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Capnobotryella isiloglui, a new rock-inhabiting fungus from Austria

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Abstract: A new rock-inhabiting fungal taxon was isolated from St. Margarethen Church in Vienna, Austria. Based on small subunit and internal transcribed regions of rDNA sequencing, the new taxon was described as *Capnobotryella isiloglui* Sert & Sterflinger. The type culture of the taxa is in the ACBR culture collection (Vienna, Austria, MA 3619, EMBL Accession Number: AM746201.1).

Key words: New taxon, black fungi, *Capnobotryella*, rock fungi

Avusturya'dan yeni bir taş mantarı, *Capnobotryella isiloglui*

Özet: Viyana (Avusturya)'da bulunan St. Margarethen kilisesinden taşta yaşayan yeni bir fungal takson izole edildi. rDNA'nın küçük alt birimi ve ITS bölgelerinin dizi analizi yapılarak yeni takson *Capnobotryella isiloglui* Sert & Sterflinger olarak tanımlandı. *C. isiloglui*'nin tip örneği ACBR Kültür Koleksiyonu'nda (Viyana/Avusturya, MA 3619, EMBL No: AM746201.1) saklanmaktadır.

Anahtar sözcükler: Yeni takson, siyah mantar, *Capnobotryella*, taş mantarı

Introduction

Biodeterioration may be defined as any undesirable change in the properties of a material caused by the vital activities of living organisms. This definition distinguishes biodeterioration from fields of study such as corrosion and the wearing of materials that relate to undesirable changes in the properties of a material brought about by chemical, mechanical, and physical influences (Leznicka et al., 1988; Urzi & Krumbein, 1994; Kumar & Kumar, 1999; Varol et al., 2010; Işık, 2011).

The development of specific biological species on a particular stone surface is determined by the nature and properties of the stone, viz. the mineral constituents, pH, relative percentage of various minerals, salinity, moisture content, and texture (Sterflinger & Krumbein, 1997; Sterflinger et al., 1998; Sterflinger, 2000; Selbmann et al., 2004; Sert et al., 2007a). It also depends on certain environmental factors, viz. the temperature, relative humidity, light conditions, atmospheric pollution levels, wind, and rainfall. In other words, the response of living

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organisms to a potentially colonisable surface depends on the ecological and physiological requirements of the biological species involved (Caneva & Salvadori, 1988; Köstler & Vedral, 1991; Krumbein & Urzi, 1993; Gorbushina et al., 1994; Diakumaku et al., 1995).

During an investigation of the biodeterioration of historical monuments, we isolated numerous strains of fungi, the majority of which belonged to the black yeasts. Among these isolates, we found a dematiaceous hyphomycete in a Roman quarry. It formed brown to black, torulose hyphae on the natural substrate, and dark brown to black moriform colonies in culture. It is highly pleomorphic and hence it is difficult to classify in any hyphomycete genus. Because of its morphological and genetic features, the genus *Capnobotryella* Sugiyama was judged to be most suitable for its accommodation. It is described below as a new taxon.

Materials and methods

Isolation and morphological characterisation

The strains were isolated from the northern face of the Roman quarry of St. Margarethen Church (Vienna, Austria) in July 2007 and conserved in the Austrian Centre of Biological Resources and Applied Mycology (ACBR) culture collection for further investigation.

Rock samples were taken with a scalpel previously cleaned with 70% alcohol, transferred to sterile petri dishes, and stored at room temperature. The samples were collected during spring and early summer. The fungi were isolated from samples under a stereomicroscope by dissecting or lifting fungal colonies using needles (Sterflinger & Krumbein, 1997). The black colonies were transferred to petri dishes. Malt extract agar (2%; 20 g malt extract, 1.0 g peptone, 20 g glucose, 20 g agar in 1 L water), and dichloran rose bengal agar (Merck, Darmstadt, Germany) were used. The plates were incubated at room temperature. The isolates were purified in 2 or 3 steps by transfer to fresh medium [malt extract agar and Czapek agar (Merck, Darmstadt, Germany)]; purity was checked and maintained by repeated light microscopic observation. For morphological characterisation, mounts of the mycelium were prepared and observed using a light microscope.

