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Updating the taxonomy of *Aspergillus* in South Africa

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Abstract: The taxonomy and nomenclature of the genus *Aspergillus* and its associated sexual (teleomorphic) genera have been greatly stabilised over the last decade. This was in large thanks to the accepted species list published in 2014 and associated metadata such as DNA reference sequences released at the time. It had a great impact on the community and it has never been easier to identify, publish and describe the missing *Aspergillus* diversity. To further stabilise its taxonomy, it is crucial to not only discover and publish new species but also to capture infraspecies variation in the form of DNA sequences. This data will help to better characterise and distinguish existing species and make future identifications more robust. South Africa has diverse fungal communities but remains largely unexplored in terms of *Aspergillus* with very few sequences available for local strains. In this paper, we re-identify *Aspergillus* previously accessioned in the PPRI and MRC culture collections using modern taxonomic approaches. In the process, we re-identify strains to 63 species, describe seven new species and release a large number of new DNA reference sequences.

Key words: Beta-tubulin, DNA barcoding, Calmodulin, GCPSR, Multigene phylogenies, RPB2, Secondary identification markers.
Taxonomic novelties: New species: *Aspergillus elsenburgensis* Visagie, S.M. Romero & Houbraken, *Aspergillus heldtiae* Visagie, *Aspergillus krugeri* Visagie, *Aspergillus magaliesburgensis* Visagie, *Aspergillus purpureocrustaceus* Visagie, *Aspergillus seifertii* Visagie & N. Yilmaz, *Aspergillus sigurros* Visagie.

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INTRODUCTION

Aspergillus is cosmopolitan fungi occurring on a wide range of substrates. Here they fulfil many different functions and have a wide-ranging influence on human and animal life. Even though most species occur as saprophytes living on dead organic material, various species have an (economic) impact on humans (Raper & Fennell 1965).

Human infections caused by *Aspergillus* are some of the most widely reported for all filamentous fungi (Gianni & Romano 2004, Balajee *et al.* 2007). *Aspergillus fumigatus, A. flavus* and *A. terreus* attract special interest as human pathogens causing widespread aspergillosis (fungus ball) or bad allergies (Raper & Fennell 1965, Steinbach *et al.* 2004, Sugui *et al.* 2012, de Hoog *et al.* 2014, Frisvad & Larsen 2015b), while a much broader spectrum of species is known to cause less invasive and/or superficial infections (Kaur *et al.* 2000, Zotti & Corte 2002, Hubka *et al.* 2012, de Hoog *et al.* 2014). *Aspergillus* causes widespread losses for agriculture where they spoil food or grow in agricultural produce, leading to mycotoxin contamination (Perrone *et al.* 2007, Pitt & Hocking 2009, Samson *et al.* 2010, Frisvad & Larsen 2015a). *Aspergillus* isolates produce three of the five agriculturally important mycotoxins, including aflatoxins, ochratoxins and fumonisins (Miller 1995, Frisvad & Larsen 2015a). The global cost of aflatoxin alone is huge and represents a major problem in developing countries where stunting in children is of major concern (Wu *et al.* 2008, Pitt *et al.* 2012, Wu 2015). Aflatoxin is most commonly produced by *A. flavus* and *A. parasiticus*, but many other Aspergilli can produce this devastating mycotoxin. Ochratoxins are commonly produced by *Aspergillus* species classified in sections *Circumdati* and *Nigri* (Frisvad *et al.* 2004, Frisvad *et al.* 2011, Davolos *et al.* 2012, Visagie *et al.* 2014b), while some sect *Nigri* species can also produce fumonisins (Frisvad *et al.* 2011, Frisvad & Larsen 2015a). On a more positive note, species have industrial applications as producers of enzymes, drugs, organic acids or are used in food fermentations. For example, *A. oryzae* (the domesticated form of *A. flavus*) is used in a koji fermentation important for the production of a wide variety of oriental foods (Raper & Fennell 1965, Varga *et al.* 2000, Samson *et al.* 2010, Hong *et al.* 2013, Kim *et al.* 2014).

The taxonomy of *Aspergillus* and its nine associated sexual (or teleomorphic) genera has been greatly stabilised over the last decade. Based on a multigene phylogenetic study, Kocsube *et al.* (2016) confirmed that *Aspergillus* is monophyletic and sister to *Penicillium* as originally shown by Houbraken & Samson (2011). Furthermore, they showed that the genus can be subdivided into six subgenera and several sections, which to a large degree corresponds to associated sexual states. The nomenclatural review and "accepted species list" published by Samson *et al.* (2014) played a significant role in stabilizing the taxonomy of *Aspergillus*. It created an "open access" model in the sense that all metadata associated with species names, such as ex-type culture collection accession numbers, sectional classifications, MycoBank numbers and GenBank accession numbers to reference sequences generated from ex-type cultures, were released in the public domain. Calmodulin was proposed as a secondary identification marker to the formal, but rather conserved, ITS DNA barcode (Schoch *et al.* 2012). All the released data resulted in more reliable species identifications, and new species discovery and its subsequent description are

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Table 1. Strains sequenced during the course of this project.

Species	Strains[1]	Section	Location collected / isolated, year	Host	GenBank nr			
					ITS	BenA	CaM	RPB2
Aspergillus chevalieri	PPRI13427 = CMV011F5	*Aspergillus*	South Africa, KwaZulu-Natal, Pinetown, 2013	Soil	–	–	MK451336	–
A. chevalieri	PPRI26000 = CMV003I3	*Aspergillus*	South Africa, 2017	Animal feed	–	MK450979	MK451332	–
A. chevalieri	PPRI26033 = CMV012H5	*Aspergillus*	South Africa, Gauteng, Pretoria, 2018	Dog food	–	–	MK451338	–
A. chevalieri	PPRI26034 = CMV012H6	*Aspergillus*	South Africa, Gauteng, Pretoria, 2018	Dog food	–	–	MK451339	–
A. chevalieri	PPRI26348 = CMV016E5	*Aspergillus*	South Africa, Gauteng, Pretoria, 2019	Dog food	–	–	MN031422	–
A. chevalieri	PPRI26554 = CMV016D7	*Aspergillus*	South Africa, Gauteng, Pretoria, 2019	Dog food	–	–	MK951911	–
A. chevalieri	PPRI3791 = CMV011B6	*Aspergillus*	South Africa, 1986		–	–	MK451333	–
A. chevalieri	PPRI4908 = CMV011B7	*Aspergillus*	South Africa, Kwazulu Natal, 1993	Maize kernels *(Zea mays)*	–	–	MK451334	–
A. chevalieri	PPRI5410 = CMV012B1	*Aspergillus*	South Africa, Western Cape, Clanwilliam, 1994	Rooibos tea *(Aspalathus linearis)*	–	–	MK451337	–
A. chevalieri	PPRI6331 = CMV011B9	*Aspergillus*	South Africa, Gauteng, Pretoria, 1996	Dried sausage	–	–	MK451335	–
A. montevidensis	CMV012H4	*Aspergillus*	South Africa, Gauteng, Pretoria, 2018	Dog food	–	–	MK451446	–
A. montevidensis	MRC1250 = CMV017A6	*Aspergillus*	South Africa, Western Cape, Ceres, 1975	Apple juice concentrate	–	–	MK951923	–
A. montevidensis	PPRI26035 = CMV012H7	*Aspergillus*	South Africa, Gauteng, Pretoria, 2018	Dog food	–	–	MK451447	–
A. montevidensis	PPRI4851 = CMV011G2	*Aspergillus*	South Africa, Gauteng, Johannesburg, 1993	Air sample	–	–	MK451445	–
A. montevidensis	PPRI6330 = CMV011B8	*Aspergillus*	South Africa, Gauteng, Pretoria, 1996	Dried sausage	–	–	MK451443	–
A. montevidensis	PPRI8674 = CMV011C2	*Aspergillus*	South Africa, Gauteng, Johannesburg, 2007	Wheat *(Triticum sp)*	–	–	MK451444	–
A. porosus	PPRI3419a = CMV012A8 = CSIR980	*Aspergillus*	South Africa, 1988		–	–	MK451494	–
A. porosus	PPRI3419b = CMV012A9 = CSIR980	*Aspergillus*	South Africa, 1988		–	–	MK451495	–
A. proliferans	PPRI6735 = CMV011C1	*Aspergillus*	South Africa, Mpumalanga, Piet Retief, 1988	Bee larvae *(Apis mellifera)*	–	–	MK451496	–
A. pseudoglaucus	MRC1231 = CMV017A3	*Aspergillus*	South Africa, Western Cape, Elgin, 1975	Apple	–	–	MK951920	–
A. pseudoglaucus	MRC455 = CMV017E9	*Aspergillus*	South Africa, unknown		–	–	MN031425	–
A. pseudoglaucus	MRC462 = CMV017A1	*Aspergillus*	South Africa, Pretoria, unknown		–	–	MK951918	–

Table 1. (Continued).

Species	Strains[1]	Section	Location collected / isolated, year	Host	GenBank nr			
					ITS	BenA	CaM	RPB2
A. pseudoglaucus	PPRI26346 = CMV016D9	*Aspergillus*	South Africa, Gauteng, Pretoria, 2019	Dog food	–	–	MK951912	–
A. zutongqii	PPRI3429 = CMV011F7	*Aspergillus*	South Africa, Gauteng, Pretoria, 1988	Lab contaminant	–	–	MK451575	–
A. species	PPRI6060 = CMV004E8	*Candidi*	South Africa, Free State, Bloemfontein, 1995	Dung	MK450633	MK451000	MK451330	–
A. tritici	MRC3080 = CMV017B1	*Candidi*	South Africa, Mpumalanga, 1982	Maize *(Zea mays)*	–	–	MK951927	–
A. tritici	MRC418 = CMV016I7	*Candidi*	South Africa, North West Province, Brits, 1971	Sorghum malt	–	–	MK951916	–
A. ochraceus	PPRI26013 = CMV006D9	*Circumdati*	South Africa, Western Cape, 2018	Wheat *(Triticum sp)*	–	–	MK451474	–
A. ochraceus	PPRI6335 = CMV007B6	*Circumdati*	South Africa, Mpumalanga, Nelspruit, 1997	Cochecille insects	–	–	MK451476	–
A. ochraceus	PPRI6816 = CMV007B5	*Circumdati*	South Africa, North West, Potchefstroom, 1999	Cowpea *(Vigna ungiculata)*	–	–	MK451475	–
A. pallidofulvus	CMV012D2	*Circumdati*	South Africa, Limpopo, Groblersdal, 2018	Soil	MK450639	–	MK451477	–
A. sclerotiorum	PPRI8357 = CMV007B4	*Circumdati*	South Africa, 2006	Rat food	–	–	MK451507	–
A. westerdijkiae	PPRI5061 = CMV007B2	*Circumdati*	South Africa, Limpopo, Vaalwater, 1993	Chrysomelid beetle	–	–	MK451571	–
A. westerdijkiae	PPRI8700 = CMV007B7	*Circumdati*	South Africa, Limpopo , Kruger National Park, 2005	Mopane twigs and leaves *(Colophospermum mopane)*	–	–	MK451572	–
A. clavatus	PPRI13831 = CMV008F4	*Clavati*	South Africa, Gauteng, Bapsfontein, 2014	Barley seedling *(Hordeum vulgare)*	–	–	MK451347	–
A. clavatus	PPRI13832 = CMV005I8	*Clavati*	South Africa, Gauteng, Bapsfontein, 2014	Barley seedling *(Hordeum vulgare)*	–	–	MK451344	–
A. clavatus	PPRI14650 = CMV005I9	*Clavati*	South Africa, North Weat, Potchefstroom, 2014	Animal feed	–	–	MK451345	–
A. clavatus	PPRI17069 = CMV001F9	*Clavati*	South Africa, Gauteng, Near Delmas, 2014	Animal feed, maize kernels	–	–	MK451341	–
A. clavatus	PPRI21896 = CMV006A1	*Clavati*	South Africa, Western Cape, Malmesbury, 2016	Barley sprouted seed *(Hordeum vulgare)*	–	–	MK451346	–
A. clavatus	PPRI26042 = CMV013A3	*Clavati*	South Africa, 2018	Dragon fruit plant	–	–	MK451349	–
A. clavatus	PPRI26045 = CMV013B4	*Clavati*	Swaziland, 2018	Pig feed	–	–	MK451351	–
A. clavatus	PPRI26493 = CMV013A9	*Clavati*	Swaziland, 2018	Pig feed	–	–	MK951883	–
A. clavatus	PPRI26495 = CMV013B2	*Clavati*	Swaziland, 2018	Pig feed	–	–	MK451350	–

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Table 1. (Continued).

