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Phaeotheca triangularis, a new meristematic black yeast from a humidifier

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Abstract

Two strains of a new species of meristematic fungi, described as *Phaeotheca triangularis,* were isolated from moisteners of air-conditioning systems. The species is believed to be related to dothideaceous black yeasts. Its morphology, ultrastructure and nutritional physiology are reported.

Introduction

In the course of a study of fungi occurring in humidifiers of air-conditioning systems by one of the authors (H.B.), a meristematic black yeast was encountered which produced triangular endoconidia. It could not be identified as any known species.

Black yeast-like fungi commonly inhabit water which is poor in nutrients. Members of the genus *Exophiala* Carmichael have been found in humidifiers (Nolard et al. 1986; Nishimura & Miyaji 1982) and occur relatively often, though in low abundance, in bathing facilities (Nishimura et al. 1987). Human infection, particularly in patients with cystic fibrosis, may take place through inhalation of moist aerosols packed with fungal propagules (Haase et al. 1994). Black yeasts classified in *Aureobasidium* Viala & Boyer are also regularly found on damp walls (Adan 1994). The two genera are known to be unrelated, judging from the taxonomic position of Ascomycete teleomorphs: *Exophiala* is linked to the family Herpotrichiellaceae (Untereiner 1994), and *Aureobasidium* to the Dothideaceae (De Hoog & Yurlova 1994). On the basis of ribosomal DNA phylogeny, the two families are found at a large distance from one another and have recently been classified in two separate orders, viz. the Chaetothyriales and Dothideales, respectively (Spatafora et al. 1995; Haase et al. 1995).

The taxonomy and identification of meristematic black yeasts is problematic due to their pleoanamorphism and poor differentiation. Meristematic growth forms have been reported from both the Herpotrichiellaceae and the Dothideaceae. Conversion towards isodiametric expansion can be induced in hyphal or yeast-like thalli of Herpotrichiellaceae by environmental factors such as acidification of the culture medium (Mendoza et al. 1993); similar observations have been made in Dothideaceae (De Hoog et al. 1997). In some cases these may be stable mutants (Matsumoto et al. 1986). Thus, black meristematic fungi may belong to both groups of black yeasts, but their poor differentiation does not allow assignment to either family on the basis of morphology alone. De Hoog et al. (1997) introduced additional molecular and physiological methods for the classification of little-differentiated black fungi, and found that one of the key characters for distinguishing herpotrichiellaceous from dothideaceous black yeasts is the production of extracellular DNAse.

The present fungus has been compared to type strains of meristematic black yeasts using morphological and physiological criteria, supplemented with data on PCR-RFLP of amplified parts of the ribosomal repeat reported by Wollenzien et al. (1997). It was found to be a new species. Morphologically the species fits the genus *Phaeotheca* Sigler et al. (1981). It is described below as *Phaeotheca triangularis.*

Materials and methods

Isolation, morphology, cultural characteristics and identification. Strains were swab-isolated from the walls of two humidifiers in an office building. Swabs were inoculated onto 2% malt extract agar (MEA) with 0.05% chloramphenicol and incubated at 24 °C. Subcultures were grown on potato carrot agar (PCA), 4% MEA and oatmeal agar (OA) at room temperature. Slides were made in water, in mucicarmine and Shaffers jet-black ink for the visualization of capsules and in Melzer's reagent for the detection of starch-like compounds. Identification of strains was also done by comparison of RFLP patterns of ribosomal amplicons with morphologically similar strains; these results are reported by Wollenzien et al. (1997).

Physiology. Nutritional physiology and tolerance tests were based on the methods described by Van der Walt & Yarrow (1984) and adapted for black yeasts by de Hoog et al. (1995) and Wollenzien et al. (1997).

Ultrastructure. Cells were fixed with aqueous $KMnO_4$ for 30 min at room temperature. The pellets, centrifuged in Beem capsules, were dehydrated in an ethanol series, stained with 1.5% uranyl acetate for 2 h at the 50% ethanol step and finally embedded in Spurr's resin. Ultrathin sections were post-stained with Reynold's lead citrate for 10 sec and examined with a Philips EM 201 electron microscope at 60 kV.

