Mucor renisporus sp. nov., a new coprophilous species from Southern Africa

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Two unique mucoralean strains were isolated from canine dung in the Western Cape. These strains could be recognized by short as well as tall sporangiophores, reaching lengths of up to 80 mm. Morphologically, they are similar to *M. piriformis,* which is a postharvest pathogen of stone and pome fruit. The strains also resemble *M. grandis* but can be distinguished from the latter by the presence of short sporangiophores. The coprophilous strains were also compared to other mucorelean fungi on the basis of genetic characteristics with special emphasis on species described in *Mucor*. For comparisons, we used the ITS region of the ribosomal DNA gene region as well as the Elongation factor 1-α and actin genes. Our results confirmed results from previous studies that species in *Mucor* do not form a monophyletic group when compared on a genetic level with other Zygomycetes. The ITS region proved to be unreliable in distinguishing between closely related species. The Elongation factor 1-α and actin genes, was found to be more informative as the different species could be distinguished from one another. Based on the morphological and molecular evidence we describe the coprophilous strain as a distinct taxon, namely *Mucor renisporus* sp. nov.

Key words: actin gene, Elongation 1-α gene, morphology, phylogeny, Zygomycetes

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Introduction

Species in the genus *Mucor* (*Mucorales*, *Mucoraceae*) have a cosmopolitan distribution and can be isolated from almost any organic material that is in contact with air. *Mucor* spp. have been isolated from substrates such as soil, decaying plant material, dung, from air samples and in one case as a parasite on other fungi (Hesseltine, 1955). *Mucor* spp. are mostly known as spoilage organisms, and only one species, *M. piriformis,* has been reported as a pathogen on stone and pome fruit (Michailides and Spotts, 1988). *Mucor circinelloides* has been described as the causal agent of cutaneous zygomycosis in humans (Chandra and Woodgyer, 2002).

There are currently more than 300 species names for *Mucor* described in literature, although a little more than 50 species are known and described from cultures (Schipper, 1978). The majority of species can be placed into one of three groups based on their morphology. These are the *M. hiemalis* group, which consists of a number of morphological similar species. In cases where there were no mating reactions between morphological similar strains, formae speciales were designated for closely related taxa (Schipper, 1973). The other two groups are the *M. circinelloides*-group characterised by rather small species with reddish-brown zygospores (Schipper, 1976) and the *M. mucedo* group, characterised by the tall species, which generally show optimal development at temperature below 20° C (Schipper, 1975).

Traditionally, *Mucor* spp. were grouped according to morphological similarities, although these groups did not necessarily reflect the phylogenetic relationship among strains (Schipper, 1973). This was confirmed by O'Donnell *et al.* (2001) and White *et al.* (2006) who found that the monophyletic groups based on the Elongation 1-α gene

sequences does not reflect family relationships within the *Mucorales*. Although species of *Mucor* grouped in different clades, data from this gene region can effectively be used to identify undescribed species.

Two strains of a rather tall species of *Mucor* were isolated from the dog faeces on the senior author's lawn. These strains were identified using a published key to species in *Mucor* (Schipper, 1978), but did not correspond to other species described in this genus. It is, therefore, described here as new to science.

Materials and methods

Morphological comparisons

All measurements and microscopic observations were made from fungal structures grown on 2% malt extract agar (MEA) (20 g Biolab malt extract, 20 g agar, 1000 ml distilled water) and oatmeal agar (OA) (Gams *et al.*, 1998) and incubated in incident light at 25°C. Fungal structures were mounted on slides in 85% lactic acid and examined using phase or differential interference contrast microscopy. Fifty measurements were made for each morphological character and the averages and standard deviations were calculated. The optimal growth temperature of strains was determined on MEA at 4, 15, 20, 26 and 30°C, respectively.

Phylogenetic analyses

The strains from canine droppings as well as additional *Mucor* strains (Table 1) were grown on commercial potato dextrose agar (PDA, Biolab, Johannesburg, South Africa) for 10 d at 25°C. DNA was extracted from pure cultures using the method described by Möller *et al.* (1992) and modified by Jacobs *et al.* (2004). The presence of DNA was confirmed on a 1% agarose gel stained with ethidium bromide.

