (Zalar *et al.*, 1999).

Hyphospora Ramaley

Hyphospora Ramaley, Mycologia 88: 133. 1996.

Colonies restricted, initially pale pigmented, becoming olive-brown to black, slimy or dry, smooth or cerebriform. Hyphae absent or present. Meristematic thalli dark- and thick-walled, producing pale to dark brown endoconidia by irregular rupture of the mother cell wall. Hyphal thalli initially (sub)hyaline, soon becoming dark- and thick-walled, producing similar endoconidia.
Type species: *Hyphospora agavaciensis* Ramaley.

Hyphospora agavaciensis Ramaley – Plate 1g-k, Fig. 2a-e

Hyphospora agavaciensis Ramaley, Mycologia 88: 133. 1996.

Teleomorph: *Comminutispora agavaciensis* Ramaley, Mycologia 88: 132. 1996.

Cultural characteristics. – Colonies on MEA pink, gradually becoming dark brown, moist and slimy, growing superficially, central part 2 mm elevated above the medium; reverse deep pink, becoming brown; margin sharp, marginal hyphae growing into the medium present. Daily growth rate 1.5 mm, maximum diam (2 months) 30 mm.

Colonies on OA yellow-orange, becoming orange-green, later dark greenish-brown, then producing concentric zones; colonies flat; reverse greenish-yellow, becoming greenish-brown; margin sharp. Daily growth rate 1 mm, maximum diam (2 months) 45 mm.

Colonies on PDA pale red, becoming dark brown to olive-black, with moist and wrinkled appearance; colony centre elevated; reverse reddish, becoming brown; margin regular, consisting of marginal hyphae growing into the medium. Daily growth rate 3 mm, maximum diam (2 months) 30 mm.

MICROSCOPY.– Description based on cultures grown on MEA at 25°C.

Hyphae hyaline, later becoming brown, thick- and smooth-walled, 2-4 µm wide. Hyphae septate, irregularly branched, with intercalary or terminal swellings which develop transverse and longitudinal septation, multicellular clumps being separated from each other by clear isthmi. Cellular clumps measuring 20-80 × 10-30 µm, releasing numerous endoconidia after rupture of the cell wall. Endoconidia hyaline, unicellular, smooth- and thin-walled, variable in shape, mostly 1.5-3.8 × 1.5-3.0 µm directly after liberation, swelling to spherical before becoming septate. Occasionally hyphal cells develop inside mature hyphae.

DEVIATIONS ON OTHER MEDIA. – OA: laterally formed conidiogenous initials may be present.

PDA: hyphae more pigmented near conidiogenous cells, and are slightly rough-walled.

PHYSIOLOGY. – Physiological properties are listed in Table 1. Fermentation absent. Cardinal temperatures: slow growth at 6°C, optimal growth at 30°C, no growth at 35°C. The fungus forms restricted colonies (diam 4 mm) on MEA with 6% additional salt.

MATERIAL EXAMINED. – CBS 619.95, type strain, isolated from dead leaves of *Dasylirion leiophyllum*, Texas, U.S.A.

DISCUSSION. – The species produces small endoconidia from meristematically enlarging hyphal cells which may remain pale for several weeks. The change of colour and texture of colonies is strongly reminiscent of *Aureobasidium* and *Hormonema*, which are also anamorph members of *Dothideales*. The species is further distinguishable from *P. fissurella* and *P. triangularis* by assimilation of D-glucosamine, arbutin and ribitol, by no growth with sucrose, and by the ability to grow with up to 5% of NaCl added.

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