Results and discussion

Phaeotheca triangularis *de Hoog & Beguin, sp. nov. – Figures 1–7*

Coloniae in agaro farina avenae confecto olivaceae ad atrae, punctiformes, madidae, lucidae, post 5 dies minus quam 1 mm expandentes. In agaro maltoso (4%) coloniae atrae, mucidae, minute lobatae, in medio pulvinatae; deinde exsiccantes; margo acutus, lobatus; ad 7 mm diam. post 10 dies, post 60 dies non magis quam 10 mm (in agaro farinae) vel 20 mm (in agaro maltoso) diametro attingentes. Cellulae germinantes inflatae, halteriformes, mox septatae, primum transverse deinde undique divisae, acervos hyalinos, multicellulares, verrucosos, 40–70 μm diam formantes, deinde corpora conspicue lobata et in granula minora ca 20 μm diam dilabentia. Acervi in maculis olivaceo-brunneis maturantes, rupta endoconidia liberantia. Conidia levia et fere crassitunicati, olivaceo-viridia, late ellipsoidea, primum 5.5–7.0 × 4.5–5.5 μm, saepe inaequilateraliter applanata vel triangularia, deinde ad formam globosam inflata et septata.

Holotypus CBS 471.90, exsiccatus et vivus, isolatus a H. Beguin ex apparatu humidificationis Bruxelliae in Belgio, Mart. 1990.

Cultures on OA olivaceous to jet-black, punctiform, moist, glistening with oily shimmer, lying on the surface of the substrate, with less than 1 mm expansion growth in 5 d; on 4% MEA jet-black, slimy, finely lobed, sometimes becoming cushion-shaped at the centre, later dull, dry; margin sharp; attaining 7 mm diam in 10 d; maximum diam after 2 month incubation 10 (OA) - 20 (4% MEA) mm. Cells swelling after inoculation, becoming dumbbell-shaped, soon becoming septate, first transversely and soon afterwards in all directions, enlarging to hyaline, multicellular, cauliflower-like clumps, average diam 40–70 μm on 4% MEA, becoming strongly lobed and repeatedly disarticulating into smaller clumps about 20 μm in diam. Cellular clumps maturate by some cells becoming olivaceous-brown, the cells soon rupturing and liberating endoconidia. Conidia smooth- and rather thick-walled, olivaceous green, broadly ellipsoidal, 5.5–7.0 × 4.5–5.5 μm directly after liberation, often unilaterally flattened up to triangular, soon swelling to spherical before becoming septate. Physiological properties are listed in Table 1.

Holotype: CBS 471.90, dried culture preserved at Centraalbureau voor Schimmelcultures, Baarn, The Netherlands; type culture CBS 471.90, isolated by H. Beguin from humidifier of air-conditioning system, Brussels, Belgium, March 1990.

Liberated conidia of *Phaeotheca triangularis* swell and develop a median septum, having a *Schizosaccharomyces*-like appearance. Further swelling and the development of oblique septa is noted until a maximum diam of 40–70 μm is attained. The resulting multicellular clumps are first hyaline and have septa in all directions. Subsequently, dark brown, unilaterally flattened or triangular endoconidia become visible which are liberated by deterioration of the mother cell wall. This justifies classification of the species in *Phaeotheca* Sigler et al. (1981). The meristematic clumps in *Phaeotheca fissurella* Sigler et al. are smaller and liberate spherical endoconidia (de Hoog & Rubio 1982).

The hyaline meristematic cell clumps are similar to those of *Botryomyces* de Hoog & Rubio (1982), but that species does not produce endoconidia. Unlike *Sarcino-*