The same gene regions used by Voigt and Wöstemeyer (2000) and O'Donnell *et al*. (2001) for phylogenetics in this groups was used. These are the internal transcribed spacer (ITS) regions of the rDNA operon as well as the actin and elongation factor 1-α (EF 1-α) genes. PCR reactions were performed in 25 µl volumes (2.5 mM $MgCl_2$, 1X PCR buffer, 0.2 mM dNTP, 0.2 mM of each primer and 2.5 U *Taq*-polymerase enzyme). Primers used in the amplification reactions and for cycle sequencing were ITS1 and ITS4 (White *et al,*. 1990) for the ITS, Act-1 and Act-5R (Voigt and Wöstemeyer, 2000) for the actin gene and EF1F and MEFR (5'-TCTCCTTG TTCCAT CCCTTGAAC-3') for the elongation factor 1-α gene (Jacobs *et al.* 2004). PCR products were purified using the Qiaquick PCR purification kit (Qiagen) and sequenced using the Big Dye terminator cycle sequencing premix kit (Applied Biosystems) on an ABI PRISM 3100 automatic sequencer (Perkin Elmer Applied Biosystems). Sequence contigs were assembled using Seqman™ II (DNASTAR Inc., WI, USA), aligned in ClustalX (Thompson *et al.,* 1997) and manually adjusted in Se-Al (Rambaut, 1996).

Phylogenetic relationships for the taxa were inferred using distance analysis in PAUP* v.4.0b10 (Swofford, 2001). Characters were treated as unweighted in the analysis and gaps were treated as missing data. A single tree for each dataset was obtained using neighbour-joining analysis with an uncorrected P-distance and rooted to midpoint. A bootstrap analysis (1000 replicates using the neighbour-joining option) was performed to determine the confidence levels of the nodes. For all the datasets, ambiguously aligned regions were coded and step matrices to assign different weights to these codes were computed using INAASE 2.3b (Lutzoni *et al*., 2000). These weighted codes were used in the analysis to replace the ambiguous aligned regions. Datasets were treated separately, as the strains were not duplicated in all the sets.

Results

Phylogenetic analyses

Amplification of the ITS gene region resulted in fragments of approximately 600 base pairs (bp). The aligned data set consisted of 721 characters. Nine ambiguously aligned regions (492 bp) were identified and excluded from the analysis. These regions were replaced by weighted, coded characters (Lutzoni *et al.,* 2000). The *Mucor* species did not form a monophyletic clade and considerable variation could be seen even within species (Fig. 1). The

Table 1. Strains used in this study

Species	Genbank accession numbers			
	Strain no	ITS	Actin	Elongation factor 1-α
Mucor rouxii	CBS416.77	EF203695	n.d.	EF203704
Mucor plumbeus	A220	EF203696	n.d.	EF203701
	A162	EF203697	EF203691	EF203705
	CBS111.08	n.d.*	EF203690	n.d.
Mucor renisporus	KJ1119	EF203698	EF203693	EF203699
	PREM 5859	n.d.	EF203694	EF203700
Mucor flavus	CBS234.35	n.d.	EF203689	EF203702
Mucor mucedo	CBS109.16	n.d.	EF203692	EF203703

* not determined

strains were found to be related to *M. circinelloides* and in the case of DQ118989 (*M. circinelloides*) the sequences were found to be identical. However, these species are morphologically very different.

Amplification of a part of the actin gene resulted in fragments of approximately 700 bp. The aligned data set consisted of 573 characters. One ambiguously aligned region (96 bp) were identified and excluded from the analysis. As in the case of the ITS dataset, the strain from canine droppings formed a distinct clade although in this case it appears to be closer related to *M. plumbeus* (Fig. 2).