Species	Strains[1]	Section	Location collected / isolated, year	Host	GenBank nr			
					ITS	BenA	CaM	RPB2
A. clavatus	PPRI4976 = CMV010D7	*Clavati*	South Africa, Gauteng, Magaliesburg, 1994	Soil	–	–	MK451348	–
A. clavatus	PPRI8552 = CMV005I6	*Clavati*	South Africa, Mpumalanga, Lydenburg, 2007	Sunflower seed *(Helianthus annuus)*	–	–	MK451342	–
A. clavatus	PPRI9818 = CMV005I7	*Clavati*	South Africa, Free State, 2008	Sunflower soil	–	–	MK451343	–
A. giganteus	MRC453 = CMV016I9	*Clavati*	South Africa, Pretoria, unknown		–	–	MN031424	–
A. giganteus	PPRI26019 = CMV008C9	*Clavati*	South Africa, Limpopo, 2018	Chicken feed	MK450637	MK451147	MK451418	–
A. seifertii	PPRI26025 = CMV011E3	*Clavati*	South Africa, Free State, Golden Gate, unknown	Soil	MK450648	MK451205	MK451510	MK450801
A. seifertii	PPRI3211 = CMV006F5 (ex-type)	*Clavati*	South Africa, Free State, Golden Gate, 1988	Grassroots	MK450647	MK451093	MK451509	MK450800
A. dimorphicus	CMV012C9	*Cremei*	South Africa, Limpopo, Groblersdal, 2018	Soil	MK450634	MK451246	MK451357	–
A. dimorphicus	PPRI26031 = CMV012G4	*Cremei*	South Africa, Limpopo, Groblersdal, 2018	Soil	MK450646	MK451263	MK451508	MK450799
A. wentii	PPRI25999 = CMV003I2	*Cremei*	South Africa, 2017	Animal feed	–	–	MK451569	–
A. wentii	PPRI26048 = CMV013F6	*Cremei*	South Africa, Mpumalanga, Barberton, 2018	Wood in mine	–	–	MK451570	–
A. wentii	PPRI26349 = CMV016E7	*Cremei*	South Africa, Mpumalanga, Barberton, 2018	Wood in mine	–	–	MK951914	–
A. alliaceus	PPRI6826 = CMV007B1	*Flavi*	South Africa, Eastern Cape, Port Elizabeth, 1999	Moth larvae *(Cryptophlebia leucotreta)*	–	–	MK451307	–
A. flavus	CMV015C6 = 2019-M44	*Flavi*	South Africa, 2019	Wood pallet	–	–	MK951894	–
A. flavus	CMV015C7 = 2019-M44	*Flavi*	South Africa, 2019	Wood pallet	–	–	MK951895	–
A. flavus	CMV015C9 = 2019-M44	*Flavi*	South Africa, 2019	Wood pallet	–	–	MK951896	–
A. flavus	MRC1317 = CMV017A2	*Flavi*	South Africa, Western Cape, Somerset West, 1977	Lemon *(Citrus limon)*	–	–	MK951919	–
A. flavus	MRC1366 = CMV017A7	*Flavi*	South Africa, Western Cape, Ceres, 1978	Maize *(Zea mays)*, pathotoxicity to sheep	–	–	MK951924	–
A. flavus	MRC1745 = CMV017A8	*Flavi*	South Africa, North West Province, Potchefstroom, 1979	Sorghum malt	–	–	MK951925	–
A. flavus	MRC2526 = CMV017A9	*Flavi*	South Africa, unknown	Biltong	–	–	MK951926	–
A. flavus	MRC3732 = CMV017B3	*Flavi*	South Africa, Western Cape, Ceres, 1984	Apple	–	–	MK951929	–
A. flavus	MRC6979 = CMV017B5	*Flavi*	South Africa, Mpumalanga, Kruger National Park, unknown	Soil	–	–	MK951931	–
A. flavus	PPRI13141 = CMV002B4	*Flavi*	South Africa, Kwazulu Natal, Pietermaritzburg, unknown	Maize *(Zea mays)*	–	–	MK451376	–

Table 1. (Continued).

Species	Strains[1]	Section	Location collected / isolated, year	Host	GenBank nr			
					ITS	BenA	CaM	RPB2
A. flavus	PPRI18143 = CMV001I2	*Flavi*	South Africa, 2015	Rooibos *(Aspalathus linearis)*	–	–	MK451365	–
A. flavus	PPRI18144 = CMV001I8	*Flavi*	South Africa, 2015	Rooibos *(Aspalathus linearis)*	–	–	MK451370	–
A. flavus	PPRI18161 = CMV002B3	*Flavi*	South Africa, Free State, Bethlehem, 2015	Wheat (*Triticum sp*)	–	–	MK451375	–
A. flavus	PPRI18711 = CMV001I4	*Flavi*	South Africa, Northwest, Sannieshof, 2015	Frass of moth *(Busseola fusca)* feeding inside maize stems	–	–	MK451367	–
A. flavus	PPRI18712 = CMV001I5	*Flavi*	South Africa, Northwest, Sannieshof, 2015	Frass of moth *(Busseola fusca)* feeding inside maize stems	–	–	MK451368	–
A. flavus	PPRI18713 = CMV001I9	*Flavi*	South Africa, Northwest, Coligny, 2015	Frass of moth *(Busseola fusca)* feeding inside maize stems	–	–	MK451371	–
A. flavus	PPRI18714 = CMV001I1	*Flavi*	South Africa, Northwest, Coligny, 2015	Frass of moth *(Busseola fusca)* feeding inside maize stems	–	–	MK451364	–
A. flavus	PPRI18715 = CMV001I6	*Flavi*	South Africa, Northwest, Coligny, 2015	Frass of moth *(Busseola fusca)* feeding inside maize stems	–	–	MK451369	–
A. flavus	PPRI20581 = CMV002B1	*Flavi*	South Africa, Western Cape, Grabouw, 2015	Insect	–	–	MK451374	–
A. flavus	PPRI22482 = CMV001I3	*Flavi*	South Africa, Limpopo, Atlanta, 2016	Soya beans *(Glycine max)*	–	–	MK451366	–
A. flavus	PPRI23389 = CMV002A1	*Flavi*	South Africa, Western Cape, Stellenbosch, 2016	Animal feed	–	–	MK451372	–
A. flavus	PPRI25992 = CMV003A4	*Flavi*	South Africa, Western Cape, Knysna, 2017	Hominy chop animal feed	–	–	MK451379	–
A. flavus	PPRI26001 = CMV003I5	*Flavi*	South Africa, 2017	Animal feed	–	–	MK451380	–
A. flavus	PPRI26002 = CMV003I6	*Flavi*	South Africa, 2017	Animal feed	–	–	MK451381	–
A. flavus	PPRI26003 = CMV003I7	*Flavi*	South Africa, 2017	Animal feed	–	–	MK451382	–
A. flavus	PPRI26004 = CMV003I8	*Flavi*	South Africa, 2017	Animal feed	–	–	MK451383	–
A. flavus	PPRI26007 = CMV005E1	*Flavi*	South Africa, Gauteng, Sunninghill, 2017	Groundnut	–	–	MK451384	–
A. flavus	PPRI26022 = CMV008E3	*Flavi*	South Africa, Limpopo, 2018	Chicken feed	–	–	MK451385	–
A. flavus	PPRI26032 = CMV012H1	*Flavi*	South Africa, Gauteng, Pretoria, 2018	Dog food	–	–	MK451387	–
A. flavus	PPRI26036 = CMV012H8	*Flavi*	South Africa, Gauteng, Pretoria, 2018	Dog food	–	–	MK451388	–
A. flavus	PPRI26044 = CMV013B3	*Flavi*	Swaziland, 2018	Pig feed	–	–	MK451389	–
A. flavus	PPRI26345 = CMV016D6	*Flavi*	South Africa, Gauteng, Pretoria, 2019	Dog food	–	–	MK951910	–

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Table 1. (Continued).

Species	Strains[1]	Section	Location collected / isolated, year	Host	GenBank nr			
					ITS	BenA	CaM	RPB2
A. flavus	PPRI26347 = CMV016E4	*Flavi*	South Africa, Gauteng, Pretoria, 2019	Dog food	–	–	MK951913	–
A. flavus	PPRI26486 = CMV010D4	*Flavi*	South Africa, Limpopo, Groblersdal, 2015	Soil	–	–	MK451386	–
A. flavus	PPRI3274 = CMV002A5	*Flavi*	South Africa, Gauteng, Pretoria, 1988		–	–	MK451373	–
A. flavus	PPRI7977 = CMV002B5	*Flavi*	South Africa, 2005		–	–	MK451377	–
A. flavus	PPRI8551 = CMV002B7	*Flavi*	South Africa, Mpumalanga, Lydenburg, 2007	Maize *(Zea mays)*	–	–	MK451378	–
A. krugeri	PPRI8986 = CMV006G4 (ex-type)	*Flavi*	South Africa, Limpopo, Kruger National Park, 2005	Mopane debris *(Colophospermum mopane)*	MK450655	MK451098	MK451517	MK450808
A. krugeri	PPRI9280 = CMV002C8	*Flavi*	South Africa, Limpopo, Kruger National Park, 2005	Mopane debris *(Colophospermum mopane)*	MK450654	MK450928	MK451516	MK450807
A. magaliesburgensis	PPRI6165 = CMV007A3 (ex-type)	*Flavi*	South Africa, Gauteng, Magaliesburg, 1996	Antlion *(Myrmeleontidae)*	MK450649	MK451116	MK451511	MK450802
A. nomius	PPRI3753 = CMV002B2	*Flavi*	South Africa, Gauteng, Rietondale, 1989	Termites dead colony	–	MK450926	MK451473	–
A. parasiticus	PPRI14636 = CMV001H8	*Flavi*	South Africa, Gauteng, Bapsfontein, 2014	Spawnrun on grass	–	–	MK451478	–
A. parasiticus	PPRI14642 = CMV001H9	*Flavi*	South Africa, Gauteng, Bapsfontein, 2014	Spawnrun on grass	–	–	MK451479	–
A. parasiticus	PPRI23021 = CMV002C7	*Flavi*	South Africa, Dinaka game reserve, 2016	Animal feed	–	–	MK451483	–
A. parasiticus	PPRI26046 = CMV013B6	*Flavi*	Zambia, Mpangwe, Mpangwe, 2018	Wheat *(Triticum sp)*	–	–	MK451489	–
A. parasiticus	PPRI2885 = CMV007A7	*Flavi*	South Africa, 1990	Seed *(Watsonin marginata)*	–	–	MK451487	–
A. parasiticus	PPRI3754 = CMV007A5	*Flavi*	South Africa, Gauteng, Pretoria, 1989	Termites	–	–	MK451485	–
A. parasiticus	PPRI5183 = CMV007A6	*Flavi*	South Africa, Western Cape, Clanwilliam, 1993	Rooibos tea *(Aspalathus linearis)*	–	–	MK451486	–
A. parasiticus	PPRI7978 = CMV010B6	*Flavi*	South Africa, 2005		–	–	MK451488	–
A. parasiticus	PPRI9511 = CMV002B8	*Flavi*	South Africa, North West , 2008	Soil	–	–	MK451480	–
A. parasiticus	PPRI9513 = CMV002G1	*Flavi*	South Africa, North West , 2008	Soil	–	–	MK451484	–
A. parasiticus	PPRI9532 = CMV002C1	*Flavi*	South Africa, North West , 2008	Soil	–	–	MK451481	–
A. parasiticus	PPRI9534 = CMV002C2	*Flavi*	South Africa, North West , 2008	Soil	–	–	MK451482	–
A. pseudonomius	PPRI5063 = CMV002B6	*Flavi*	South Africa, Limpopo, Vaalwater, 1992	Chrysomelid beetle	–	–	MK451505	–

Table 1. (Continued).