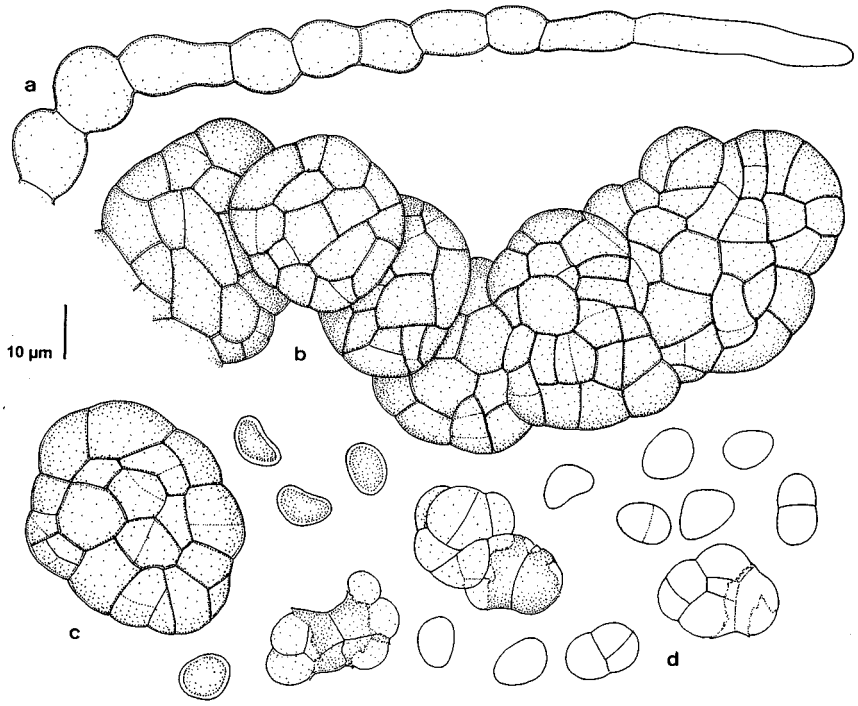


Figure 1. *Sarcinomyces triangularis*, CBS 471.90. a. Young hyphal element, 10-day-old culture on OA; b. Hypha with meristematic conversion, 10-day-old culture on OA; c. Liberated meristematic clump of cells, 10-day-old culture on MEA; d. Endoconidia in various stages of meristematic conversion; 10-day-old culture on MEA.

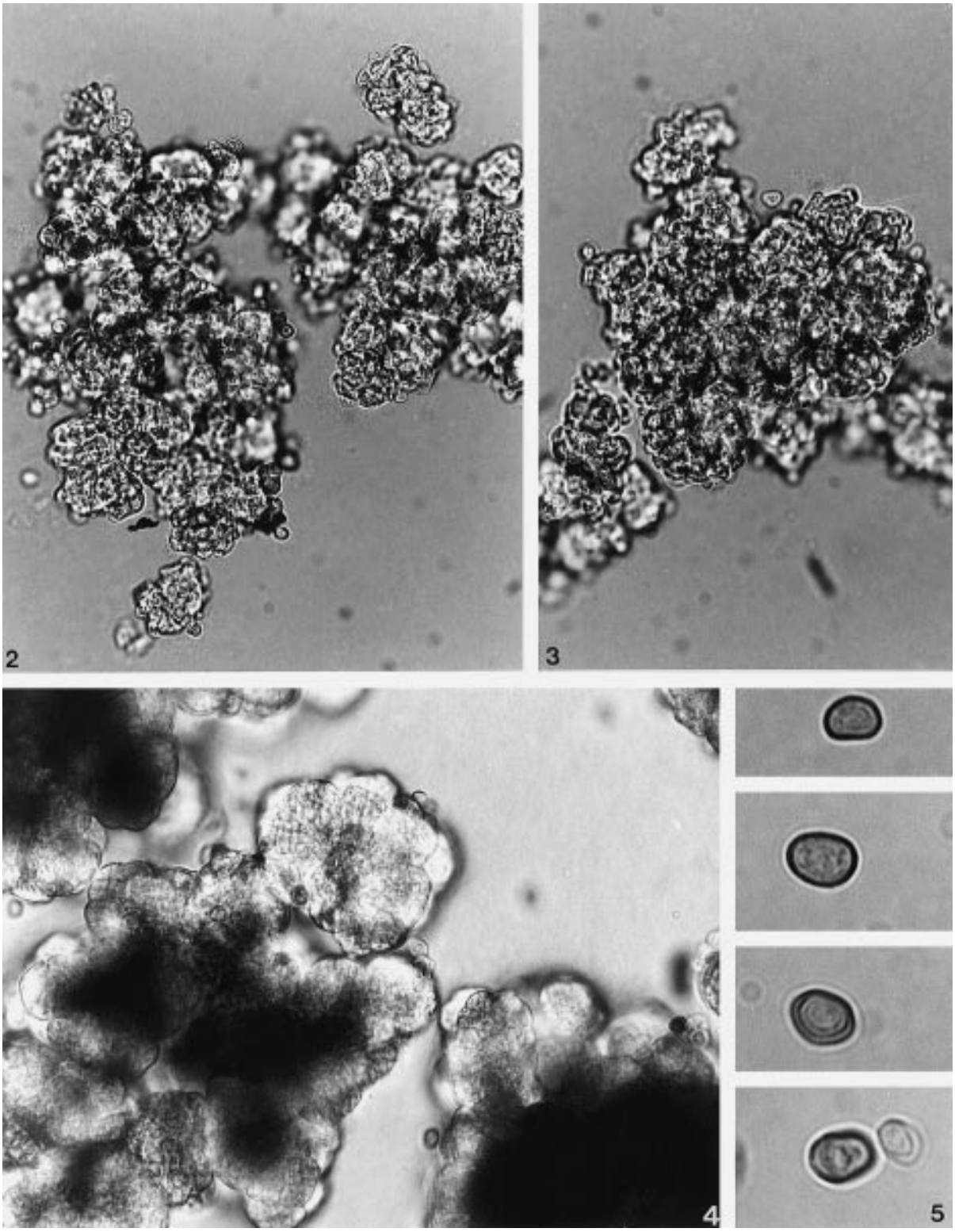
myces crustaceus Lindner (Sigler et al. 1981), *P. triangularis* does not produce blastic conidia. *Phaeosclera dematioides* Sigler et al. (1981) is strictly hyphal with intercalary cell clumps and without conidia.