DNA extraction, PCR, and sequencing reactions

DNA extraction, PCR reaction, and the sequencing of the 18S, ITS I, 5.8S, ITS II regions of the rDNA, including alignment and phylogenetic analysis were performed as described in Sert et al. (2007b). Sequences were deposited in the EMBL databank (Barber, 2011).

Results

Capnobotryella isiloglui sp. nova was isolated from the Roman quarry of St. Margarethen Church (Vienna, Austria). The taxonomic and phylogenetic position is clarified based on the SSU and ITS regions of rDNA sequencing data.

Capnobotryella isiloglui Sert & Sterflinger sp. nova

Coloniae in agaro maltoso 12.05 mm diam post 28 dies; atrae brunneae, hirsutae, aridae, durae, punctiformae, cerebriformae in temporis transitis. Hyphae dilutae-atrae atris brunneis, pectinatae. Conidia brunnea, parietes conidiae laeves et tenues, pyriforma vel reniforma, 6-11.5 × 3-5.5 µm.

Type: Austria, Vienna: St. Margarethen, Roman quarry, north face (MA 3619 holotype; GenBank no: AM746201.1).

Colonies on malt extract agar attaining about 12.05 mm dia. in 4 weeks; black to light-brown, hairy, dry, hard, punctiform, cerebriform with age; margin lobed. Colonies on Czapek agar attaining up to 5.5 mm dia. in 4 weeks; black to brown, hairy, dry, initially soft, later hard; margin lobed. Hyphae pale-dark brown, branched, consisting subspherical and cylindrical cells with thick wall, cells about 5-13 × 6-11 µm and developing 2 or more longitudinal and oblique septa. Cracks or fissures occurred in the outer cell wall of these cells. Conidia scarce in culture, pale to dark brown, smooth, thick-walled, pyriform or reniform, 6-11.5 × 3-5.5 µm, mostly with a median septum (Figure 1).