Species	Strains[1]	Section	Location collected / isolated, year	Host	GenBank nr			
					ITS	BenA	CaM	RPB2
A. tamarii	PPRI26008 = CMV005E2	*Flavi*	South Africa, Gauteng, Sunninghill, 2017	Groundnut	–	–	MK451528	–
A. tamarii	PPRI26010 = CMV005E4	*Flavi*	South Africa, 2017	Soil	–	–	MK451529	–
A. tamarii	PPRI26023 = CMV008E4	*Flavi*	South Africa, Limpopo, 2018	Chicken feed	–	–	MK451531	–
A. tamarii	PPRI2812 = CMV003E1	*Flavi*	South Africa, 1991	Soya beans *(Glycine max)*	–	–	MK451527	–
A. tamarii	PPRI7392 = CMV007B3	*Flavi*	South Africa, 2004		–	–	MK451530	–
A. transmontanensis	PPRI14275 = CMV011A5	*Flavi*	Zambia, 2013	Soil	MK450657	MK451183	MK451519	MK450810
A. iizukae	PPRI4965 = CMV007B8	*Flavipedes*	South Africa, Gauteng, Pretoria, 1993	Chrysomelid beetle	–	–	MK451428	–
A. arcoverdensis	PPRI7491 = CMV003C4	*Fumigati*	South Africa, 2004		–	–	MK451311	–
A. arcoverdensis	PPRI7514 = CMV003C3	*Fumigati*	South Africa, 2004		–	–	MK451310	–
A. aureolus	PPRI11297 = CMV008A9	*Fumigati*	South Africa, Kwazulu Natal, Pinetown, 2011	Air sample	–	–	MK451321	–
A. aureolus	PPRI3451 = CMV011F8	*Fumigati*	South Africa, 1988		–	–	MK451322	–
A. elsenburgensis	DTO015G7	*Fumigati*	Argentina, La Pampa Province, Chacharramendi	Soil	MT110301	MT108410	MT108412	–
A. elsenburgensis	DTO380H5	*Fumigati*	Argentina, Catamarca Province	Soil	–	MT108411	MT108413	–
A. elsenburgensis	DTO381D3	*Fumigati*	Argentina, Catamarca Province	Soil	–	–	MT108414	–
A. elsenburgensis	DTO381D8	*Fumigati*	Argentina	Soil	MT110302	–	MT108415	–
A. elsenburgensis	PPRI2994 = CMV011G4 = CSIR1013 (ex-type)	*Fumigati*	South Africa, Western Cape, Elsenburg, 1986	Soil	MK450651	MK451215	MK451513	MK450804
A. fischeri	PPRI26026 = CMV011H6	*Fumigati*	South Africa, Gauteng, Pretoria, 2018	Lab contaminant	–	–	MK451359	–
A. fischeri	PPRI3418 = CMV012A7 = CSIR978	*Fumigati*	South Africa, 1988		–	–	MK451363	–
A. fischeri	PPRI3428 = CMV011I5 = CSIR990	*Fumigati*	South Africa, 1988		–	–	MK451361	–
A. fischeri	PPRI3488 = CMV012A6 = CSIR1039	*Fumigati*	South Africa, 1988		–	–	MK451362	–
A. fischeri	PPRI4507 = CMV011I4 = CSIR1094	*Fumigati*	South Africa, Eastern Cape, Butterworth, 1986	Soil	–	–	MK451360	–
A. fumigatiaffinis	PPRI13089 = CMV001G1	*Fumigati*	South Africa, Succulent karoo area , unknown	Soil	MK450636	MK450913	MK451390	–
A. fumigatiaffinis	PPRI13090 = CMV010I7	*Fumigati*	South Africa, Succulent karoo area , unknown	Soil	–	–	MK451392	–
A. fumigatiaffinis	PPRI3210 = CMV004C3	*Fumigati*	South Africa, Western Cape, Beaufort West, 1988	Grass	–	–	MK451391	–
A. fumigatus	CMV015C1 = 2019-M44	*Fumigati*	South Africa, 2019	Wood pallet	–	–	MK951890	–

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Table 1. (Continued).

Species	Strains[1]	Section	Location collected / isolated, year	Host	GenBank nr			
					ITS	BenA	CaM	RPB2
A. fumigatus	CMV015C2 = 2019-M44	*Fumigati*	South Africa, 2019	Wood pallet	–	–	MK951891	–
A. fumigatus	CMV015C3 = 2019-M44	*Fumigati*	South Africa, 2019	Wood pallet	–	–	MK951892	–
A. fumigatus	CMV015C5 = 2019-M44	*Fumigati*	South Africa, 2019	Wood pallet	–	–	MK951893	–
A. fumigatus	CMV015D8 = 2019-M44	*Fumigati*	South Africa, 2019	Wood pallet	–	–	MK951904	–
A. fumigatus	CMV015D9 = 2019-M44	*Fumigati*	South Africa, 2019	Wood pallet	–	–	MK951905	–
A. fumigatus	MRC435 = CMV016I8	*Fumigati*	South Africa, Port Health, 1971	Rice	–	–	MK951917	–
A. fumigatus	PPRI10161 = CMV002G6	*Fumigati*	South Africa, Eastern Cape, 2009	Silage	–	–	MK451396	–
A. fumigatus	PPRI10162 = CMV002G2	*Fumigati*	South Africa, Eastern Cape, 2009	Silage	–	–	MK451393	–
A. fumigatus	PPRI10498 = CMV003D5	*Fumigati*	South Africa, Eastern Cape, Port Elizabeth, 2010	Maize silage *(Zea mays)*	–	–	MK451406	–
A. fumigatus	PPRI10499 = CMV002G5	*Fumigati*	South Africa, Eastern Cape, Port Elizabeth, 2010	Maize silage *(Zea mays)*	–	–	MK451395	–
A. fumigatus	PPRI12665 = CMV002G3	*Fumigati*	South Africa, Free State, Luchhof, 2012	Rye seed *(Secale cereale)*	–	–	MK451394	–
A. fumigatus	PPRI13084 = CMV002G7	*Fumigati*	South Africa, Gauteng, Pretoria, 2013	Pear	–	–	MK451397	–
A. fumigatus	PPRI13252 = CMV003D6	*Fumigati*	South Africa, 2013		–	–	MK451407	–
A. fumigatus	PPRI20934 = CMV008B7	*Fumigati*	South Africa, 2016		–	MK451141	MK451412	–
A. fumigatus	PPRI25993 = CMV003A5	*Fumigati*	South Africa, Western Cape, Knysna, 2017	Hominy chop animal feed	–	–	MK451398	–
A. fumigatus	PPRI25998 = CMV003H8	*Fumigati*	South Africa, 2017	Animal feed	–	–	MK451409	–
A. fumigatus	PPRI26006 = CMV005D8	*Fumigati*	South Africa, Gauteng, Bedfordview, 2018	Potting soil	–	–	MK451411	–
A. fumigatus	PPRI3283 = CMV003C6	*Fumigati*	South Africa, North West, Pella, 1993	Soil	–	–	MK451399	–
A. fumigatus	PPRI3478 = CMV004C2	*Fumigati*	South Africa, Gauteng, Bapsfontein, 1988	Straw	–	–	MK451410	–
A. fumigatus	PPRI3479 = CMV003C7	*Fumigati*	South Africa, Gauteng, Johannesburg, 1988	Compost	–	–	MK451400	–
A. fumigatus	PPRI3505 = CMV010B9	*Fumigati*	South Africa, Gauteng, Denneboom, 1987	Pine	–	MK451153	MK451416	–
A. fumigatus	PPRI4975 = CMV003C8	*Fumigati*	South Africa, Mpumalanga, Malelane, 1993	Bagasse	–	–	MK451401	–
A. fumigatus	PPRI5090 = CMV003H1	*Fumigati*	South Africa, Mpumalanga, Malelane, 1993	Decayed mineola	–	MK450976	MK451408	–

Table 1. (Continued).

Species	Strains[1]	Section	Location collected / isolated, year	Host	GenBank nr			
					ITS	BenA	CaM	RPB2
A. fumigatus	PPRI7394 = CMV003C9	*Fumigati*	South Africa, 2004		–	–	MK451402	–
A. fumigatus	PPRI8522 = CMV003D1	*Fumigati*	South Africa, Mpumalanga, Vlakfontein, 2006	Chickens *(Gallus domesticus)*	–	–	MK451403	–
A. fumigatus	PPRI8523 = CMV003D2	*Fumigati*	South Africa, Mpumalanga, Vlakfontein, 2006	Chickens *(Gallus domesticus)*	–	–	MK451404	–
A. fumigatus	PPRI8525 = CMV008F9	*Fumigati*	South Africa, Mpumalanga, Vlakfontein, 2006	Chickens *(Gallus domesticus)*	–	–	MK451414	–
A. fumigatus	PPRI8527 = CMV008G1	*Fumigati*	South Africa, Mpumalanga, Vlakfontein, 2006	Chickens *(Gallus domesticus)*	–	–	MK451415	–
A. fumigatus	PPRI8558 = CMV003D3	*Fumigati*	South Africa, 2007	Chickens *(Gallus domesticus)*	–	–	MK451405	–
A. fumigatus	PPRI8560 = CMV008F8	*Fumigati*	South Africa, 2007	Chickens *(Gallus domesticus)*	–	–	MK451413	–
A. hiratsukae	PPRI3260 = CMV012G1 = CSIR1064	*Fumigati*	South Africa, 1988		–	–	MK451422	–
A. hiratsukae	PPRI9172 = CMV008F5	*Fumigati*	South Africa, Limpopo, Kruger National Park, 2005	Soil	–	–	MK451421	–
A. hiratsukae	PPRI9185 = CMV004E7	*Fumigati*	South Africa, Limpopo, Kruger National Park, 2005	Soil	–	–	MK451420	–
A. hiratsukae	PPRI9190 = CMV004E4	*Fumigati*	South Africa, Limpopo, Kruger National Park, 2005	Mopane twigs and leaves *(Colophospermum mopane)*	–	–	MK451419	–
A. laciniosus	PPRI3197 = CMV011I6 = CSIR1050	*Fumigati*	South Africa, 1988		–	–	MK451440	–
A. laciniosus	PPRI3247 = CMV011G5	*Fumigati*	South Africa, North West, Pella, 1988		–	MK451216	MK451439	–
A. laciniosus	PPRI3417 = CMV010F8 = CSIR638	*Fumigati*	South Africa, 1988		–	MK451163	MK451437	–
A. laciniosus	PPRI3847 = CMV011F6	*Fumigati*	South Africa, Kwazulu Natal, Greytown, 1985	Maize kernels *(Zea mays)*	–	–	MK451438	–
A. lentulus	PPRI6170 = CMV007I3	*Fumigati*	South Africa, Northern Cape, Loffiesdraai, 1996	Sand	–	MK451134	MK451442	–
A. lentulus	PPRI7532 = CMV003C5	*Fumigati*	South Africa, 2004		–	MK450952	MK451441	–
A. udagawae	PPRI11324 = CMV010I9	*Fumigati*	South Africa, Eastern Cape, Port Elizabeth, 2011	Mealy bug on Citrus	–	MK451179	MK451543	–
A. udagawae	PPRI26030 = CMV012F7	*Fumigati*	South Africa, Limpopo, Groblersdal, 2018	Soil	–	MK451259	MK451544	–
A. wyomingensis	PPRI5178 = CMV007I4	*Fumigati*	South Africa, Western Cape, Clanwilliam, 1993	Rooibos tea *(Aspalathus linearis)*	–	–	MK451574	–
A. wyomingensis	PPRI5573 = CMV007I2	*Fumigati*	South Africa, Western Cape, Clanwilliam, 1994	Rooibos tea *(Aspalathus linearis)*	–	–	MK451573	–
A. amoenus	PPRI26021 = CMV008E2	*Nidulantes*	South Africa, Limpopo, 2018	Chicken feed	–	–	MK451308	–
A. amoenus	PPRI26047 = CMV013F4	*Nidulantes*		Wood in mine	–	–	MK451309	–

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Table 1. (Continued).