Physiologically (Table 1; compare also De Hoog et al. 1997) the species differs from *Phaeotheca fissurella* by assimilation of L-rhamnose, *meso*-erythritol, ethanol and nitrite. *Botryomyces caespitosus* assimilates *myo*-inositol and citrate but not D-gluconate and DL-lactate. *Sarcinomyces crustaceus* assimilates glucono-δ-lactone and D-glucuronate. *Phaeosclera dematioides* does not assimilate melibiose and D-gluconate.

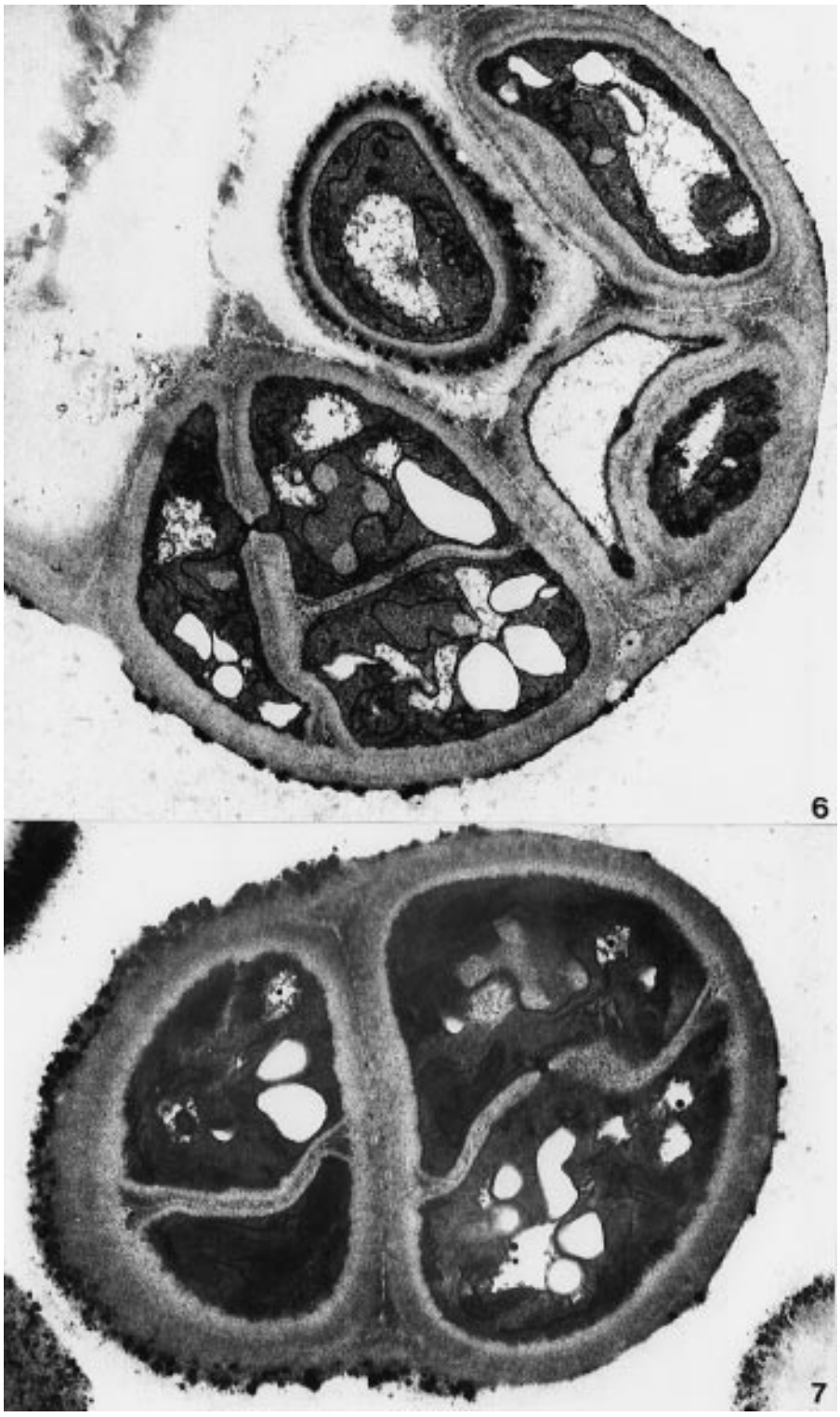
Patterns of PCR-RFLP of SSU amplicons of *P. triangularis* deviated from all those derived from comparable type strains having NS1-NS24 amplicons about 1800 bp in length (Wollenzien et al. 1997).