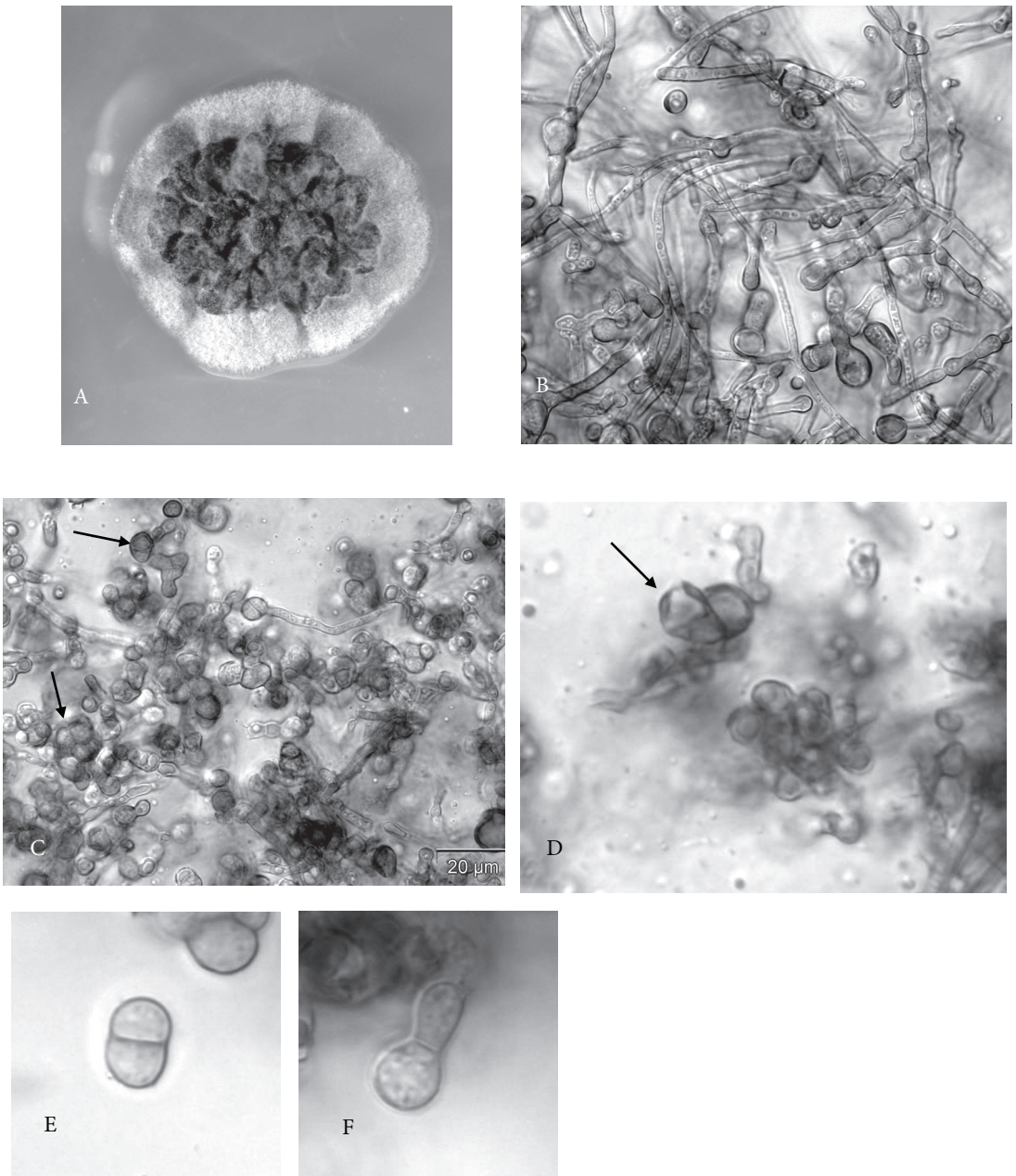

Figure 1. **A** - Fungal growth after 1 month incubation on MEA (×6); **B** - Hyphae on MEA after 1 month incubation (×1200); **C** - Enlarged cells with longitudinal and oblique septa (×1200); **D** - Enlarged cells with cracks or splits (×3000); **E, F** - Mature conidia (×3000).

Etymology: This taxa is named in honour of Prof. Mustafa IŞILOĞLU (Muğla University, Mushroom Research Centre, Turkey) who published many papers on Turkish macrofungi.

Discussion

The genus *Capnobotryella* Sugiyama & Amano contains 2 species, *C. renispora* Sugiyama & Amano and *C. antalyensis* Sert & Sterflinger. Sugiyama and Amano (1987) found *C. renispora* in association with *Capnobotrys neesii* S.Hughes on *Abies veitchii* Veitch Fir branches in Japan. Titze and de Hoog (1990) found it in association with the lichen *Lecidea fuscoatra* (L.) Ach., the non-lichenised ascomycete *Lichenothelia convexa* Hennsen (Dothideales), and a green alga of the order Chlorococcales. Sert and Sterflinger found *C. antalyensis* on marble monument surfaces in Turkey, but it was not associated with lichens or algae (Sert et al., 2007b).

The taxonomic and phylogenetic position of the third species of *Capnobotryella* genus, *C. isiloglui*, is clarified based on morphological characters and the SSU and ITS regions of rDNA sequencing data.