Species	Strains[1]	Section	Location collected / isolated, year	Host	GenBank nr			
					ITS	BenA	CaM	RPB2
			South Africa, Mpumalanga, Barberton, 2018					
A. creber	PPRI13168 = CMV002A9	*Nidulantes*	South Africa, North West, Mafikeng, 2013	Chicken house bedding	–	–	MK451352	–
A. creber	PPRI3737 = CMV002G9	*Nidulantes*	South Africa, Gauteng, Pretoria, 1989	Orange *(Citrus sinensis)*	–	–	MK451353	–
A. creber	PPRI3869 = CMV011F9	*Nidulantes*	South Africa, Free State, Bloemfontein, 1990	Honey flower seed *(Melianthus comosus)*	–	–	MK451356	–
A. creber	PPRI5081 = CMV002H1	*Nidulantes*	South Africa, Mpumalanga, Hazyview, 1993	Lemon *(Citrus limon)*	–	–	MK451354	–
A. creber	PPRI9900 = CMV008C2	*Nidulantes*	South Africa, Kwazulu Natal, Pinetown, 2008		–	–	MK451355	–
A. jensenii	PPRI13238 = CMV001F7	*Nidulantes*	South Africa, KwaZulu-Natal, Pinetown, 2013	Environmental sample	–	–	MK451433	–
A. jensenii	PPRI2806 = CMV011G1	*Nidulantes*	South Africa, 1991		–	–	MK451436	–
A. jensenii	PPRI5384 = CMV007A9	*Nidulantes*	South Africa, 1993	Flower *(Gladiolus corms)*	–	–	MK451435	–
A. jensenii	PPRI6329 = CMV003H2	*Nidulantes*	South Africa, Kwazulu Natal, 1996	Contaminant bioproduct	–	MK450977	MK451434	–
A. nidulans	PPRI20935 = CMV010I1	*Nidulantes*	South Africa, 2016		–	–	MK451456	–
A. protuberus	PPRI26350 = CMV016F8	*Nidulantes*	South Africa, Mpumalanga, Barberton, 2018	Wood in mine	–	–	MK951915	–
A. protuberus	PPRI5575 = CMV008B2	*Nidulantes*	South Africa, 1994	Diesel fuel filters	–	–	MK451497	–
A. purpureocrustaceus	PPRI3840 = CMV008B3 (ex-type)	*Nidulantes*	South Africa, Limpopo, 1990	Plant debris	MK450653	MK451138	MK451515	MK450806
A. purpureocrustaceus	PPRI5548 = CMV008B1	*Nidulantes*	South Africa, Western Cape, Cape Town, 1994	Spider *(Palystes castaneus)*	MK450652	MK451137	MK451514	MK450805
A. quadrilineatus	PPRI26342 = CMV015B3	*Nidulantes*	South Africa, Mpumalanga, Marble Hall, 2019		–	–	MK951887	–
A. recurvatus	PPRI3165 = CMV010C4	*Nidulantes*	South Africa, 1988		MK450645	MK451157	MK451506	–
A. rugulosus	MRC3329 = CMV017B2	*Nidulantes*	South Africa, Free State, Clocolan, 1983	Oats	–	–	MK951928	–
A. sydowii	CMV008E1 = 2018-M76/352	*Nidulantes*	South Africa, Limpopo, 2018	Chicken feed	–	–	MK451524	–
A. sydowii	CMV015B9 = 2019-M44	*Nidulantes*	South Africa, 2019	Wood pallet	–	–	MK951889	–
A. sydowii	PPRI12668 = CMV008C6	*Nidulantes*	South Africa, KwaZulu-Natal, Pinetown, 2012	Environmental sample	–	–	MK451523	–
A. sydowii	PPRI13067 = CMV008B4	*Nidulantes*	South Africa, KwaZulu-Natal, Pinetown, 2012	Environmental sample	–	–	MK451521	–
A. sydowii	PPRI13241 = CMV001D6	*Nidulantes*	South Africa, KwaZulu-Natal, Pinetown, 2013	Environmental sample	–	–	MK451520	–

Table 1. (Continued).

Species	Strains[1]	Section	Location collected / isolated, year	Host	GenBank nr			
					ITS	BenA	CaM	RPB2
A. sydowii	PPRI3810 = CMV008F1	*Nidulantes*	South Africa, Free State, Bloemfontein, 1990	Honey flower seed *(Melianthus comosus)*	–	–	MK451525	–
A. sydowii	PPRI3839 = CMV008F2	*Nidulantes*	South Africa, 1990	*Watsonia marginata*	–	–	MK451526	–
A. sydowii	PPRI6542 = CMV008C5	*Nidulantes*	South Africa, Kwazulu Natal, Pinetown, 1997	Lab shelf	–	–	MK451522	–
A. alabamensis	PPRI25994 = CMV003A6	*Terrei*	South Africa, Western Cape, Knysna, 2017	Hominy chop animal feed	–	MK450947	MK451300	MK450758
A. alabamensis	PPRI25996 = CMV003A9	*Terrei*	South Africa, Western Cape, Knysna, 2017	Hominy chop animal feed	–	MK450948	MK451301	MK450759
A. alabamensis	PPRI26028 = CMV012E2	*Terrei*	South Africa, Limpopo, Groblersdal, 2018	Soil	–	–	MK451299	–
A. aureoterreus	PPRI13096 = CMV010F6	*Terrei*	South Africa, Succulent karoo area , unknown	Soil	–	MK451161	MK451323	MK450772
A. carneus	PPRI13094 = CMV010F7	*Terrei*	South Africa, Succulent karoo area , unknown	Soil	–	MK451162	MK451331	MK450778
A. cf alabamensis	PPRI7492 = CMV004A7	*Terrei*	South Africa, 2004		–	MK450983	MK451312	MK450765
A. cf alabamensis	PPRI8696 = CMV004D7	*Terrei*	South Africa, Limpopo , Kruger National Park, 2005	Soil	–	MK450993	MK451318	MK450770
A. cf alabamensis	PPRI8741 = CMV004C9	*Terrei*	South Africa, Limpopo, Kruger National Park, 2005	Soil	–	MK450990	MK451315	MK450768
A. cf alabamensis	PPRI8747 = CMV004D2	*Terrei*	South Africa, Limpopo, Kruger National Park, 2005	Soil	–	MK450991	MK451316	MK450769
A. cf alabamensis	PPRI8979 = CMV004D5	*Terrei*	South Africa, Limpopo, Kruger National Park, 2005	Soil	–	MK450992	MK451317	–
A. cf alabamensis	PPRI9150 = CMV004C8	*Terrei*	South Africa, Limpopo, Kruger National Park, 2005	Mopane debris *(Colophospermum mopane)*	–	MK450989	MK451314	MK450767
A. cf alabamensis	PPRI9184 = CMV004C7	*Terrei*	South Africa, Limpopo, Kruger National Park, 2005	Soil	–	MK450988	MK451313	MK450766
A. cf alabamensis	PPRI9189 = CMV004E1	*Terrei*	South Africa, Limpopo, Kruger National Park, 2005	Mopane twigs and leaves *(Colophospermum mopane)*	–	MK450995	MK451320	–
A. cf alabamensis	PPRI9206 = CMV004D9	*Terrei*	South Africa, Limpopo, Kruger National Park, 2005	Mopane twigs and leaves *(Colophospermum mopane)*	–	MK450994	MK451319	MK450771
A. cf allahabadii	PPRI5574 = CMV004C1	*Terrei*	South Africa, Western Cape, Clanwilliam, 1994	Rooibos tea *(Aspalathus linearis)*	–	MK450987	MK451302	MK450760
A. cf allahabadii	PPRI7534 = CMV004E6	*Terrei*	South Africa, Limpopo, Kruger National Park, 2003	Soil	–	MK450999	MK451306	MK450764
A. cf allahabadii	PPRI8751 = CMV004E3	*Terrei*	South Africa, Limpopo, Kruger National Park, 2005	Soil	MK450629	MK450997	MK451304	MK450762

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Table 1. (Continued).

Species	Strains[1]	Section	Location collected / isolated, year	Host	GenBank nr			
					ITS	BenA	CaM	RPB2
A. cf allahabadii	PPRI8987 = CMV004E2	*Terrei*	South Africa, Limpopo, Kruger National Park, 2005	Mopane debris *(Colophospermum mopane)*	MK450628	MK450996	MK451303	MK450761
A. cf allahabadii	PPRI9194 = CMV004E5	*Terrei*	South Africa, Limpopo, Kruger National Park, 2005	Mopane debris *(Colophospermum mopane)*	–	MK450998	MK451305	MK450763
A. citrinoterreus	PPRI7464 = CMV004A6	*Terrei*	South Africa, North West, Welwitschia, 2004		–	–	MK451340	–
A. heldtiae	PPRI4229 = CMV004A2 (ex-type)	*Terrei*	South Africa, 1991	Millet seed	MK450656	MK450981	MK451518	MK450809
A. hortai	PPRI25995 = CMV003A8	*Terrei*	South Africa, Western Cape, Knysna, 2017	Hominy chop animal feed	–	–	MK451423	–
A. hortai	PPRI5864 = CMV004A5	*Terrei*	South Africa, Gauteng, Onderstepoort, 1995	Animal tissue	–	–	MK451424	–
A. hortai	PPRI7533 = CMV004A9	*Terrei*	South Africa, Limpopo, Kruger National Park, 2003	Soil	–	MK450985	MK451425	–
A. hortai	PPRI8707 = CMV004C5	*Terrei*	South Africa, Limpopo , Kruger National Park, 2005	Mopane *(Colophospermum mopane)*	–	–	MK451427	–
A. hortai	PPRI9902 = CMV004B1	*Terrei*	South Africa, Gauteng, Pretoria, 2008	Industrial food colourant	–	–	MK451426	–
A. species CBS142751	PPRI9903 = CMV004A8	*Terrei*	South Africa, Gauteng, Pretoria, 2008	Industrial food colourant	MK450635	MK450984	MK451358	–
A. terreus	PPRI10373 = CMV007A8	*Terrei*	South Africa, Eastern Cape, Port Elizabeth, 2010	Maize silage *(Zea mays)*	–	–	MK451535	–
A. terreus	PPRI13086 = CMV011A4	*Terrei*	South Africa, Succulent karoo area , unknown	Soil	–	–	MK451537	–
A. terreus	PPRI20932 = CMV004B3	*Terrei*	South Africa, 2016		–	–	MK451533	–
A. terreus	PPRI25997 = CMV003H4	*Terrei*	South Africa, 2017	Animal feed	–	–	MK451532	–
A. terreus	PPRI26027 = CMV012E1	*Terrei*	South Africa, Limpopo, Groblersdal, 2018	Soil	–	–	MK451538	–
A. terreus	PPRI26029 = CMV012E3	*Terrei*	South Africa, Limpopo, Groblersdal, 2018	Soil	–	–	MK451539	–
A. terreus	PPRI8282 = CMV010F9	*Terrei*	South Africa, 2006		–	–	MK451536	–
A. terreus	PPRI8672 = CMV004B9	*Terrei*	South Africa, 2007	Kenaf *(Hibiscus cannabinus)*	–	–	MK451534	–
A. calidoustus	PPRI15353 = CMV006B3	*Usti*	South Africa, KwaZulu-Natal, Pinetown, 2014		–	–	MK451329	–
A. insuetus	MRC5597 = CMV017B4	*Usti*	South Africa, Western Cape, Cape Town, unknown	Direct scraping off Castle wall	–	–	MK951930	–
A. insuetus	PPRI3456 = CMV006F8	*Usti*	South Africa, 1988	Grass	–	–	MK451429	–
A. pseudodeflectus	PPRI5177a = CMV006F9	*Usti*	South Africa, Western Cape, Clanwilliam, 1993	Rooibos tea *(Aspalathus linearis)*	MK450644	MK451096	MK451503	–

Table 1. (Continued).

