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ARTICLE

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 pycnidial condiomata, 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*) trees suffered from a serious leaf spot disease, which appears to eventually kill the 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. macrosperma*, a coelomycetous fungus that occurs on dead branches of *Carpinus betulus* in Europe. *Stilbospora macrosperma* has been linked to the sexual morph *Prosthecium 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 1. Collection details and GenBank accession numbers of isolates for which novel sequences were generated in this study.

Species	Strain no.[1]	Substrate	Country	Collector	GenBank accession no.[2]	
					ITS	LSU
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	DQ286770
	CBS 118146; CMW 17948	*Cannomois virgata*, dead culm	South Africa: Jonkershoek	S. Lee	DQ286765	DQ286769
	CBS 118152; CMW 18025	*Thamnochortus spicigerus*, dead culm	South Africa: Kirstenbosch	S. Lee	JX517283	JX517292
	CBS 118224; CMW 18063	*Ischyrolepis subverticellata*, dead culm	South Africa: Kirstenbosch	S. Lee	JX517284	JX517293
Stilbospora macrosperma (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, Wasserg-spreng	H. Voglmayr	JX517289	JX517298
	CBS 121883	*Carpinus betulus*, dead corticated twig	Austria: Oberösterreich, Leithen-bachtal	H. Voglmayr	JX517290	JX517299

[1]CBS: 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.

[2]ITS: 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 LR5 (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 stereomicroscope, 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

Phylogenentic 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 topology and lasted 506 000 generations, after which the split frequency reached less than 0.01. Trees were saved each 1 000 generations, resulting in 1 012 saved trees. Burn-in was set at 25 %, leaving 760 trees from which the consensus tree and posterior probabilities (PP's) were calculated (Fig. 1).

Neighbour-joining analyses using three substitution models on the same LSU sequence alignment yielded trees with identical topologies and differed mainly with regard to the arrangement of the clades representing *Umbilicariales* and *Teloschistales* compared to that obtained from the Bayesian analysis (Fig. 1).

Parsimony analysis of the LSU alignment yielded 88 equally most parsimonious trees (data not shown; TL = 795 steps; CI = 0.540; RI = 0.885; RC = 0.478). Similar to the tree generated by MrBayes, the clades representing the *Umbilicariales* and *Teloschistales* were differently ordered in the parsimony phylogeny compared to the neighbour-joining and Bayesian analyses. Also, the *Stilbospora*-like strain isolated in this study moved to a basal position in *Botryosphaeriales* as sister to *Phyllosticta* in the parsimony analyses (data not shown). However, its position in *Botryosphaeriales* was not supported in the bootstrap analysis (data not shown).

A megablast search of the ITS sequence failed to reveal any high similarity hits in the general nucleotide database of GenBank. Highest levels of similarity were observed with *Bagnisiella examinans* (GenBank EU167562; Identities = 522/628 (83 %), Gaps = 54/628 (9 %)), *Botryosphaeria dothidea* (GenBank DQ008327; "Identities" = 497/600 (83 %), Gaps = 58/600 (10 %)) and *Sclerotinia homoeocarpa* (GenBank GU002301; "Identities" = 515/622 (83 %), Gaps = 58/622 (9 %)). The *Stilbospora*-like strain isolated in this study is described in a new genus below.

Taxonomy

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

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

Hormotomyces resembles *Stilbospora* (*Melanoconidaceae, Diaporthales*), but is distinguished from that genus by having pycnidial condiomata, 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.

