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

Pedro W. Crous¹, Johannes Z. Groenewald¹, Lorenzo Lombard¹ and Michael J. Wingfield¹

¹CBS-KNAW Fungal Biodiversity Centre, Uppsalaalan 8, 3584 CT Utrecht, The Netherlands; corresponding author e-mail: p.crous@cbs.knaw.nl
²Department of Microbiology and Plant Pathology, DST/NRF Centre of Excellence in Tree Health Biotechnology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20 Hatfield, Pretoria 0028, Pretoria, South Africa
³Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa

**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 pycnidial 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

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**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 africanus*, 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*), a species that gives rise to brown, transversely distoseptate conidia, 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.

**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 1. Collection details and GenBank accession numbers of isolates for which novel sequences were generated in this study.

| Species                     | Strain no. | Substrate                        | Country                          | Collector                     | GenBank accession no. |
|-----------------------------|------------|----------------------------------|----------------------------------|-------------------------------|-----------------------|
| *Homortomyces combreti*     | CBS 132554; CPC 19800 | Combretum erythrophyllum, leaves | South Africa: Maropeng          | P.W. Crous & M.J. Wingfield  | JX517280              |
|                             | CBS 132555; CPC 19808 | Combretum erythrophyllum, leaves | South Africa: Maropeng          | P.W. Crous & M.J. Wingfield  | JX517281 JX517291     |
| *Sclerostagonospora* sp.    | CBS 118142; CMW 18281 | *Elegia equisetacea*, dead culm | South Africa: Kirstenbosch      | S. Lee                        | DQ286766 / JX517282   |
|                             | CBS 118146; CMW 17948 | Cannomois virgata, dead culm    | South Africa: Jonkershoek       | S. Lee                        | DQ286765 DQ286769     |
|                             | CBS 118152; CMW 18025 | Thamnochorus spicigerus, dead culm | South Africa: Kirstenbosch    | S. Lee                        | JX517283 JX517292     |
|                             | CBS 118224; CMW 18063 | Ischyrolepis subverticillata, dead culm | South Africa: Kirstenbosch | S. Lee                        | JX517284 JX517293     |
| *Stilbospora macrospema* (syn. Prosthecium ellipsosporum) | CBS 121692 | Carpinus betulus, dead corticated twig | Austria: Niederösterreich | H. Voglmayr                   | JX517285 JX517294     |
|                             | CBS 121693 | Carpinus betulus, dead corticated twig | Austria: Niederösterreich | H. Voglmayr                   | JX517286 JX517295     |
|                             | CBS 121694 | Carpinus betulus, dead corticated twig | Austria: Oberösterreich | H. Voglmayr                   | JX517287 JX517296     |
|                             | CBS 121695 | Carpinus betulus, dead corticated twig | The Netherlands: Utrecht     | H. Voglmayr                   | JX517288 JX517297     |
|                             | CBS 121882 | Carpinus betulus, dead corticated twig | Austria: Niederösterreich, Wassergspreng | H. Voglmayr                   | JX517289 JX517298     |
|                             | CBS 121883 | Carpinus betulus, dead corticated twig | Austria: Oberösterreich, Leitenthbachtal | H. Voglmayr                   | JX517290 JX517299     |

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 rDNA; LSU: 28S rDNA; 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 LSU1F (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) and 850 characters including alignment gaps. Conidia characterising the species were described as being), “orto or origo” (origin) and “-myces” (fungus).

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

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

Description: Foliicolous, associated with leaf spots. Conidiodoma 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...
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 

(C27–)32–38–(40) µm diam after 1 mo. Surfaceumber to

**Cultural characteristics**: Colonies on MEA on 25 ºC spreading, or displaced towards the side, 3–3.5 µm diam.

**Paraphyses**

intermingled among conidiogenous cells, 1–4 inconspicuous percurrent proliferations at their apex. 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 *Stegonsporium Corda 1827 conidial morphs*. Although *Stegonsporium* resembles *Stilbospora*, it differs from that genus in that conidia have longitudinal septa. Furthermore, taxa with *Stegonsporium* morphs cluster adjacent to *Stilbospora s.str.* (Voglmayr & Jaklitsch 2006), and represent a different morphological and phylogenetic entity, to which the name *Stegonsporium* 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 paraphyses and conidiogenous cells with percurrent proliferation. However, the description of *Homortomyces* as a coelomycetous genus characterised by diosspore 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.

**DISCUSSION**

In a recent phylogenetic study, the type species of the genus *Stilbospora, S. macrosporuma* was linked to a *Prosthecium* sexual state, *P. ellipsosporum* (Voglmayr & Jaklitsch 2006). *Stilbospora macrosporuma* 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 *Stegonsporium Corda 1827 conidial morphs*. Although *Stegonsporium* resembles *Stilbospora*, it differs from that genus in that conidia have longitudinal septa. Furthermore, taxa with *Stegonsporium*morphs cluster adjacent to *Stilbospora s.str.* (Voglmayr & Jaklitsch 2006), and represent a different morphological and phylogenetic entity, to which the name *Stegonsporium* applies. *Prosthecium*, however, is a later synonym of *Stilbospora* (*Melanconidaceae, Diaporthales*) in this taxonomy.

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