Species	Strains[1]	Section	Location collected / isolated, year	Host	GenBank nr			
					ITS	BenA	CaM	RPB2
A. pseudodeflectus	PPRI5177b = CMV010G3	*Usti*	South Africa, Western Cape, Clanwilliam, 1993	Rooibos tea *(Aspalathus linearis)*	–	–	MK451504	–
A. pseudodeflectus	PPRI8971 = CMV005H9	*Usti*	South Africa, Limpopo, Kruger National Park, 2005	Mopane debris *(Colophospermum mopane)*	MK450642	MK451064	MK451498	–
A. pseudodeflectus	PPRI8976 = CMV005I1	*Usti*	South Africa, Limpopo, Kruger National Park, 2005	Soil	–	–	MK451499	–
A. pseudodeflectus	PPRI9168 = CMV006A6	*Usti*	South Africa, Limpopo, Kruger National Park, 2008	Mopane twigs and leaves *(Colophospermum mopane)*	–	–	MK451502	–
A. pseudodeflectus	PPRI9203 = CMV005I2	*Usti*	South Africa, Limpopo, Kruger National Park, 2005	Mopane twigs and leaves *(Colophospermum mopane)*	MK450643	MK451065	MK451500	–
A. pseudodeflectus	PPRI9404 = CMV005I3	*Usti*	South Africa, Limpopo, Kruger National Park, 2005	Soil	–	–	MK451501	–
A. pseudoustus	MRC1233 = CMV017A5	*Usti*	South Africa, Western Cape, Drakenstein, 1975	Apple juice concentrate	–	–	MK951922	–
A. pseudoustus	MRC1234 = CMV017A4	*Usti*	South Africa, Western Cape, Drakenstein, 1975	Apple juice concentrate	–	–	MK951921	–
A. sigurros	PPRI15889 = CMV005I4 (ex-type)	*Usti*	South Africa, KwaZulu-Natal, Pinetown, 2014	Environmental sample	MK450650	MK451066	MK451512	MK450803

[1] Acronyms of culture collections: PPRI, culture collection of the National Collections of Fungi, housed at the Agricultural Research Council - Plant Health and Protection (ARC), Roodeplaat, South Africa; MRC, culture collection of the Medical Research Council housed at PPRI; CSIR, culture collection of the Council for Scientific and Industrial Research; CMV, working collection housed at the PPRI; DTO, working collection of the Applied and Industrial Mycology group housed the Westerdijk Institute, Utrecht, the Netherlands.

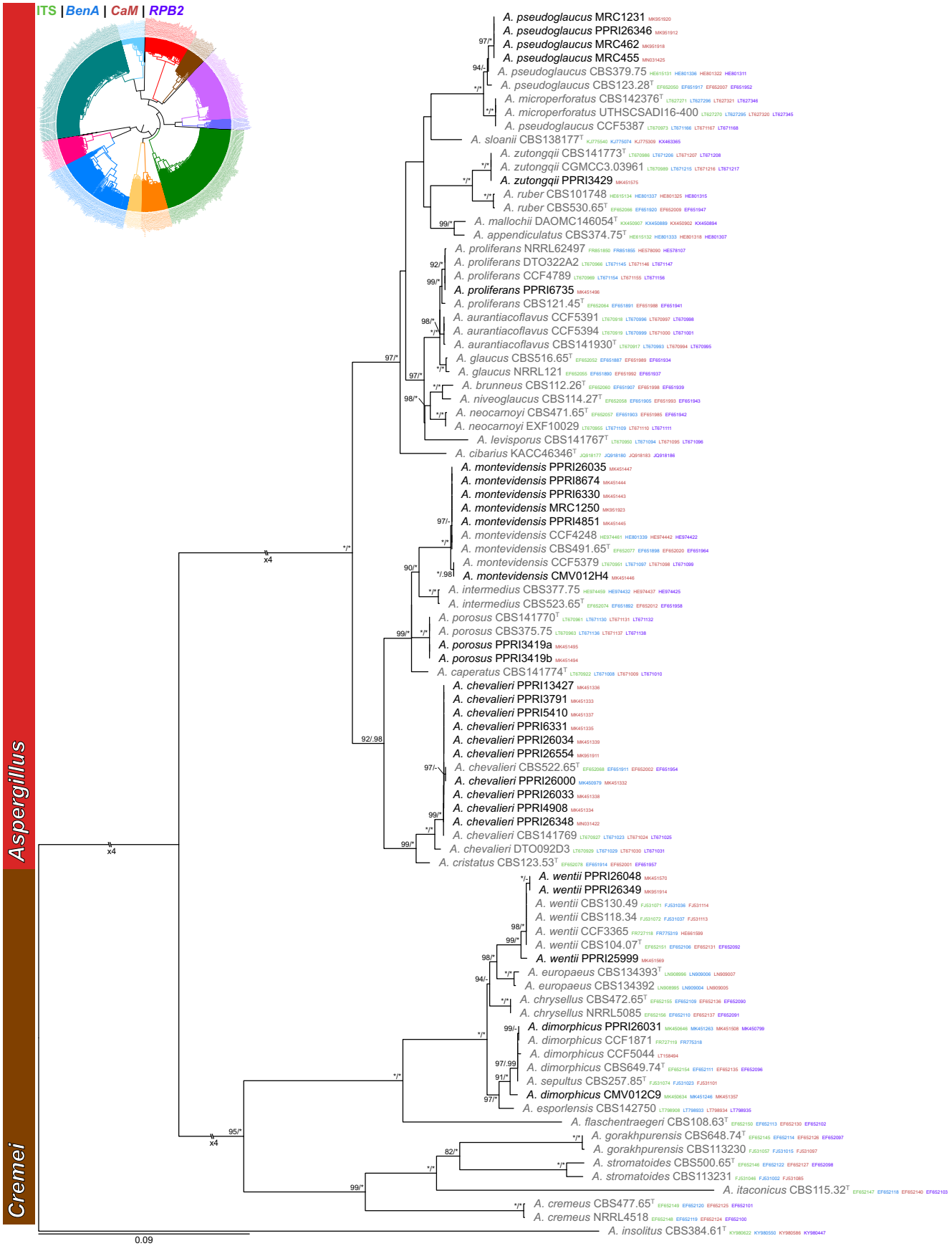

Fig. 1. Multigene phylogeny of *Aspergillus* sect *Aspergillus* and *Cremei* based on a combined ITS, *BenA*, *CaM* and *RPB2* dataset. Strains identified during this study are shown in black text and reference strains in grey text. GenBank accession numbers for ITS (green), *BenA* (blue), *CaM* (maroon) and *RPB2* (purple) are given behind strain numbers. Branch support in nodes higher than 80 % bs and/or 0.95 pp are indicated above thickened branches (^{T} = ex-type; * = 100 % bs or 1.00 pp; - = support lower than 80 % bs and/or 0.95 pp).

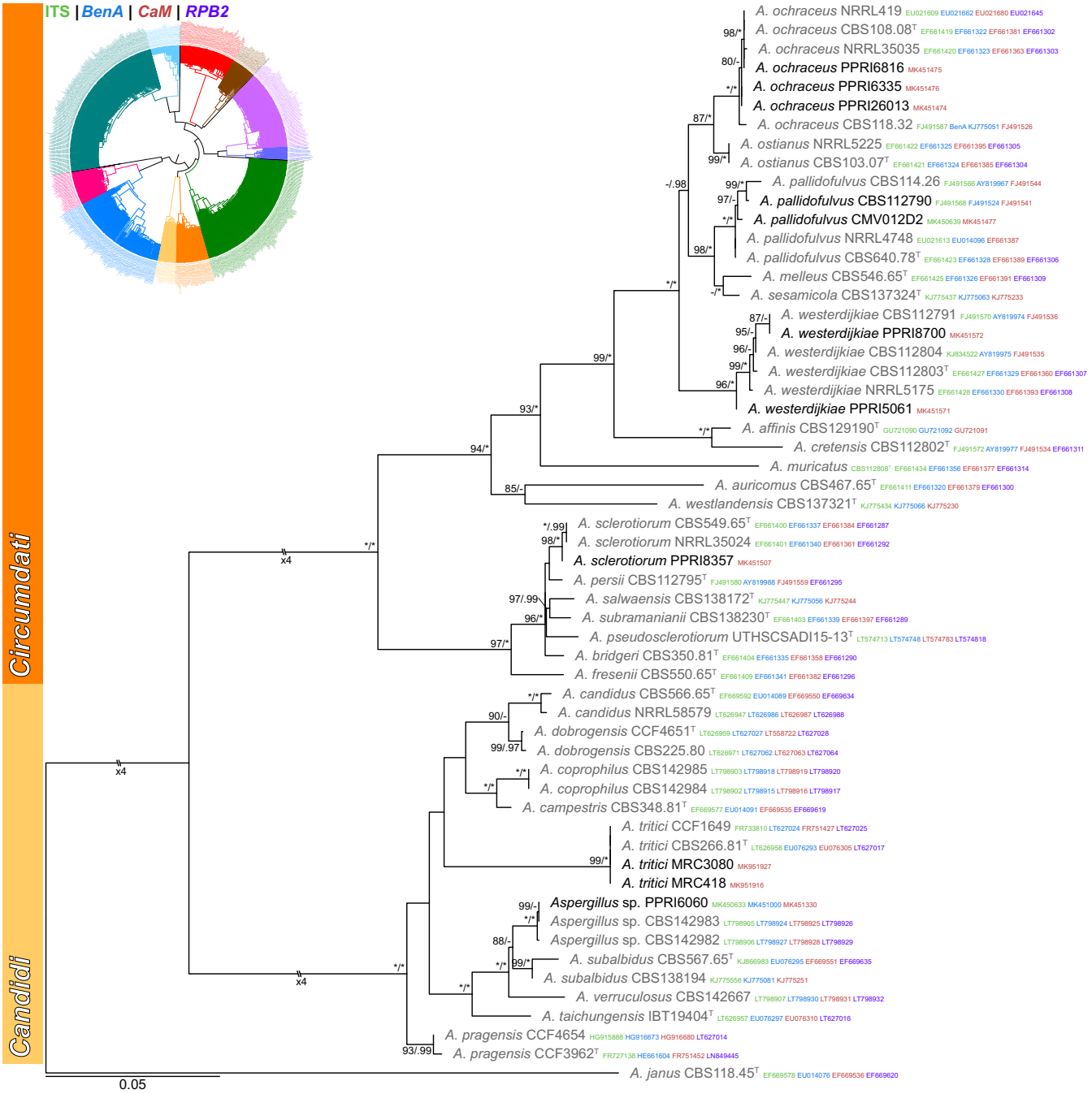

Fig. 2. Multigene phylogeny of *Aspergillus* sect *Circumdati* and *Candidi* based on a combined ITS, *BenA*, *CaM* and *RPB2* dataset. Strains identified during this study are shown in black text and reference strains in grey text. GenBank accession numbers for ITS (green), *BenA* (blue), *CaM* (maroon) and *RPB2* (purple) are given behind strain numbers. Branch support in nodes higher than 80 % bs and/or 0.95 pp are indicated above thickened branches (^{T} = ex-type; * = 100 % bs or 1.00 pp; - = support lower than 80 % bs and/or 0.95 pp).

easier and more accurate than ever. Almost anybody with a bit of background knowledge can describe their new species. As a result, the accepted species list grew with more than 100 taxa in the space of 5 years and resulted in the so-called "broad" *Aspergillus* that has mostly been accepted by the community (Pitt & Taylor 2014, 2016, Samson *et al.* 2014, Kocsube *et al.* 2016, Samson *et al.* 2017).