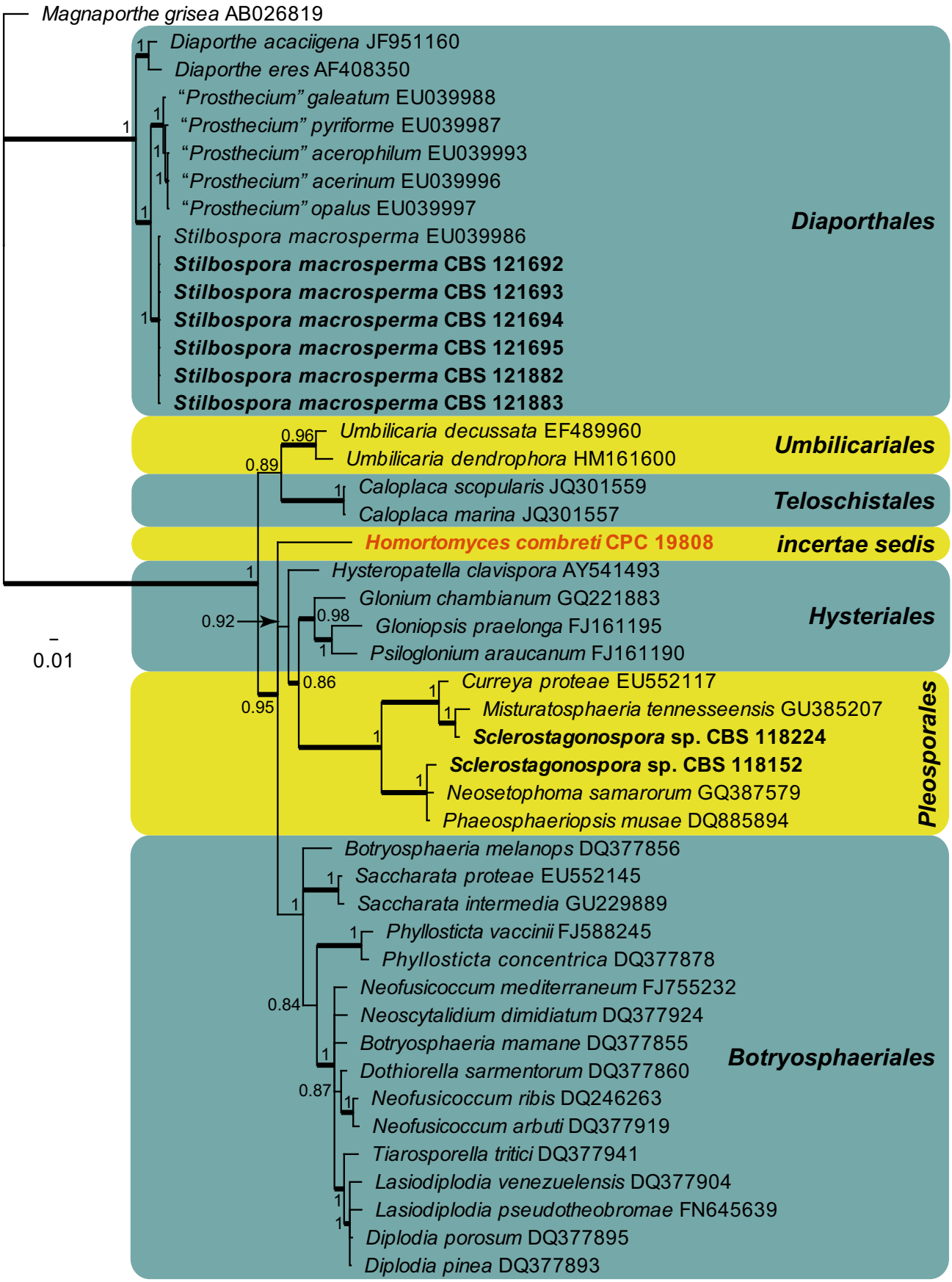

Fig. 1. Bayesian consensus phylogeny obtained from the analysis of the LSU sequence alignment. The scale bar represents the average number of substitutions per site, and posterior probability values are shown at the nodes. The novel species treated in this study is shown in red and novel sequences in **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).