Amplification of a part of the EF 1-α gene resulted in fragments of approximately 700 bp. The aligned data set consisted of 433 characters with no ambiguously aligned regions. As with the ITS and actin datasets, the strains from canine droppings formed a distinct clade but in this case as a sistergroup to *M. recurvus* and *M. rouxii.*

Morphology

The strains isolated from canine droppings could easily be seen sporulating as it produced tall sporangiophores. Closer examination showed characters typically associated with *Mucor* spp. The sporangiophores were characterized by columella with various shapes ranging from cylindrical to pyriform. The most distinguishing character of these strains was the length of the conidiophores. In most instances it was up to 8 cm with a number of shorter sporangiophores at the base of the colony (Table 2). Based on these characters and comparisons with known species in the genus, the strains are here described as new to science.

Mucor renisporus K. Jacobs & Botha, **sp. nov.** (Figs 4-11)

MycoBank: 511205

Etymology: Named after the reniform sporrangiospores.

Zygosporae nec in cultu nec in substrato. *Sporangiophorae* longae plerumque irramosae; sporangiophorae breves, ramosae sympodiales. *Sporangia* candida nigrescunt aetate. *Columellae* vel cylindratae vel pyriformes. *Columellae* parvae plerumque conicae, (50-)104-180(-250) µm diametro. *Guttae* flavae pingues in columellis plerisque. Collares prominentes in sporangiophoris longis; non in sporangiophoris brevibus. *Sporangiosporae* ellipsoidales, aliquando reniformes e latere visae, (8-)13-19(-23) × 5-10 µm. *Colonia* pervenit altum 80 mm. *Colonia* novella candida est; fit ravida aetate. Non crescit apud 4°C, crescit restrictim apud 16°C, bene crescit apud 20-25°C. Minus crescit temperatura surgente.

Zygospores not seen in culture or on substrate. Tall sporangiophores mostly unbranched, short sporangiophores branched sympodially, sporangia white, turning black with age. *Columellae* ranging from cylindrical to pyriform, smaller columellae mostly conical, (50-)104-180(-250) µm in diameter. Yellow lipid droplets present in most columellae. Prominent collarettes on tall sporangiophores, absent on short sporangiophores. *Sporangiospores* ellipsoidal, occasionally kidney-shape in sideview, (8-)13-19(-23) × 5-10 µm. Colony up to 80 mm in heigth, whitish when young turing dark grey with age. No growth at 4ºC, restricted growth at 16ºC, good growth between 20-26ºC, restricted growth above 30ºC.

Teleomorph: not seen

Habitat: on domestic dog faeces

Known distribution: Western Cape, South Africa.