South Africa has great fungal diversity and makes significant contributions to international understanding of a wide range of fungi. *Aspergillus* is very commonly isolated across South Africa and unlike *Penicillium* (Schutte 1992), local mycologists were not afraid to attempt identifications down to species level (Cohen 1950, Swart 1959, Eicker 1969, 1970a, b, 1972, 1973, 1974, 1976, 1980, van der Merwe *et al.* 1979, Rabie & Lübben 1984, Allsop *et al.* 1987, Watson *et al.* 1990, Schutte 1994, Roux & van Warmelo 1997). These identifications were all based on morphology, meaning that diversity could easily be misidentified due to the known complexities in distinguishing between closely related species without DNA sequence data. Considering the modern methods required to identify species (Samson *et al.* 2014), we consider *Aspergillus* to be grossly understudied in South Africa. To our knowledge, the only modern studies reported 23 species isolated from house dust (Visagie *et al.* 2014a) and seven species from abalone feed collected in the Western Cape (Greeff-Laubscher *et al.* 2018). The PPRI culture collection housed at the Agricultural Research Council – Plant Health and Protection, Roodeplaat, Pretoria is the biggest repository of *Aspergillus* in South Africa with close to 500

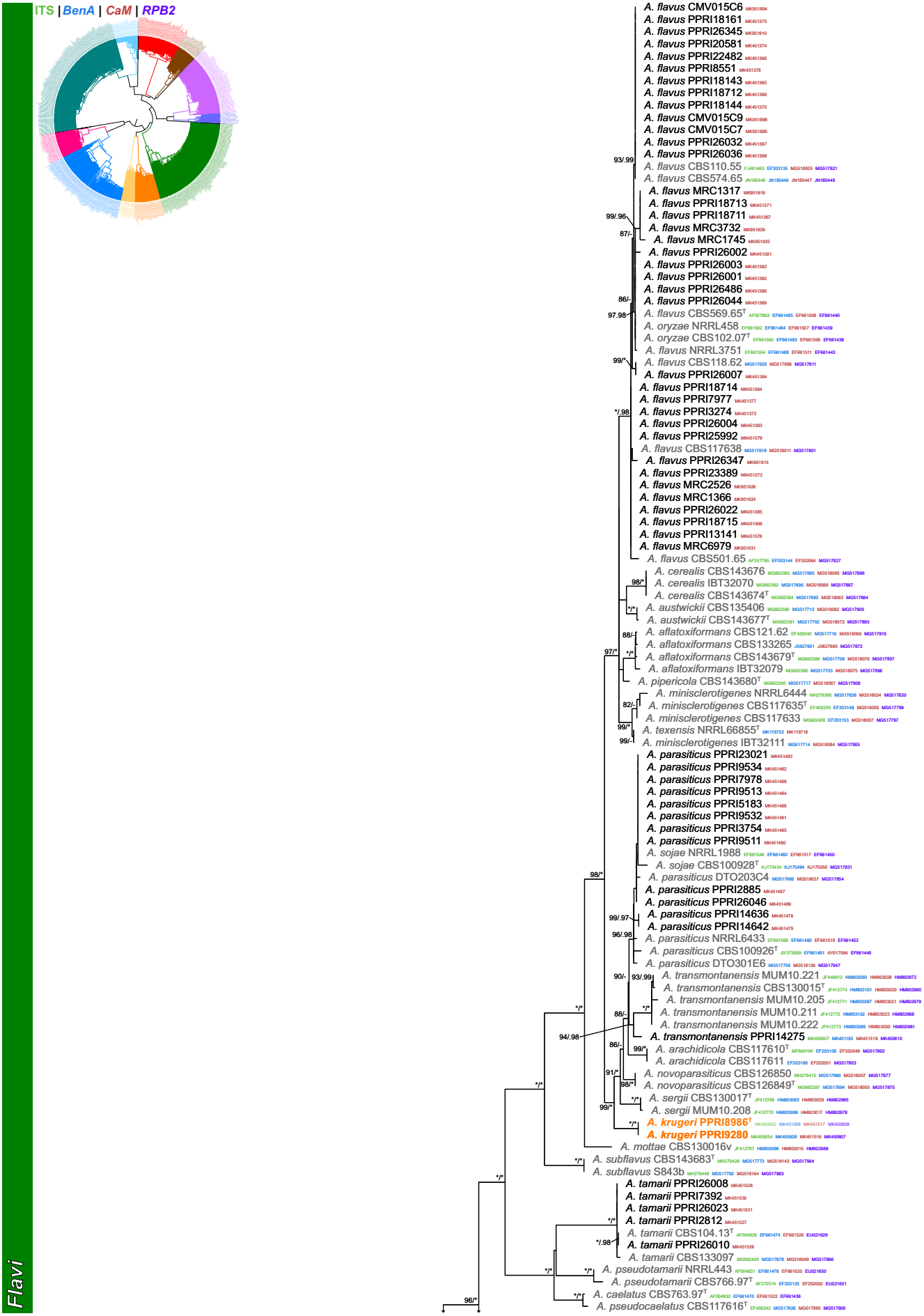

Fig. 3. Multigene phylogeny of *Aspergillus* sect *Flavi* based on a combined ITS, *BenA*, *CaM* and *RPB2* dataset. Strains from new species are shown in orange text, strains identified during this study in black text and reference strains in grey text. GenBank accession numbers for ITS (green), *BenA* (blue), *CaM* (maroon) and *RPB2* (purple) are given behind strain numbers. Branch support in nodes higher than 80 % bs and/or 0.95 pp are indicated above thickened branches (^{T} = ex-type; * = 100 % bs or 1.00 pp; - = support lower than 80 % bs and/or 0.95 pp).

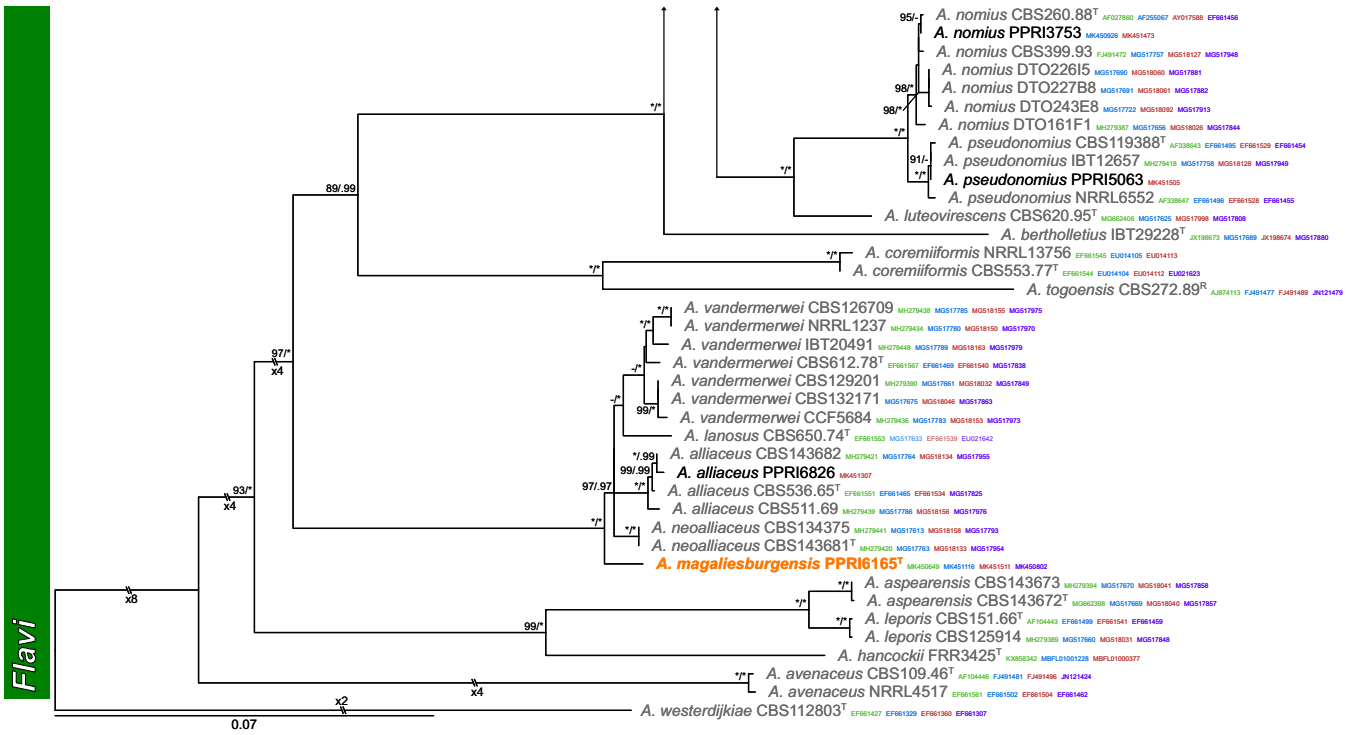

Fig. 3. (*Continued*).

accessioned strains. The PPRI also houses the old MRC (Medical Research Council) culture collection that contains several *Aspergillus*. Strains from these collections mostly originate from agricultural sources, but plenty was sourced from environmental collection trips across the country. The aim of this project was to recover as many strains as possible and re-identify them using modern DNA sequencing approaches in order to obtain a baseline knowledge on the diversity of *Aspergillus* in the country. In this paper, we report on the diversity discovered, formally introduce seven new species and release a large number of valuable DNA reference sequences in the NCBI nucleotide sequence database (GenBank).

MATERIALS & METHODS

Strains

Strains were recovered from the South African National Collection of Fungi (PPRI) and the Medical Research Council (MRC) collection, both housed at the Agricultural Research Council (ARC; Plant Health and Protection, Roodeplaat). New isolates were obtained during routine identification services provided at PPRI. These originate from a wide range of sources across the country and were deposited into a working collection (CMV) and PPRI. Isolations were made using potato dextrose agar (PDA) or dichloran 18 % glycerol agar (DG18; Oxoid CM0729). Strains and its collection data are summarised in Table 1.

DNA extraction, sequencing, and phylogenetic analysis

DNA was extracted from 7 d old colonies grown on Blakeslee's (1915) malt extract agar (MEAbl) using the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, CA, USA). The 5.8S rDNA internal transcribed spacer regions (ITS), beta-tubulin (*BenA*), calmodulin (*CaM*) and RNA polymerase II second largest subunit (*RPB2*) genes were amplified in a 25 µl PCR master mix containing 12.5 µl OneTaq® 2X Master Mix with GC Buffer (New England Biolabs$_{inc}$, MA, USA), 0.5 µl for each primer (10 µM), 10.5 µl milliQ H_2O, and 1 µl template DNA. PCR conditions and primers were used as suggested by Samson *et al.* (2014). Automated sequencing was done at Inqaba Biotechnical Industries (Pty) Ltd (Pretoria, South Africa) using the same primers used for PCR amplification. For *RPB2*, additional sequencing reactions were performed with internal sequencing primers RPB2-527R (Peterson 2008), RPB2-388F (Peterson 2008), RPB2-F311 (Houbraken & Samson 2011) and RPB2-R310 (Houbraken & Samson 2011).

Contigs were assembled and edited in Geneious Prime v. 2019.2.1 (BioMatters Ltd., Auckland, New Zealand), and new sequences deposited to GenBank (www.ncbi.nlm.nih.gov/genbank/). Accession numbers are listed in Table 1. Sequences were compared to a locally curated reference sequence dataset based on the ex-type sequences published in Samson *et al.* (2014). Preliminary identifications were made using this dataset in a local BLAST search tool in Geneious. Subsequent reference sequences were selected (Supplementary Table 1) based on these results, with GenBank accession numbers also shown on phylogenetic trees.

