Homortomyces gen. nov., a new dothidealean pycnidial fungus from the Cradle of Humankind

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Abstract: Homortomyces is introduced as a new coelomycetous genus associated with leaf spots on Combretum erythrophyllum trees growing near and around the Sterkfontein caves, Maropeng, South Africa. Based on its transversely septate, brown conidia, the presence of paraphyses, and percurrent proliferation of the conidiogenous cells, the genus resembles Stilbospora (Melanoconidaceae, Diaporthales). It is distinct in having pycnoidal conidiomata, conidia lacking mucoid sheaths, and becoming muriform when mature. Its morphology and phylogenetic placement based on analyses of sequence data for the large subunit nuclear ribosomal RNA gene (LSU, 28S) as well as the ITS and 5.8S rRNA gene of the nrDNA operon, show that Homortomyces represents a novel genus in Dothideomycetes, although its familial relationships remain unresolved.

Key words: coelomycetes
Combretum
Dothideomycetes
ITS
LSU
Stilbospora
systematics

INTRODUCTION

The Sterkfontein caves at Maropeng (meaning “returning to the place of origin” in the southern African language, Setswana) form part of the Cradle of Humankind, a World Heritage Site close to Johannesburg, Gauteng Province, South Africa. The site is well known for the 2.3-million year-old fossil Australopithecus africanaus, named “Mrs. Ples”, which was found there in 1947 by Robert Broom and John T. Robinson (Fleminger 2008). Although much attention has been devoted to fossils buried in the area, little is known of the fungi on the surrounding vegetation. The area is characterised by Rocky Highveld Grassland that harbours a diversity of plants and animals. During a recent visit to Maropeng, it was noted that Combretum erythrophyllum (River bushwillow; Combretaceae) trees suffered from a serious leaf spot disease, which appears to eventually kill the Combretum erythrophyllum trees. A young shoots and lead to the development of prominent stem cankers. A Stilbospora-like coelomycete was consistently found sporulating on the leaf and shoot lesions. The genus Stilbospora is based on S. macrospora, a coelomycetous fungus that occurs on dead branches of Carpinus betulus in Europe. Stilbospora macrospora has been linked to the sexual morph Pestalotiopsis ellipsoporum (Melanoconidaceae, Diaporthales) based on culture studies, and supported by DNA sequence data (Voglmayr & Jaklitsch 2008). Stilbospora is characterised by acervular conidiomata that give rise to brown, transversely distoseptate conidia with mucilaginous sheaths, formed on hyaline, percurrently proliferating conidiogenous cells, intermingled with septate and hyaline paraphyses (Sutton 1980). The genus includes more than 80 names representing many disjunct taxa, and is in urgent need of taxonomic revision. The aim of this study was to isolate and characterise the fungus associated with the leaf spots on Combretum erythrophyllum, and to compare this taxon to species in Stilbospora.

MATERIALS AND METHODS

Isolates

Single conidial colonies established from sporulating conidiomata were grown in Petri dishes containing 2 % malt extract agar (MEA; Crous et al. 2009b) as described earlier (Crous et al. 1991). Colonies were subcultured onto potato-dextrose agar (PDA), oatmeal agar (OA), MEA (Crous et al. 2009b), and pine needle agar (PNA) (Smith et al. 1996), and incubated at 25 °C under continuous near-ultraviolet light to promote sporulation. Reference strains were deposited at the CBS-KNAW Fungal Biodiversity Centre in Utrecht, The Netherlands (CBS), and taxonomic novelties were deposited in MycoBank (Crous et al. 2004).

DNA isolation, amplification and analyses

Genomic DNA was extracted from fungal colonies growing on MEA using the UltraClean™ Microbial DNA Isolation Kit (MoBio Laboratories, Inc., Solana Beach, CA, USA) following the manufacturer’s protocols. Part of the nuclear rDNA...
<table>
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<th>Species</th>
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<th>Country</th>
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<td>Homortomyces combreti</td>
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<td>Combretum erythrophyllum, leaves</td>
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<td>P.W. Crous &amp; M.J. Wingfield</td>
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<td>Elegia equisetacea, dead culm</td>
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1CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CMW: Culture Collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; CPC: Culture collection of P.W. Crous, housed at CBS.

2ITS: Internal transcribed spacers 1 and 2 together with 5.8S nrDNA; LSU: 28S nrDNA; TEF: partial translation elongation factor 1-alpha.
operon spanning the 3' end of the 18S rRNA gene, both internal transcribed spacer regions, the 5.8S rRNA gene, and the 5' end of the 28S rRNA gene (ITS) was amplified using the primers V9G (de Hoog & Gerrits van den Ende 1998) and LRS (Vilgalys & Hester 1990). The primers ITS4 (White et al. 1990) and LSU1FD (Crous et al. 2009a) were used as internal sequence primers to provide sequences of high quality over the entire length of the amplicon. The LSU sequence alignment of Voglmayr & Jaklitsch (2008) was downloaded from TreeBASE (matrix M3536; www.treebase.org/treebase/index.html) and modified with additional sequences from NCBI’s GenBank nucleotide database. The sequence alignment and subsequent phylogenetic analyses were carried out using methods described by Lombard et al. (2011); gaps were treated as “fifth state” data. Sequences derived in this study were lodged in GenBank (Table 1), the alignment in TreeBASE (www.treebase.org/treebase/index.html), and taxonomic novelties in MycoBank (www.MycoBank.org; Crous et al. 2004).