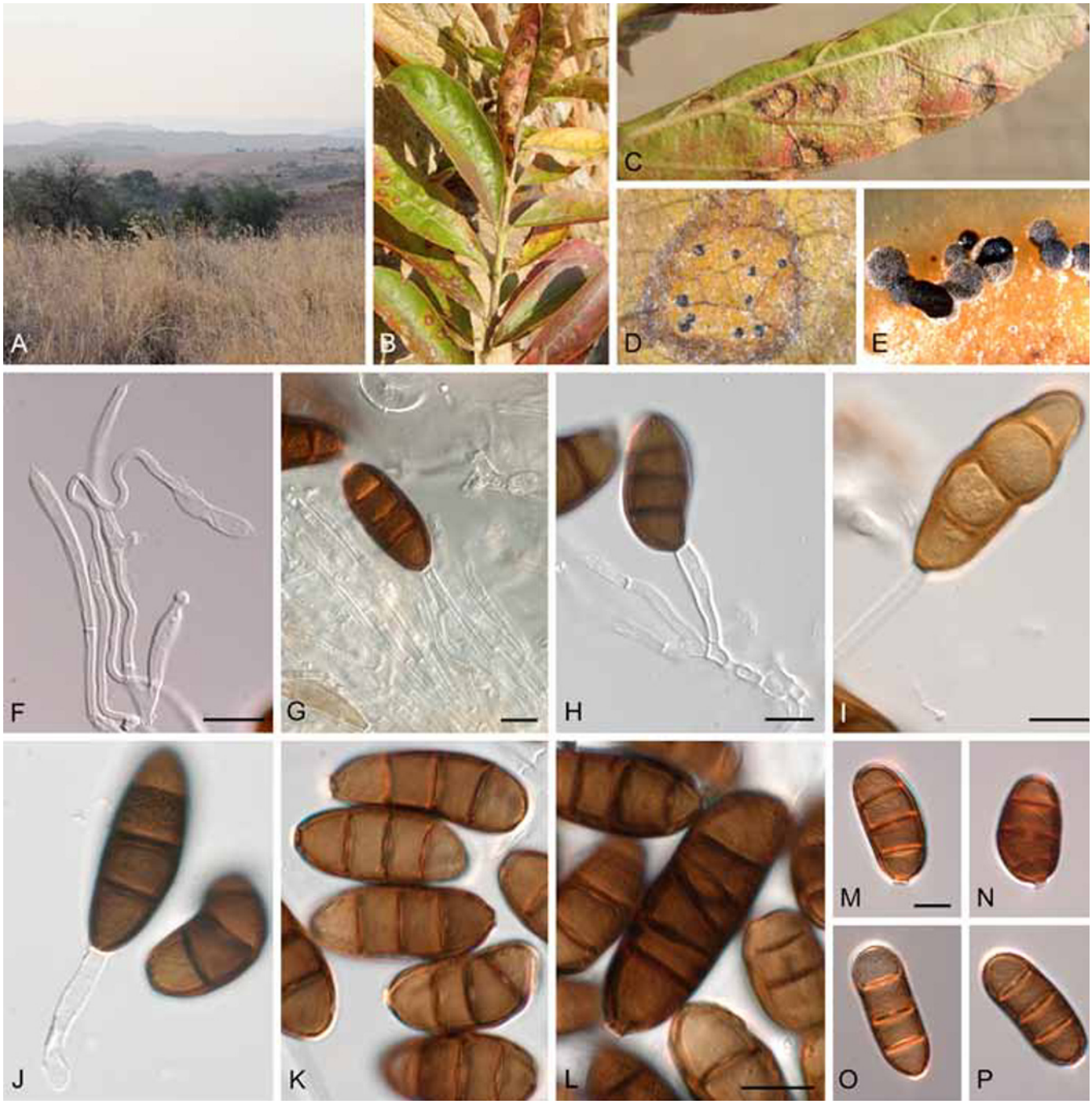

Fig. 2. *Homortomyces combreti* (CPC 19800). **A.** Rocky Highveld Grassland at Sterkfontein Caves, Maropeng. **B–D.** Prominent leaf spots on *Combretum erythrophyllum*, with black pycnidia. **E.** Sporulating pycnidial conidiomata on MEA. **F.** Paraphyses. **G–J.** Conidiogenous cells giving rise to conidia. **K–P.** Distoseptate conidia, showing septal pores, transverse septa, and flattened, eccentric, basal conidial hila. Scale bars = 10 µm.