Conidia of *C. renispora* (CBS 572.89) are reniform, 5.0-6.5 × 3.4 µm, and have a median septum (Titze & de Hoog, 1990). Morphological characters of this strain agreed well with the original description (Sugiyama & Amano, 1987). In CBS 214.90 and 215.90, conidia are absent or scarce in culture. When present, conidia are 4.5-6.5(8) × 3-4.5 µm in CBS 214.90, and 5-5.5 × 4-5 µm in CBS 215.90 (Hambleton et al., 2003). Conidia of *C. antalyensis* is 8-15 × 4-9 µm (Sert et al, 2007b), they are reniform and pyriform. *C. isiloglui* has reniform and pyriform conidia too; however, they are 6-11.5 × 3-5.5 µm in size. Colonies of *C. antalyensis* have attained about 0.7 mm dia. in 4 weeks on malt extract agar, and 2.5 mm dia. on Czapek agar. Colonies of *C. isiloglui*, on the other hand, attained about 12.05 mm dia. in 4 weeks on malt extract agar and 5.5 mm dia. on Czapek agar. Based on morphological characters, *C. isiloglui* is similar to *C. antalyensis* although it differs in terms of conidia and colony sizes.

A neighbour-joining tree based on 34 near-complete SSU rDNA sequences and, as an outgroup, *Saccharomyces cerevisiae* Meyen ex E.C Hannsen is presented in Figure 2. Figure 3 shows a Dothideales-biased neighbour-joining tree based on ITS I-II/5.8 S rDNA sequences. *Exophiala dermatidis* (Kano) de Hoog was chosen as an outgroup. According to the phylogenetic tree based on 5.8/ITS I-II sequencing data, *C. isiloglui* was found well apart from the other *Capnobotryella* species sequenced thus far. It clustered in a group with *Ascomycete* sp. IZ-1106 (EMBL Accession-number: AM921711.1), uncultured *Capnobotryella* clone IVP1-34 (EMBL: EU516821.1), uncultured *Capnobotryella* clone IVP5-27 (EMBL: EU516855.1), Fungal sp. V-F6 (EMBL: DQ068356.1), *Coniosporium* sp. MA 4597 (EMBL: AJ972792.1), uncultured soil fungus clone 137-62 (EMBL: DQ421272.1), *Capnobotryella* sp. MA 4701 (EMBL: AJ972856.1), and melanised limestone ascomycete CR-2004 strain TRN136 (EMBL: AY559368.1) as relatively close neighbours. However, based on SSU phylogeny data, the new species was clustered in a group with *Capnobotryella* sp. MA 4701 (EMBL: AJ972856.1) and *Capnobotryella* sp. NH4-3 (EMBL: AJ301706.1).

The rDNA ITS sequences did not show identity to any spacer deposited in GenBank. The length of these regions was 524 bp. Based on ITS I-II/5.8 S sequencing the strain MA 3619 (holotype) (EMBL Accession Number: AM746201.1) clustered with an unidentified Ascomycete strain (Strain IZ-1106, EMBL: AM921711) (Cooper P, 2011). Identities were 451/466 (96%).

The length of 18S regions of rDNA of *C. isiloglui* was 1774 bp. Results of 18S sequences show 98% (1748/1768) similarity with *Capnobotryella* sp. strain MA 4701 (EMBL: AJ972856.1) and 98% similarity (1744/1776) with *Capnobotryella* sp. NH4-3 (EMBL: AJ301706.1). The 18S rDNA sequences of *C. isiloglui* and *C. antalyensis* were found to differ in 10 positions, which correspond to an 18S rDNA similarity of 99% while 97% similarity (1701/1751) was observed with *Capnobotryella renispora* strains (CBS 572.89-EMBL: AY220614; CBS 215.90-EMBL: AY220613; CBS 214.90 EMBL: AY220612; UAMH 9870-EMBL: AY220611).

The phylogenetic trees show *C. isiloglui* is clearly distinct from the other *Capnobotryella* species (Figures 2 and 3). Judging from the SSU phylogeny data, *C. isiloglui* should belong to the Dothideales (Figure 2).