**Morphology**
Descriptions were based on slide preparations mounted in clear lactic acid from colonies sporulating on PNA. Observations were made with a Zeiss V20 Discovery stereo-microscope, and with a Zeiss Axio Imager 2 light microscope using differential interference contrast (DIC) illumination and an AxioCam MRc5 camera and software. Colony characters and pigment production were noted after 1 mo of growth on MEA, PDA and OA (Crous et al. 2009b) incubated at 25 ºC. Colony colours (surface and reverse) were established using the colour charts of Rayner (1970).

**RESULTS**

**Phylogenetic comparisons**
Amplicons of approximately 1 700 bases were obtained for the ITS region, including the first approximately 900 bp of LSU, for the isolates listed in Table 1. The LSU sequences were used to obtain additional sequences from GenBank, which were added to an alignment modified from that of Voglmayr & Jaklitsch (2008). The manually adjusted LSU alignment contained 46 sequences (including the outgroup sequence) and 850 characters including alignment gaps (available in TreeBASE) were used in the phylogenetic analysis; 253 of these were parsimony-informative, 36 were variable and parsimony-uninformative, and 561 were constant. The ITS sequences were used in a blast search of the GenBank nucleotide database in an attempt to identify the species.

A Bayesian analysis was conducted on the aligned LSU sequences using a general time-reversible (GTR) substitution model with inverse gamma rates and dirichlet base frequencies. The Markov Chain Monte Carlo (MCMC) analyses of two sets of 4 chains started from a random tree becoming muriformly septate in older cultures, apex obtuse, sparingly septate. 

**Taxonomy**

**Homortomyces** Crous & M.J. Wingf., **gen. nov.**
MycoBank MB801349

**Etymology:** *Homortomyces*, derived from “homo” (human being), “orto or origo” (origin) and “-myces” (fungus).

**Homortomyces** resembles *Stilbospora* (*Melanoconidaceae, Diaporthales*), but is distinguished from that genus by having pycnidial conidomata, and conidia characterised by muriform septa (in exceptional cases), and lacking mucoid sheaths.

**Description:** *Foliicolous*, associated with leaf spots. *Conidiomata* pycnidial, black, globose, with central ostiole; wall consisting of 4–7 layers of brown *textura angularis*. *Conidiophores* reduced to conidiogenous cells or one supporting cell, hyaline, cylindrical, with 1–4 inconspicuous percurrent proliferations at apex. *Paraphyses* intermingled among conidiogenous cells, extending above conidia, hyaline, smooth, cylindrical, flexuous, apex obtuse, sparingly septate. *Conidia* brown, ellipsoid to subcylindrical, verruculose, transversely euseptate, septa with visible central pore, becoming muriformly septate in older cultures, apex obtuse, base truncate with visible scar, basal or displaced towards the side.

**Type species:** *Homortomyces combreti* Crous & M.J. Wingf. 2012.
Fig. 1. Bayesian consensus phylogeny obtained from the analysis of the LSU sequence alignment. The scale bar represents the average number of substitutions per site, and posterior probability values are shown at the nodes. The novel species treated in this study is shown in red and novel sequences in **bold**. Orders are indicated in the coloured blocks. Branches also present in the strict consensus tree of the parsimony analysis are thickened and the tree was rooted on a sequence of *Magnaporthe grisea* (GenBank accession no. AB026819).
Homortomyces gen. nov. (Dothideomycetes)

**Homortomyces combreti** Crous & M.J. Wingf., sp. nov.
MycoBank MB801350
(Fig. 2)

*Etymology:* After the genus *Combretum* on which the fungus was first found.

*Type:* **South Africa:** Gauteng, Maropeng, Sterkfontein Caves, The Cradle of Humankind, on leaves of *Combretum erythrophyllum* (River bushwillow; *Combretaceae*), 4 July 2011, *P.W. Crous & M.J. Wingfield* (CBS H-21049 holotype; cultures ex-type CPC 19800 = CBS 132554, 19801, 19808 = CBS 132555, CPC 19809).