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 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. macrosperma* was linked to a *Prosthecium* sexual state, *P. ellipsosporum* (Voglmayr & Jaklitsch 2006). *Stilbospora macrosperma* 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.

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 *Botryosphaeriaceae* (results not shown). The *Botryosphaeriaceae* 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. erythrophyllum*, 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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REFERENCES

Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G (2004) MycoBank: an online initiative to launch mycology into the 21st century. *Studies in Mycology* **50**: 19–22.

Crous PW, Schoch CL, Hyde KD, Wood AR, Gueidan C, *et al*. (2009a) Phylogenetic lineages in the *Capnodiales*. *Studies in Mycology* **64**: 17–47.

Crous PW, Slippers B, Wingfield MJ, Rheeder J, Marasas WFO, *et al*. (2006) Phylogenetic lineages in the *Botryosphaeriaceae*. *Studies in Mycology* **55**: 235–253.

Crous PW, Verkley GJM, Groenewald JZ, Samson RA (eds) (2009b) *Fungal Biodiversity*. [CBS Laboratory Manual Series 1.] Utrecht: Centraalbureau voor Schimmelcultures.

Crous PW, Wingfield MJ, Park RF (1991) *Mycosphaerella nubilosa* a synonym of *M. molleriana*. *Mycological Research* **95**: 628–632.

Damm U, Fourie PH, Crous PW (2007) *Aplosporella prunicola*, a novel species of anamorphic *Botryosphaeriaceae*. *Fungal Diversity* **27**: 35–43.

Fleminger D (2008) *World Heritage Sites of South Africa: the cradle of humankind*. Johannesburg: 30 Degrees South Publishers.

Hawksworth DL, Crous PW, Redhead SA, Reynolds DR, Samson RA, *et al.* (2011) The Amsterdam Declaration on Fungal Nomenclature. *IMA Fungus* **2**: 105–112; *Mycotaxon* **116**: 491–500.

Hoog GS de, Gerrits van den Ende AHG (1998) Molecular diagnostics of clinical strains of filamentous basidiomycetes. *Mycoses* **41**: 183 –189.

Lombard L, Polizzi G, Guarnaccia V, Vitale A, Crous PW (2011) *Calonectria* spp. causing leaf spot, crown and root rot of ornamental plants in Tunisia. *Persoonia* **27**: 73–79.

Phillips AJL, Alves A, Pennycook SR, Johnston PR, Ramaley A, Akulov A, Crous PW (2008) Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the *Botryosphaeriaceae*. *Persoonia* **21**: 29–55.

Rayner RW (1970) *A Mycological Colour Chart*. Kew: Commonwealth Mycological Institute.

Rojas EI, Herre EA, Mejía LC, Chavarri P, Samuels GJ (2008) *Endomelanconium endophyticum*, a new *Botryosphaeria* leaf endophyte from Panama. *Mycologia* **100**: 760–775.

Slippers B, Wingfield MJ (2007) The *Botryosphaeriaceae* as endophytes and latent pathogens of trees: identification, ecology and potential impact. *Fungal Biology Reviews* **21**: 90–106.

Smith H, Wingfield MJ, Crous PW, Coutinho TA (1996) *Sphaeropsis sapinea* and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa. *South African Journal of Botany* **62**: 86–88.

Sutton BC (1980) *The Coelomycetes: fungi imperfecti with pycnidia, acervuli, and stromata*. Kew: Commonwealth Mycological Institute.

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246.

Voglmayr H, Jaklitsch WM (2008) *Prosthecium* species with *Stegonsporium* anamorphs on *Acer*. *Mycological Research* **112**: 885–905.

White TJ, Bruns T, Lee J, Taylor SB (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), *PCR Protocols: a guide to methods and applications*: 315–322. San Diego: Academic Press.

Wingfield MJ, de Beer ZW, Slippers B, Wingfield BD, Groenewald JZ, *et al.* (2012) One fungus, one name promotes progressive plant pathology. *Molecular Plant Pathology* **13**: 604–613.