All datasets were aligned in MAFFT v. 7.427 (Katoh & Standley 2013) selecting the G-INS-I option, with alignments manually trimmed, adjusted and concatenated in Geneious where needed or appropriate. Aligned datasets were analysed using Maximum Likelihood (ML) and Bayesian tree Inference (BI). For concatenated phylogenies, each gene was treated as separate partitions. ML was performed using IQtree v. 1.6.11 (Nguyen *et al.* 2015). For each dataset or partition, the most suitable model was calculated using Modelfinder (Kalyaanamoorthy *et al.* 2017) and ultrafast bootstrapping approximation done using UFBoot2 (Hoang *et al.* 2018), both

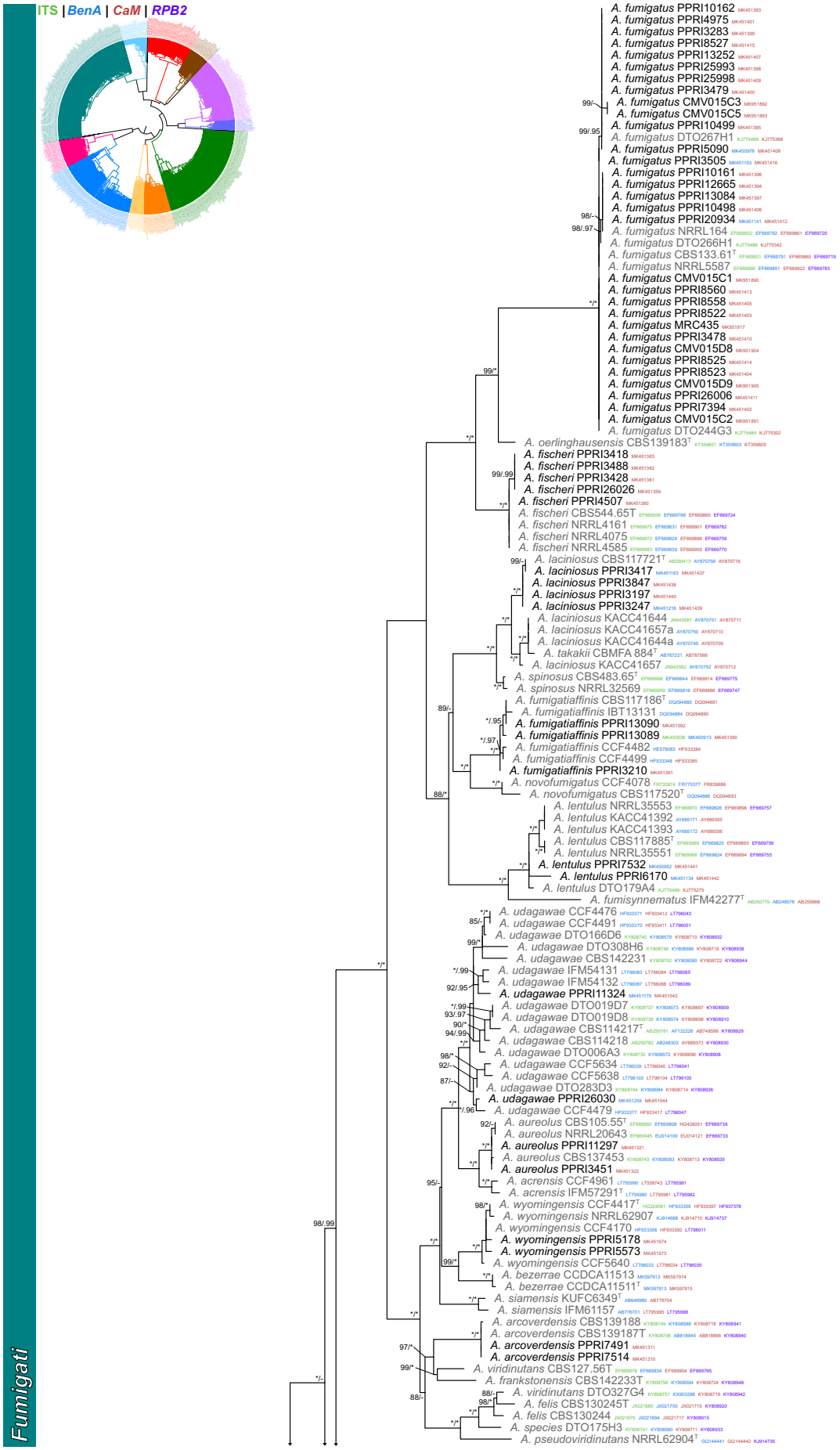

Fig. 4. Multigene phylogeny of *Aspergillus* sect *Fumigati* and *Clavati* based on a combined ITS, *BenA*, *CaM* and *RPB2* dataset. Strains from new species are shown in orange text, strains identified during this study in black text and reference strains in grey text. GenBank accession numbers for ITS (green), *BenA* (blue), *CaM* (maroon) and *RPB2* (purple) are given behind strain numbers. Branch support in nodes higher than 80 % bs and/or 0.95 pp are indicated above thickened branches (^{T} = ex-type; * = 100 % bs or 1.00 pp; - = support lower than 80 % bs and/or 0.95 pp).

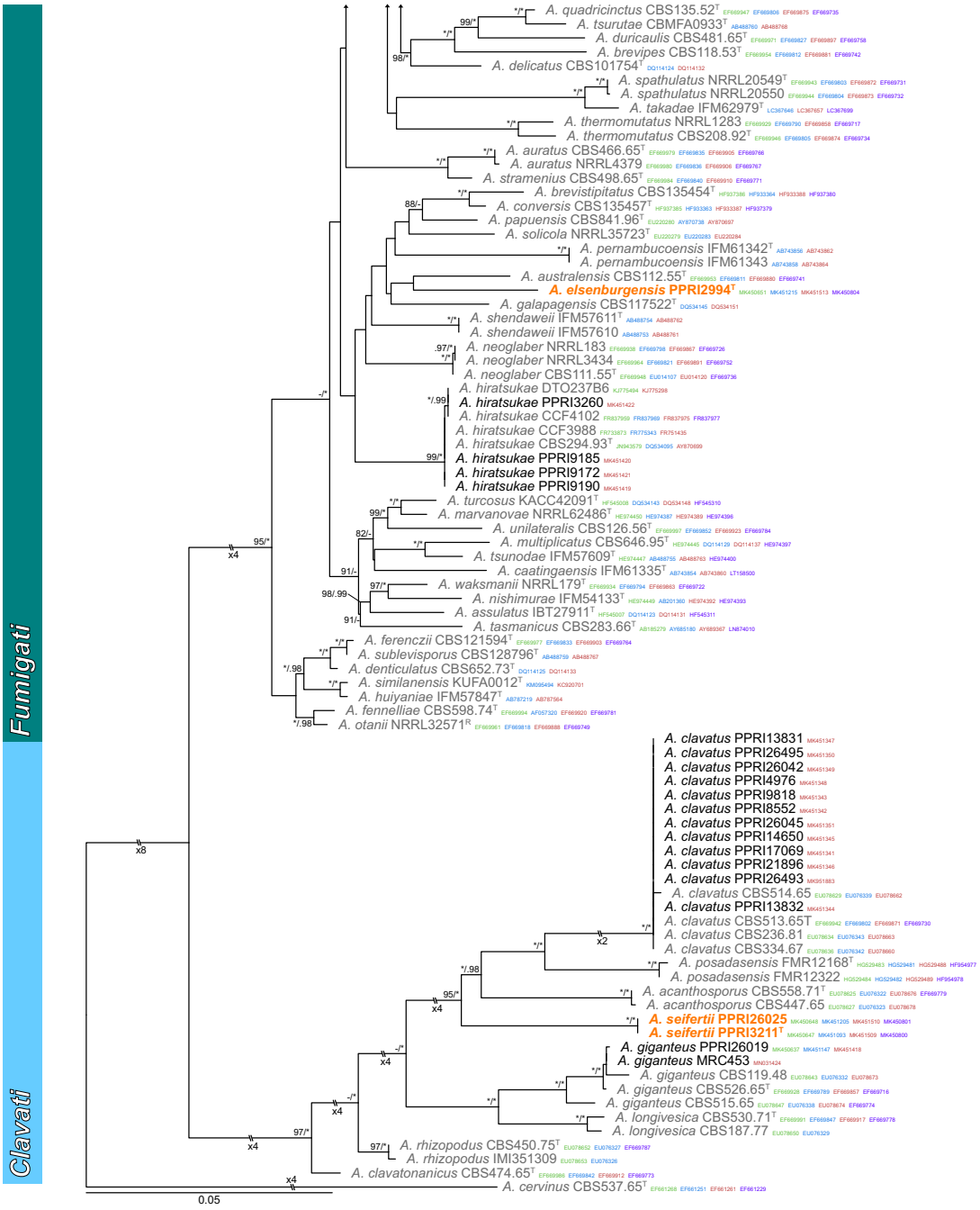

Fig. 4. (*Continued*).

integrated into IQtree. BI was performed using MrBayes v. 3.2.7 (Ronquist *et al.* 2012). The most suitable model for each dataset or partition was selected based on the Akaike information criterion (Akaike 1974) using MrModeltest v. 2.4 (Nylander 2004). Analyses were performed with three sets of four chains (1 cold and three heated) and were stopped at an average standard deviation for split frequencies of 0.01 using the stoprule. Trees were visualised in Figtree v. 1.4.4 (https://github.com/rambaut/figtree/releases) and visually prepared for publication in Affinity Designer v. 1.7.1 (Serif (Europe) Ltd, Nottingham, UK). ML and BI tree topologies did not differ, and thus the former was chosen to present results with both boostrap values and posterior probabilities shown for supported branches.

Several phylogenetic analyses were prepared. Firstly, a total phylogeny based on ITS, *BenA*, *CaM* and *RPB2* sequence data was calculated which covered all sections detected in this study. Secondly, smaller datasets were prepared based on observed relationships, which allowed for more reliable alignments and more presentable trees. Thirdly, single gene trees were calculated in the case of putative new species to apply the genealogical concordance phylogenetic species recognition concept (GCPSR) (Taylor *et al.* 2000).

Morphology

Morphological characterisation and species descriptions were made using standardised protocols published in Samson *et al.* (2014). Colony characters were captured on Czapek yeast autolysate agar (CYA), CYA with 5 % NaCl (CYAS), DG18, MEAbl (Oxoid LP0039 malt extract, Oxoid LP0034 peptone), MEA (Samson *et al.* 2010), oatmeal agar (OA), yeast extract sucrose agar (YES) and creatine sucrose agar (CREA). Strains were three-point inoculated on these media in 90 mm Petri dishes. Plates were incubated in darkness for 7 d at 25 °C, with


ITS | BenA | CaM | RPB2
Nidulantes
A. quadrilineatus CBS125.55
A. quadrilineatus PPRI26342
A. quadrilineatus CBS937.73
A. quadrilineatus CBS118.51
A. quadrilineatus CBS467.88
A. quadrilineatus CBS113684
A. quadrilineatus CBS853.96
A. quadrilineatus CBS591.65T
A. latus CBS492.65T
A. nidulans CBS240.90
A. nidulans PPRI20935
A. nidulans CBS589.65T
A. foveolatus CBS279.81T
A. corrugatus CBS191.77T
A. striatus CBS283.67T
A. rugulosus CBS117.50
A. rugulosus CBS113407
A. rugulosus MRC3329
A. rugulosus CBS130.48
A. rugulosus IBT12265
A. rugulosus CBS133.60T
A. rugulosus CBS200.75
A. pachycristatus IBT23550
A. pachycristatus IFM55265T
A. pachycristatus CBS198.88
A. spinulosporus CBS120.55T
A. jaipurensis CBS952.97T
A. omanensis CBMFA 700T
A. sulphureoviridis CBS140626T
A. violaceus CBS138.55T
A. aurantiopurpureus CBS140608T
A. navahoensis CBS351.81T
A. latilabiatus CBS426.93T
A. falconensis CBS271.91T
A. fruticulosus CBS486.65T
A. recurvatus CBS496.65T
A. recurvatus PPRI3165
A. recurvatus CBS126259
A. botswanensis CBS314.89T
A. savannensis CBS140607T
A. desertorum CBS653.73T
A. stercorarius CBS428.93T
A. pluriseminatus CBS102705
A. pluriseminatus NBRC33028
A. pluriseminatus CBS100523T
A. purpureocrustaceus PPRI5548
A. purpureocrustaceus PPRI3840T
A. tumidus CBS143587T
A. multicolor CBS133.54T
A. mulundensis IBT33104T
A. israelensis CBS140627T
A. unguis CBS132.55T
A. croceus CBS134396T
A. jensenii PPRI13238
A. jensenii PPRI5384
A. jensenii PPRI2806
A. jensenii PPRI6329
A. jensenii NRRL225
A. jensenii NRRL58600T
A. cvjetkovicii NRRL227T
A. tennesseensis NRRL13150T
A. puulaauensis NRRL35641T
A. creber PPRI13168
A. creber PPRI3869
A. creber PPRI5081
A. creber PPRI9900
A. creber NRRL58592T
A. creber NRRL58675
A. creber PPRI3737
A. venenatus NRRL13147T
A. sydowii CMV008E1
A. sydowii PPRI13067
A. sydowii PPRI3810
A. sydowii PPRI6542
A. sydowii PPRI3839
A. sydowii CMV015B9
A. sydowii PPRI12668
A. sydowii CBS593.65T
A. sydowii PPRI13241
A. sydowii NRRL4768
A. fructus NRRL239T
A. versicolor CBS583.65T
A. tabacinus CBS122718T
A. amoenus NRRL35600
A. amoenus PPRI26047
A. amoenus NRRL4838T
A. griseoaurantiacus CBS138191T
A. pepii CBS142028T
A. amoenus NRRL530
A. amoenus PPRI26021
A. austroafricanus NRRL233T
A. hongkongensis HKU49T
A. protuberus NRRL58747
A. protuberus PPRI26350
A. protuberus PPRI5575
A. protuberus CBS602.74T
A. subversicolor NRRL58999T
A. asperescens CBS110.51T
A. aureolatus CBS190.65T
A. askiburgiensis CBS134374T
A. spelunceus CBS497.65T
A. viridicatenatus CBS140629T
A. varians CBS505.65T
A. aurantiobrunneus CBS465.65T
A. purpureus CBS754.74T
A. sparsus CBS139.61T
0.05

additional CYA plates incubated at 30 and 37 °C. Colour names and codes used in descriptions follow Kornerup & Wanscher (1967). Microscopic observations were made using a Zeiss AXIO Imager.M2 compound and Zeiss AXIO Zoom.V16 microscopes equipped with AxioCaM MRc5 and 512 cameras driven by Zen Blue v. 2.3 software (Carl Zeiss CMP GmbH, Göttingen, Germany). Colonies were captured with a Sony NEX-5N camera. Extended Depth of Field analysis and stacking of colony texture micrographs were performed in Helicon Focus v. 7.5.4 (HeliconSoft, Kharkiv, Ukraine). Plates were prepared in Affinity Photo v. 1.7.1 (Serif (Europe) Ltd, Nottingham, UK). For aesthetic purposes, micrographs were adjusted using the "inpainting brush tool" without altering areas of scientific significance.