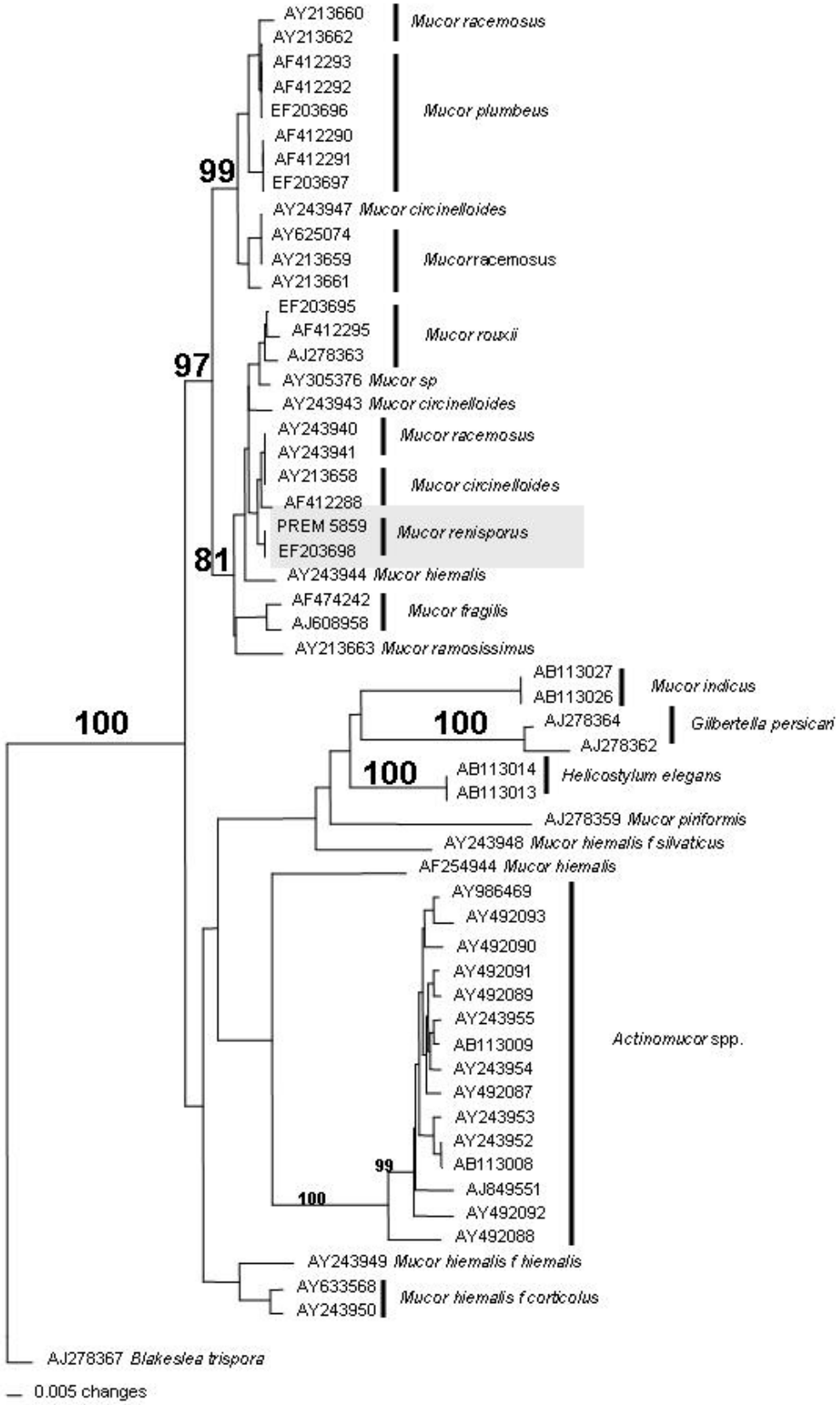


Fig. 1. Neighbour-joining tree of the ITS region of species in *Mucor* and related taxa.