*Description:* Leaf spots amphigenous, circular to subcircular, medium brown with dark brown margin, 2–7 mm diam. On MEA: Conidiomata pycnidial, amphigenous on leaves, black, globose, up to 500 µm diam with central ostiole; wall consisting of 4–7 layers of brown textura angularis. Conidiophores reduced to conidiogenous cells or one supporting cell, hyaline, cylindrical, 20–60 × 3–5 µm, with

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**Fig. 2.** *Homortomyces combreti* (CPC 19800). **A.** Rocky Highveld Grassland at Sterkfontein Caves, Maropeng. **B–D.** Prominent leaf spots on *Combretum erythrophyllum*, with black pycnidia. **E.** Sporulating pycnidial conidiomata on MEA. **F.** Paraphyses. **G–J.** Conidiogenous cells giving rise to conidia. **K–P.** Distoseptate conidia, showing septal pores, transverse septa, and flattened, eccentric, basal conidial hila. Scale bars = 10 µm.
1–4 inconspicuous percurrent proliferations at their apex. Paraphyses intermingled among conidiogenous cells, extending above the conidia, to 100 µm long, 2–4 µm diam, hyaline, smooth, cylindrical, flexuous, sparingly (1–3)-septate with obtuse apex; in old paraphyses the apical cell becoming swollen and clavate, with walls becoming thickened. Conidia 

(27–)32–38(–40) × (11–)13–16(–18) µm, brown, ellipsoid to subcylindrical, verruculose, 3(–4)-euseptate, septa with visible central pore, becoming muriformly septate in older cultures, apex obtuse, base truncate with visible scar, basal or displaced towards the side, 3–3.5 µm diam.

**Cultural characteristics:** Colonies on MEA on 25 ºC spreading, erumpent with sparse aerial mycelium and lobate, feathery margins, reaching 35 mm diam after 1 mo. Surface umber to chestnut; reverse chestnut, outer margin ochraceous.

**DISCUSSION**

In a recent phylogenetic study, the type species of the genus *Stilbospora, S. macrosporarina* was linked to a *Prosthecium* sexual state, *P. ellipsosporum* (Voglmayr & Jaklitsch 2006). *Stilbospora macrosporarina* Pers. 1794 is the type species of *Stilbospora* Pers. 1794, while *P. ellipsosporum* Fresen. 1852 is the type species of *Prosthecium* Fresen. 1852. In moving to a single nomenclature (Hawksworth et al. 2011, Wingfield et al. 2012), it would be prudent to retain *Stilbospora* over *Prosthecium*, as the former genus includes a greater number of taxa, is the older genus (thus having priority), and is the more commonly used name by plant pathologists. Other than confirming this link, Voglmayr & Jaklitsch (2006) described several other *Prosthecium*-like species, which also had *Stegonsporum* Corda 1827 conidial morphs. Although *Stegonsporum* resembles *Stilbospora*, it differs from that genus in that conidia have longitudinal septa. Furthermore, taxa with *Stegonsporum* morphs cluster adjacent to *Stilbospora s.str.* (Voglmayr & Jaklitsch 2006), and represent a different morphological and phylogenetic entity, to which the name *Stegonsporum* applies. *Prosthecium*, however, is a later synonym of *Stilbospora* (*Melanconidaceae, Diaporthales*) in this taxonomy.

*Homortomyces* closely resembles *Stilbospora* in morphology, but can be distinguished by the pycnidial conidiomata with a central ostiole, whereas *Stilbospora* has acervulate conidiomata. Conidia of *Homortomyces* also lack mucoid sheaths, and are transversely distoseptate, becoming muriformly septate in older cultures. Other genera with rather similar conidia to consider include *Endocoryneum, Hendersoniopsis, Angiopomopsis*, and *Ceratopycnis*, but none of these genera have paraphyses (Sutton 1980), and thus are easily distinguished morphologically from *Homortomyces*.

Based on our parsimony analysis, *Homortomyces* resides in *Botryosphaeriales* (*Dothideomycetes*), in which it appears to represent a family basal to *Botryosphaeriales* (results not shown). The *Botryosphaeriales* includes more than 17 genera that have *Botryosphaeria*-like ascomata (*Crous* et al. 2006, Damm et al. 2007 Phillips et al. 2008, Rojas et al. 2008), and are commonly associated with stem cankers and leaf spots of woody hosts (*Slippers* & *Wingfield* 2007). Several conidial genera in *Botryosphaeriales* have pycnidial conidiomata with paraphyses and conidiogenous cells with percurrent proliferation. However, the description of *Homortomyces* as a coelomycetous genus characterised by distoseptate conidia does not fully fit the morphological concept for this order. Both the distance and Bayesian analyses place *Homortomyces* in the backbone of the phylogenetic tree of *Dothideomycetes* (e.g. Fig. 1) and, pending collection of additional species of this genus or more closely allied genera, it is best treated as *incertae sedis* rather than referred to an any existing or a new family.

*Homortomyces combreti* is the only fungus closely associated with a destructive leaf and shoot disease of *C. erythropylhum*, and it is most likely the causal agent of this disease, though this has not yet been proven experimentally. Given the damage caused to these trees, it will be important to establish its pathogenicity and then to consider strategies to manage the disease, which is damaging large numbers of amenity trees. Although the primary infections occur on young leaves and shoots, the infections subsequently appear on larger branches and main stems, resulting in obvious stem cankers.

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