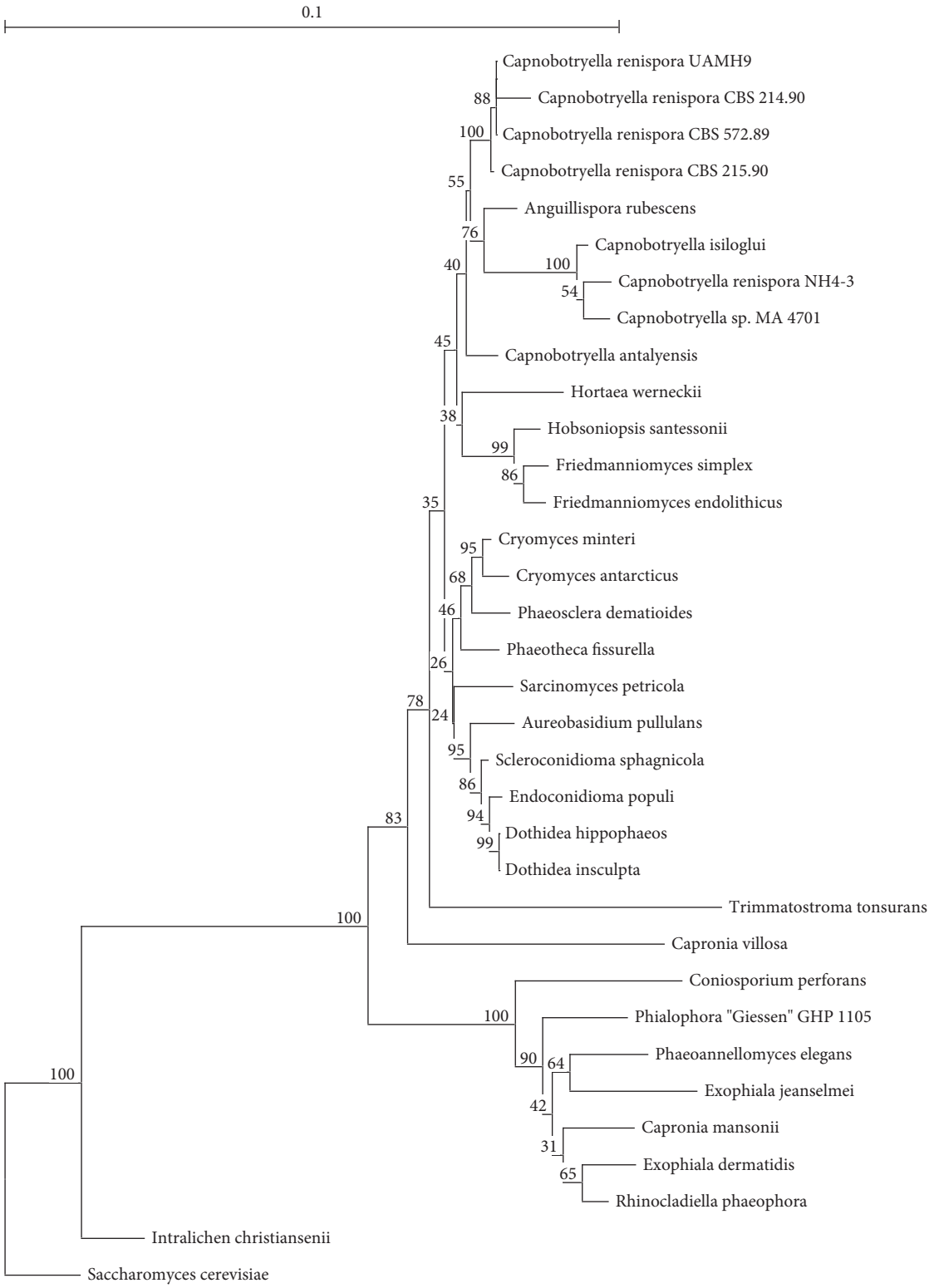

Figure 2. Dothideales-biased phylogenetic tree of 18S rDNA of 34 fungal species, *Saccharomyces cerevisiae* was used as an outgroup.