De Hoog et al. (1997) made a distinction between meristematic and other black yeast species of herpotrichiellaceous and of dothideaceous relationship. In the absence of teleomorphs, this was based on thallus karyology and maturation, coenzyme Q systems, ultrastructure, PCR-ribotyping patterns and physiology. The production of extracellular DNAse proved to be a key character for the recognition of the two groups, having nearly 100% predictive value. The present species is DNAse positive, suggesting a dothideaceous relationship. This is matched with ribotyping patterns (Wollenzien et al. 1997). In addition, the absence of Woronin bodies at the septa of the present species (Figures 8, 9) is consistent with its classification in the Dothideaceae.

The species was encountered on the wetted sides of two humidifier tanks in the same building. This suggests a moist environment poor in nutrients as its natural ecological niche. The source of isolation and the easy growth on nutritionally poor agar media allow the supposition that the species is oligotrophic. De Hoog et al. (1997) suggested a wide occurrence of oligotrophism in meristematic black yeasts on the basis of observed growth in media without additional nutrients. In negative controls cellular increase is maintained for long periods at nearly the same level as observed in glucose, differences becoming only explicit after over 30 days of incubation. This property might explain the relative abundance of black yeasts in environments



Figures 2–5. *Sarcinomyces triangularis*, CBS 471.90. (2, 3) Young meristematic clumps of cells, x 500; (4) Maturating meristematic clumps of cells, x 650; (5) Endoconidia in various stages of swelling, x 2400.



Figures 6, 7. *Sarcinomyces triangularis*, CBS 469.90. Meristematic clumps of cells, the upper one liberating an endoconidium, x 24.000.

Table 1. Physiological profile of *Phaeotheca triangularis*

	469.90	471.90		469.90	471.70
D-Glucose	+	+	keto-5-Gluconate	+	+
D-Galactose	+	+	D-Gluconate	+	+
L-Sorbose	+	+	D-Glucuronate	-	-
D-Glucosamine	-	-	D-Galacturonate	-	-
D-Ribose	+	+	DL-Lactate	+	+
D-Xylose	+	+	Succinate	+	+
L-Arabinose	+	+	Citrate	-	-
D-Arabinose	+	+	Methanol	-	-
L-Rhamnose	+	+	Ethanol	+	+
Sucrose	+	+	Nitrate	+	+
Maltose	+	+	Nitrate	+	+
α, α-Trehalose	+	+	Ethylamine	w	+
methyl-α-D-Glucoside	+	+	L-Lysine	-	w
Cellobiose	+	+	Cadaverine	+	+
Salicin	-	w	Creatine	w	w
Arbutin	-	-	Creatinine	w	w
Melibiose	+	+	5% $MgCl_2$	+	+
Lactose	+	+	10% $MgCl_2$	+	+
Raffinose	+	+	5% NaC1	+	+
Melezitose	+	+	10% NaC1	+	+
Inulin	w	w	0.01% Cycloheximide	-	-
Sol. starch	-	w	0.1% Cycloheximide	-	-
Glycerol	+	+	Mycosel	-	-
meso-Erythritol	+	+	Urease	-	-
Ribitol	+	+	Melzer	+	+
Xylitol	+	+	30C	-	-
L-Arabinitol	+	+	37C	-	-
D-Glucitol	+	+	40C	-	-
D-Mannitol	+	+	Fermentation	-	-
Galacitol	+	+	Gelatin		+
myo-Inositol	-	-	DNAse	+	+
Glucono-δ-lactone	-	-	Substrate	Water	Water

such as humidifiers and bathing facilities. The black yeast *Aureobasidium pullulans* (De Bary) Arnaud also invades moist surfaces (Adan 1995). It is common in environments with high water activity, such as lake water (Sláviková et al. 1992), but also with low water activity, for example in mattress dust (Beguin 1995). A similar species, *Hortaea werneckii* (Horta) Nishimura & Miyaji, shares this ecological spectrum in occurring on moist surfaces (Göttlich et al. 1995), in water (De Hoog & Gerrits van den Ende 1992), and in house dust (Miyaji et al. 1993). Common eco-physiological factors in these species are thus oligotrophism and tolerance of a wide range of osmotic pressures. De Hoog & Yurlova (1994) listed halotolerance as one of the predominant characters in dothideaceous black yeasts.

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