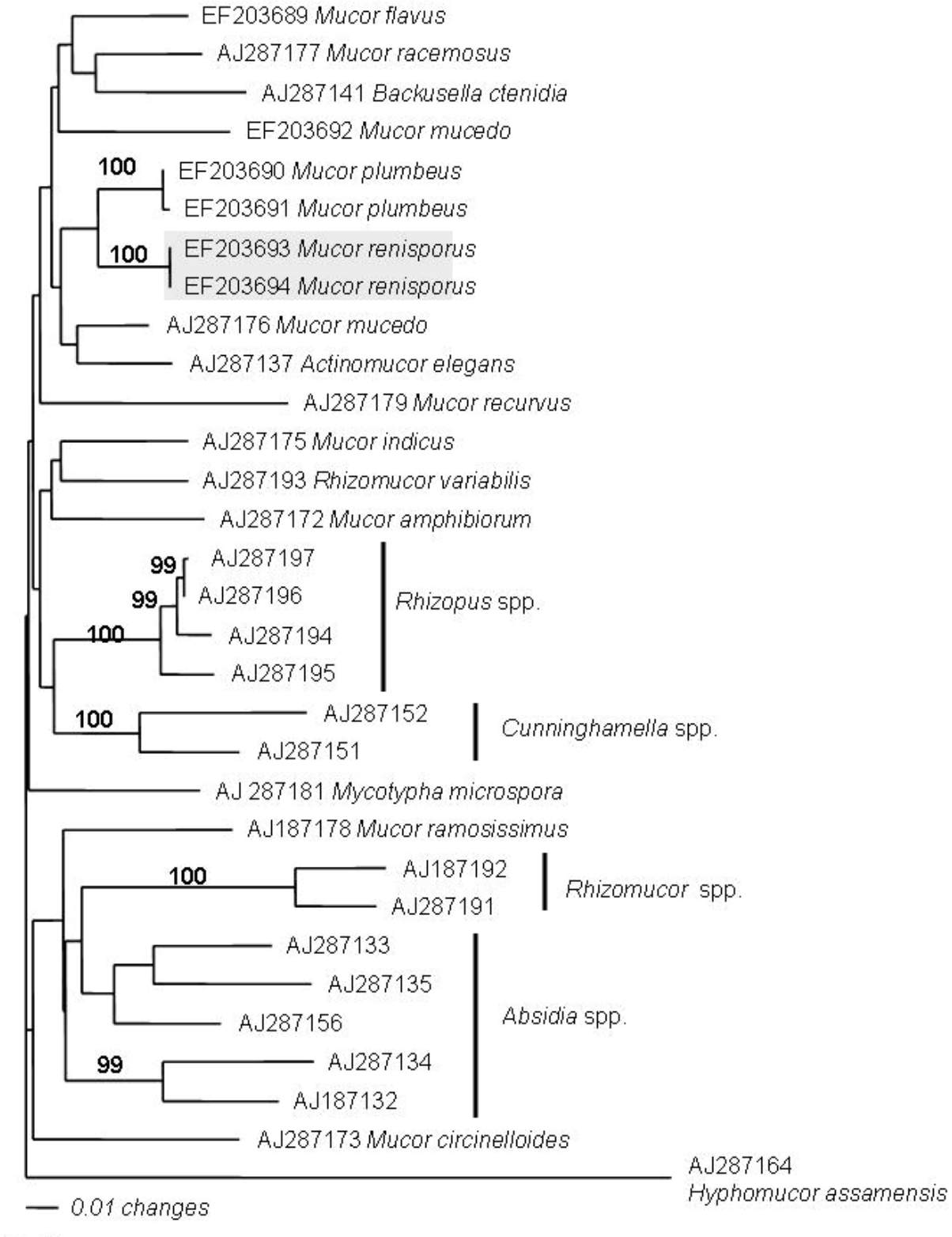


Fig. 2. Neighbour-joining tree of the actin gene region of species in *Mucor* and related taxa.