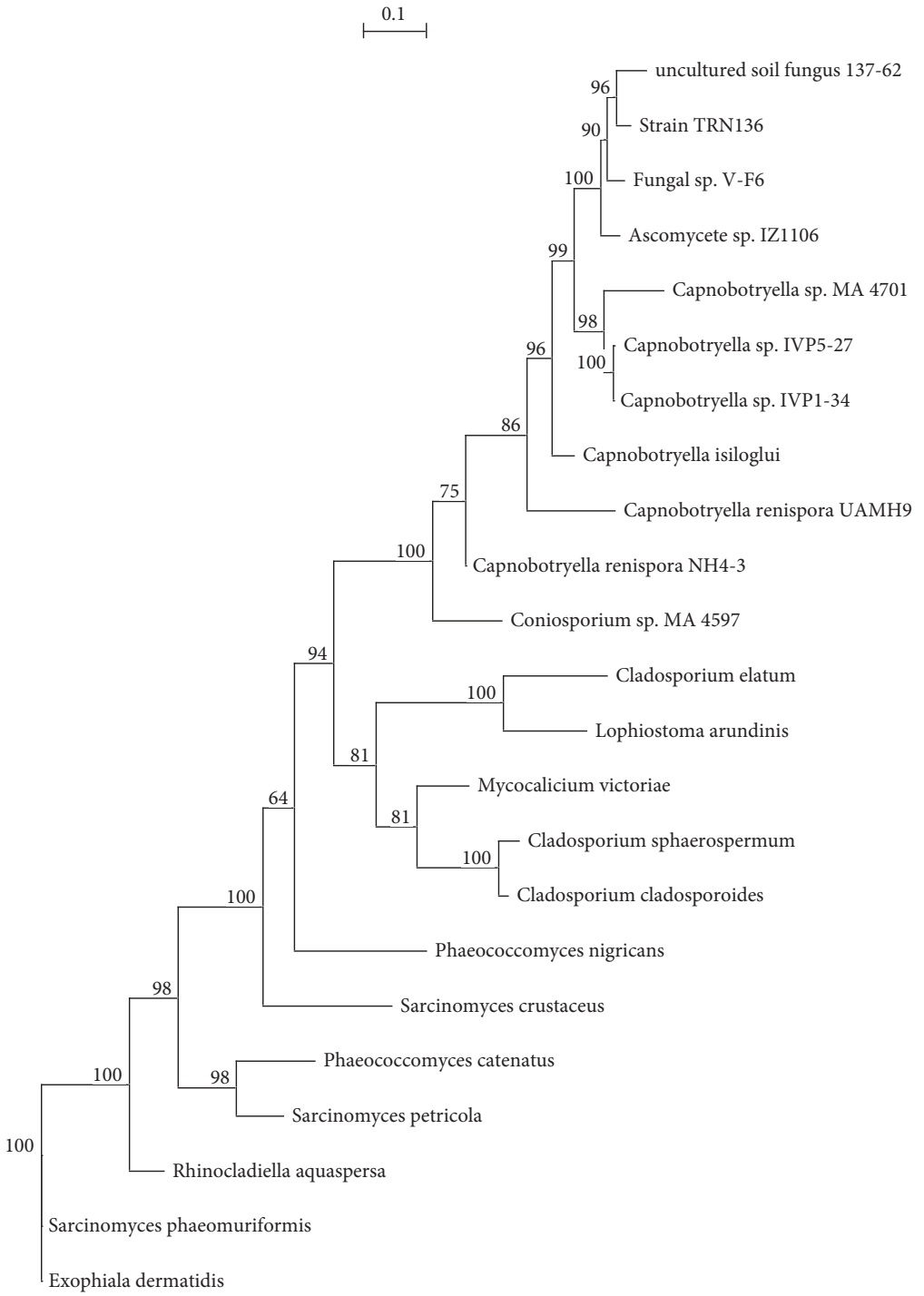

Figure 3. Consensus tree of 5.8S/ITS I and II rDNA sequencing, *Exophiala dermatidis* was used as an outgroup.

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