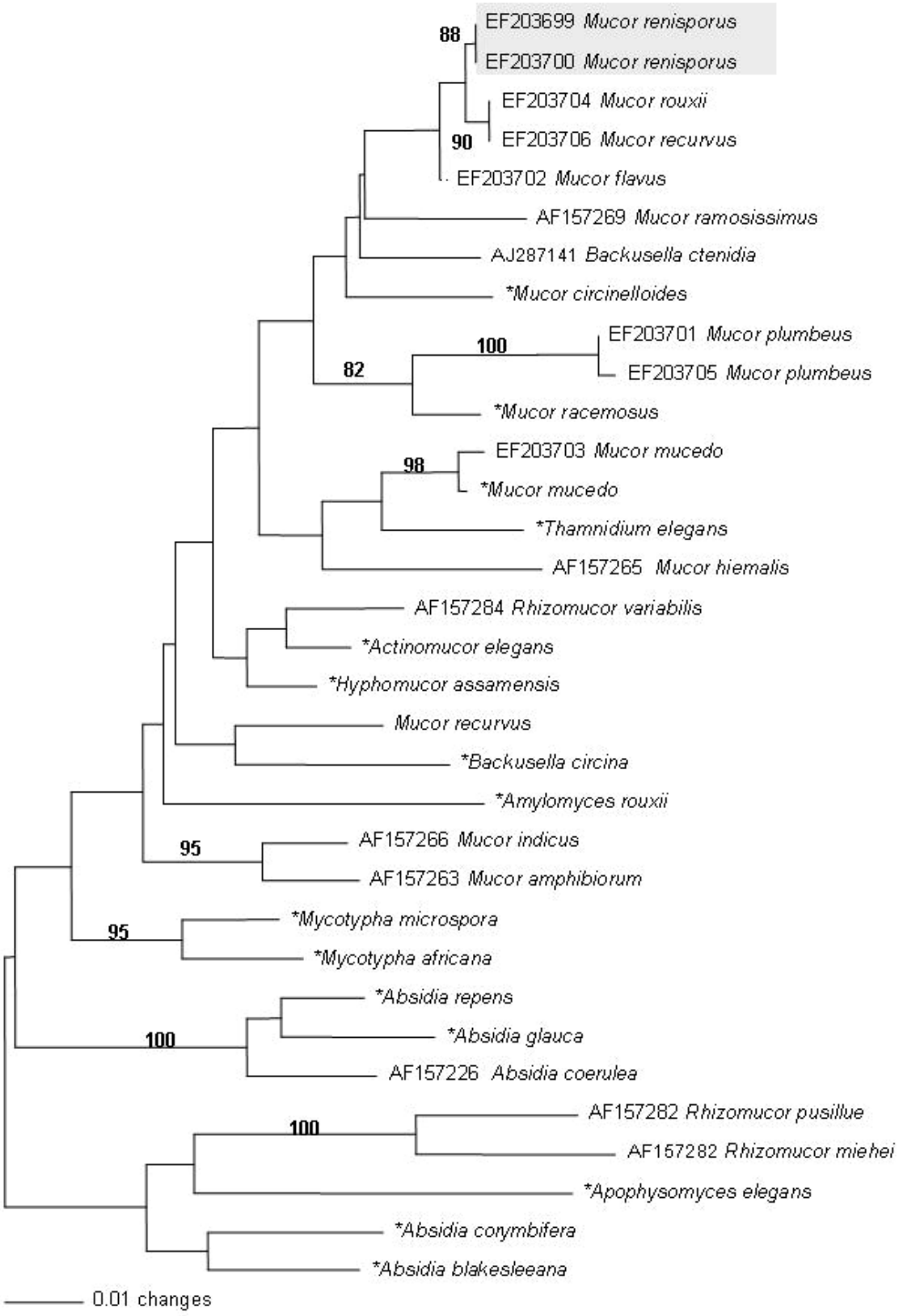
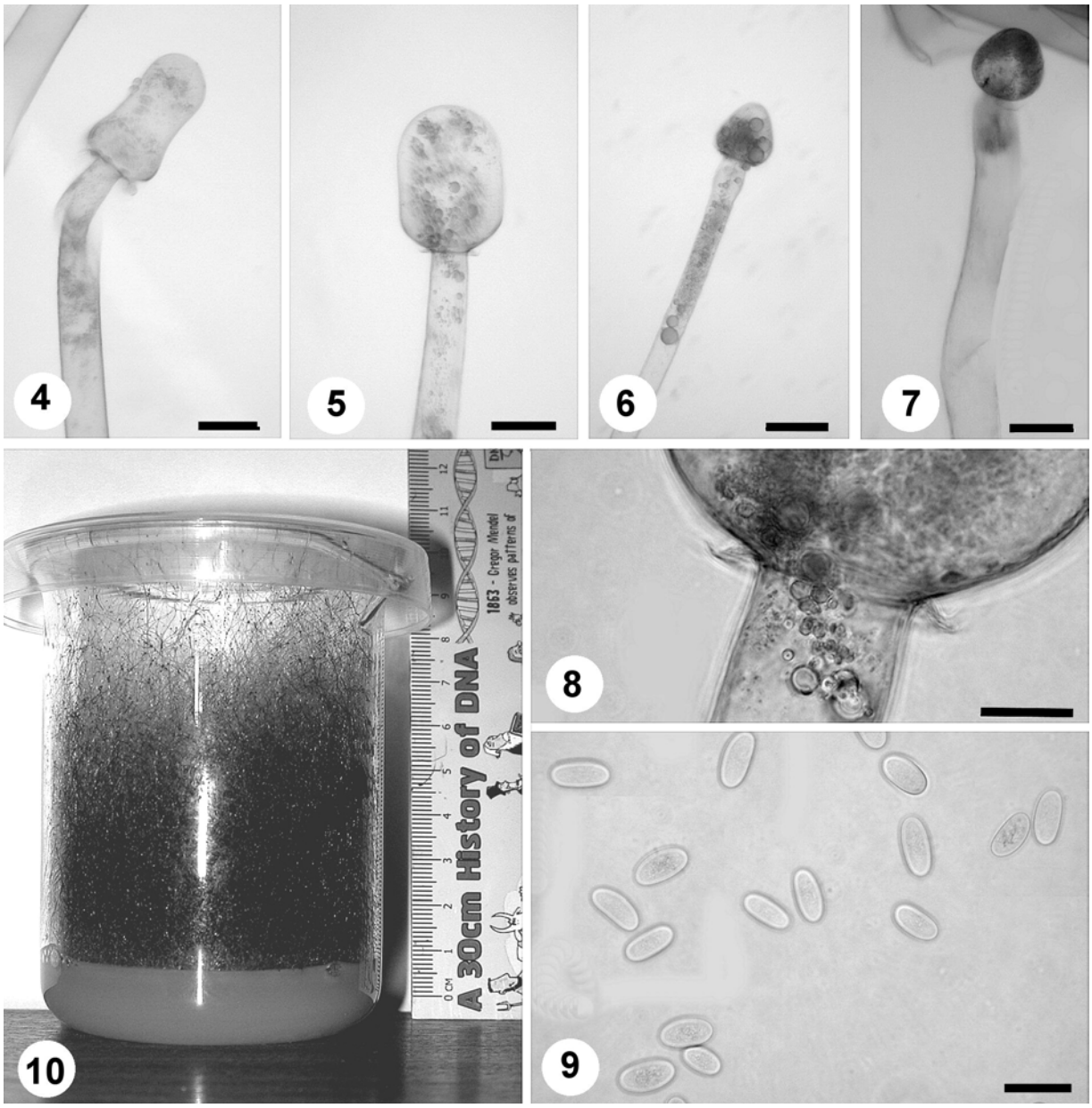


Fig. 3. Neighbour-joining tree of the Elongation 1-α gene region of species in *Mucor* and related taxa.



Figs 4-10. *Mucor renisporus* (from PREM 58590). **4-7.** Variation in columella shapes. **8.** Collar present on the sporangiophore. **9.** Ellipsoid sporangiospores, occasionally curved when viewed from the side. **10.** Colony growing in a glass beaker. Bars: 4-7 = 100 µm; 8 = 50 µm; 9: = 10 µm.

Material examined: SOUTH AFRICA, Somerset-West, canine droppings, 15 July 2004, A. Botha, (PREM 58590, **holotype**; CKJ1119, ex-type strain)

Discussion

Mucor renisporus forms part of the tall or large (cf. Schipper, 1975) species in *Mucor*. The colony height in this group is generally higher than that of the small species of *Mucor* (Schipper, 1976). This group is also characterized by optimal development below 20ºC (Schipper, 1975), although *M. renisporus* showed optimal growth development between 20 and 25ºC.

Mucor renisporus closely resembles *M. grandis* as both these species belong to the tall *Mucor* spp. (cf. Schipper, 1978). Also, both these species have been isolated from droppings (Schipper and Samson, 1994). *Mucor renisporus* is, however, distinguished from *M. grandis* by its cylindrical to often

Table 2. Comparison of *Mucor renisporus* with closely related species based on morphology and ecology.

Character	*M. renisporus*	*M. mucedo*	*M. piriformis*	*M. grandis*	*M. minutus*	*M. flavus*
Colony height	80 mm	25 mm	45 mm	90 mm	90 mm	43 mm
Sporangiophores*	Tall and short	Tall and short	Tall and short	Tall	Tall and short	Tall
Columella width	250 µm	250 µm	350 µm	115-200 µm	110-135 µm	110-135 µm
Columella shape	Cylindrical to pyriform	Obovoid to ellipsoid	Variable in shape	Cyllindrical -ellipsoid	Cylindrical to ellipsoid	Ellipsoid to pyriform
Sporangiophore recurved	No	Below 15ºC	No	Occasionally	No	No
Sporangiophore	Unbranched or sympodially branched	Usually unbranched	Sympodially	Usually unbranched	Sympodially	Sympodially
Sporangiospore shape	Ellipsoidal to kidney-shaped	Ellipsoidal to spherical	Ellipsoidal to spherical	Ellipsoidal	Subspherical	Ellipsoidal
Sporangiospore size	9-23 × 5-10 µm	10.5-13.5 × 6-7.5 µm	7-9.5 × 4-7 µm	12.5-21 × 7.5-12 µm	4-5 µm	7-12 × 4-6.5 µm
Zygospore	Absent	Present	Absent	Absent	Absent	Present
Habitat	Dung	Water, soil, dung	Soil and decaying fruit	Dung	Unknown	Soil and dung
References		Schipper, 1975	Michailides and Spotts, 1988	Schipper and Samson, 1994	Schipper, 1975	Schipper, 1975

*Sporangiophores are categorised as tall when exceeding 10mm (Schipper, 1975).

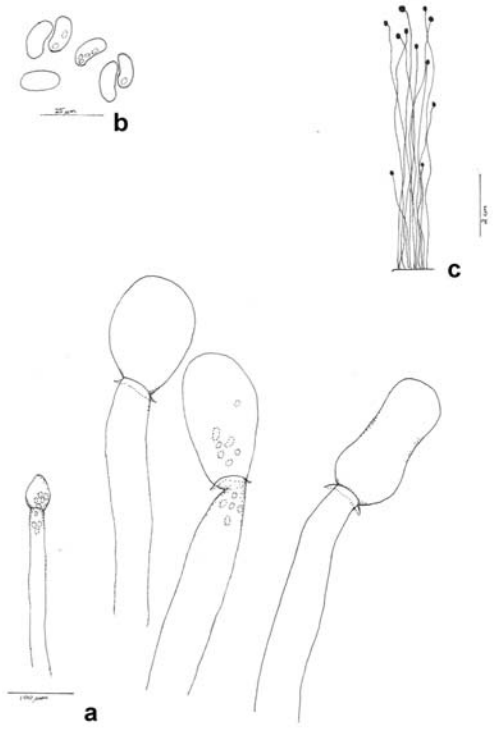


Fig. 11. *Mucor renisporus* (from PREM 58590). Line drawing showing the main morphological characters. **a.** Sporangiophores with variable columella. **b.** Sporangiospores. **c.** Habit sketch. Bars: a = 100 µm; b = 25 µm; c = 20 µm.

pyriform columellae, compared to the cylindrical to conical columellae of *M. grandis* (Schipper and Samson 1994). In addition, *M. renisporus* has a prominent collarette on its tall sporangiophores, whereas *M. grandis* only posses a short row of spines in place of a collarette. *Mucor renisporus* is further characterized by ellipsoidal sporangiospores that are often kidney-shaped when viewed from the side, while those of *M. grandis* are reported to be narrow and ellipsoidal (Schipper and Samson, 1994). In addition, *Mucor renisporus* also has a number of short sporangiophores at the base of the colony, a character not reported for *M. grandis*.

This study reflects the findings of previous phylogenetic studies of this group of fungi (Voigt and Wöstemeyer, 2001, O'Donnell *et al.,* 2004). In all three datasets, *M. renisporus* clustered in a unique and separate clade with no clear affinities to a specific sistergroup. These strains are, however, morphologically distinct and can easily be differentiated. Although this has not been the aim to determine the phylogenetic relationships within the genus *Mucor,* it was obvious from

the results that *Mucor* does not represent a monophyletic clade. However, in the absence of clear morphological differences, it will be difficult to delineate different groups within this genus. However, although the different trees showed differences in phylogenetic relatedness, *Mucor renisporus* clustered with the majority if the species in *Mucor* and also consistently formed a clade separate from other species in the genus.

Despite the availability of good morphological keys to species in *Mucor,* it is still difficult to accurately determine species names in this group. One of the main reasons for this is the variability of species, especially in the group with tall sporangiospores (Schipper, 1975). Clear species concepts can only be established if this group is studied in more detail.

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