RESULTS

Strains

Of the ±320 PPRI strains selected for this study, ±250 were viable with 218 selected for sequencing. Eighteen MRC strains were sequenced. New isolations resulted in 65 strains, of which 51 were deposited in PPRI. DNA reference sequences (350 total: 24 ITS, 52 *BenA*, 250 *CaM*, 28 *RPB2*) were generated and submitted to GenBank during this study. Identified strains belonged to 63 species, representing 11 sections of *Aspergillus*. Seven of the species were found to be novel species and are described below in the Taxonomy section.

Phylogeny

For a general overview of results, a total phylogeny was calculated including all sequences generated during this study and reference sequences summarised in Supplementary Table 1. Results were summarised as a circular tree (Supplementary Fig. 1) and subsequently used as a baseline to calculate more focused phylogenies used to confirm final identifications and show relationships of the novel species.

Sections *Aspergillus* and *Cremei* (Fig. 1) — We identified six section *Aspergillus* species including *A. chevalieri, A. montevidensis, A. porosus, A. proliferans, A. pseudoglaucus* and *A. zutongqii*. This section was reviewed recently and two recently described species *A. porosus* and *A. zutongqii* are detected here (Chen *et al.* 2017). From section *Cremei*, we identified *A. wentii* and *A. dimorphicus*. *Aspergillus dimorphicus* and *A. sepultus* are phylogenetically identical. Since *A. dimorphicus* (Mehrotra & Prasad 1969) is the older name, *A. sepultus* (Tuthill & Christensen 1986) is synonymised with the former.

Sections *Candidi* and *Circumdati* (Fig. 2) — In section *Candidi*, only two species were identified. One strain represented *A. tritici*, while PPRI6060 resolved in a unique clade closely related to *A. subalbidus,* which represents a new species that will be described in a different paper. Section *Circumdati* typically contains ochratoxin A producing species (Visagie *et al.* 2014b, Frisvad & Larsen 2015a). Our study respectively identified strains as *A. ochraceus, A. pallidofulvus, A. sclerotiorum* and *A. westerdijkiae*.

Section *Flavi* (Fig. 3) — Among strains identified during this study, section *Flavi* was well represented. Seven known (*A. alliaceus, A. flavus, A. nomius, A. parasiticus, A. pseudonomius, A. tamarii* and *A. transmontanensis*) and two new species were detected. PPRI14275 consistently grouped basal to the *A. transmontanensis* clade. This single strain was morphologically identical to latter and we, therefore, identified it as *A. transmontanensis*. PPRI8986 and PPRI9280 formed a well-supported clade basal to the *A. parasiticus* clade and is described below as *A. krugeri*. PPRI6165 represented a unique lineage in the *A. vandermerweii, A. lanosus, A. alliaceus* and *A. neoalliaceus* clade, and is described as *A. magaliesburgensis* below.

Sections *Fumigati* and *Clavati* (Fig. 4) — Section *Fumigati* was well represented amongst strains identified during this study. Strains were identified into 11 known (*A. arcoverdensis, A. aureolus, A. fischeri, A. fumigatiaffinis, A. fumigatus, A. hiratsukae, A. laciniosus, A. lentulus, A. udagawae* and *A. wyomingensis*) and one new species described below as *A. elsenburgensis*. The multigene phylogeny resolved this strain as sister species to *A. australensis*. Strains previously identified as *A. laciniosus* resolved in two distinct clades. One clade containing the ex-type (CBS 117721^{T}) for *A. laciniosus* and the other the ex-type (CBM-FA884^{T}) for *A. takakii*. Three species were identified in section *Clavati*, including *A. clavatus, A. giganteus* and a new species described below as *A. seifertii*.

Section *Nidulantes* (Fig. 5) — Ten section *Nidulantes* species were identified during this study: *A. amoenus*, *A. creber*, *A. jensenii*, *A. nidulans*, *A. protuberus*, *A. quadrilineatus*, *A. recurvatus*, *A. rugulosus*, *A. sydowii*, and one new species described below as *A. purpureocrustaceus*. The new species resolved as a close relative of *A. tumidus*.

Sections *Terrei* and *Flavipedes* (Fig. 6) — Ten section *Terrei* species were identified during this study: *A. alabamensis, A. aureoterreus, A. carneus*, *A. citrinoterreus, A. hortai*, *A. terreus* and four new species. One of these new species is described below as *A. heldtiae*, which consistently resolved as a sister species to *A. pseudoterreus.* The remaining three species or clades were temporarily named *A.* cf. *alabamensis*, *A.* cf. *allahabadii* and *Aspergillus* sp. CBS 142751 as they will be described in a separate paper. *Aspergillus iizukae* was the only species identified from section *Flavipedes.*

Section *Usti* (Fig. 7) — Five section *Usti* species were identified during this study and included *A. calidoustus, A. insuetus, A. pseudodeflectus* and *A. pseudoustus,* while one new species is described below as *A. sigurros*. The new species resolved in a clade with *A. carlsbadensis* and *A. contaminans*. Based on phylogenetic results, the more recently described *A. fuscicans* (Romero *et al.* 2018) should be considered a synonym of the older *A. pseudodeflectus* (Samson & Mouchacca 1975).

Section *Nigri* — The PPRI collection contained a large number of black *Asperillus* strains classified in section *Nigri*. Full results will be published elsewhere. Strains were identified into nine species as *A. aculeatus, A. brasiliensis, A.*

Fig. 5. Multigene phylogeny of *Aspergillus* sect *Nidulantes* based on a combined ITS, *BenA*, *CaM* and *RPB2* dataset. Strains from new species are shown in orange text, strains identified during this study in black text and reference strains in grey text. GenBank accession numbers for ITS (green), *BenA* (blue), *CaM* (maroon) and *RPB2* (purple) are given behind strain numbers. Branch support in nodes higher than 80 % bs and/or 0.95 pp are indicated above thickened branches (^{T} = ex-type; * = 100 % bs or 1.00 pp; - = support lower than 80 % bs and/or 0.95 pp).

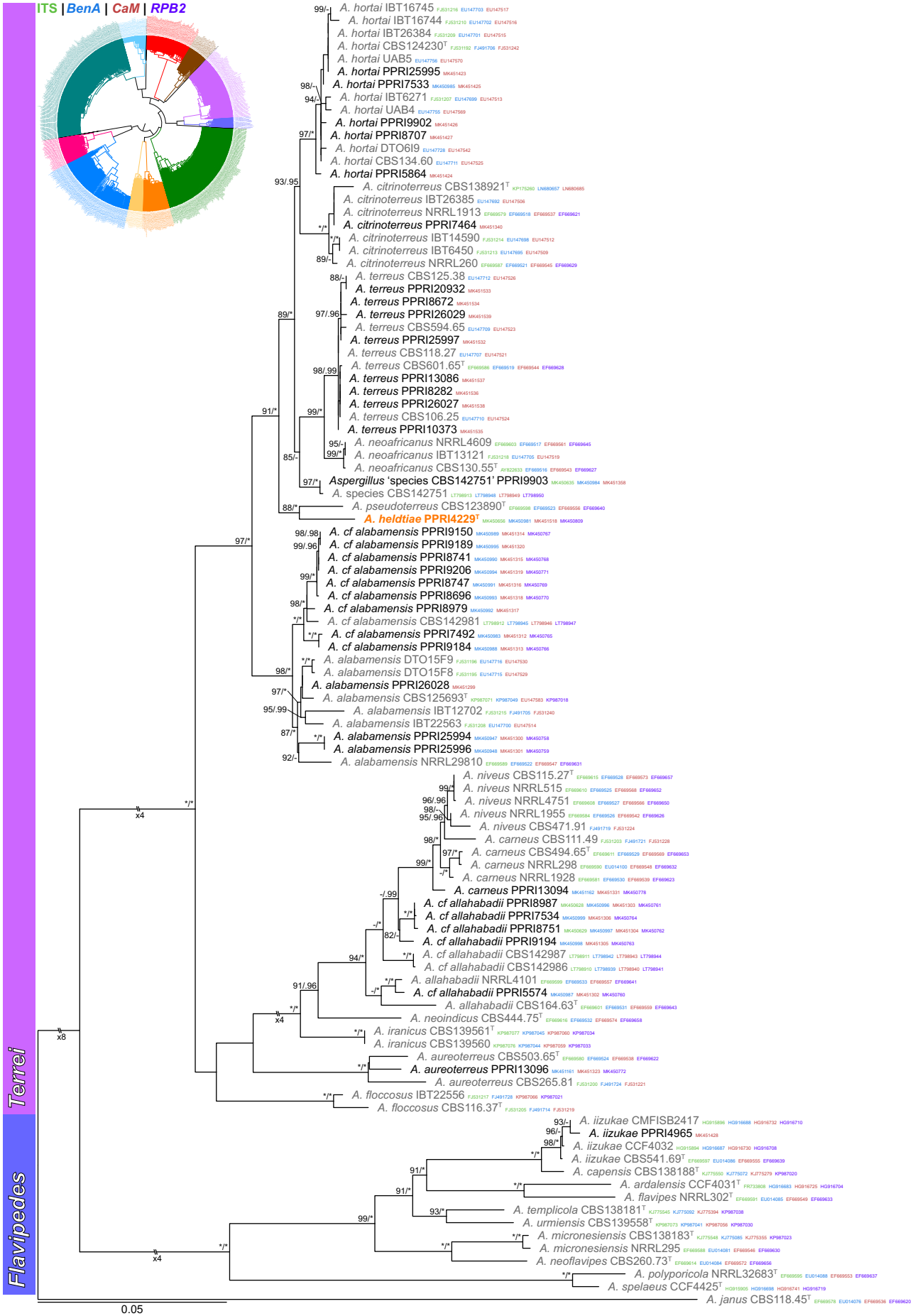

Fig. 6. Multigene phylogeny of *Aspergillus* sect *Terrei* and *Flavipedes* based on a combined ITS, *BenA*, *CaM* and *RPB2* dataset. Strains from new species are shown in orange text, strains identified during this study in black text and reference strains in grey text. GenBank accession numbers for ITS (green), *BenA* (blue), *CaM* (maroon) and *RPB2* (purple) are given behind strain numbers. Branch support in nodes higher than 80 % bs and/or 0.95 pp are indicated above thickened branches (^{T} = ex-type; * = 100 % bs or 1.00 pp; - = support lower than 80 % bs and/or 0.95 pp).

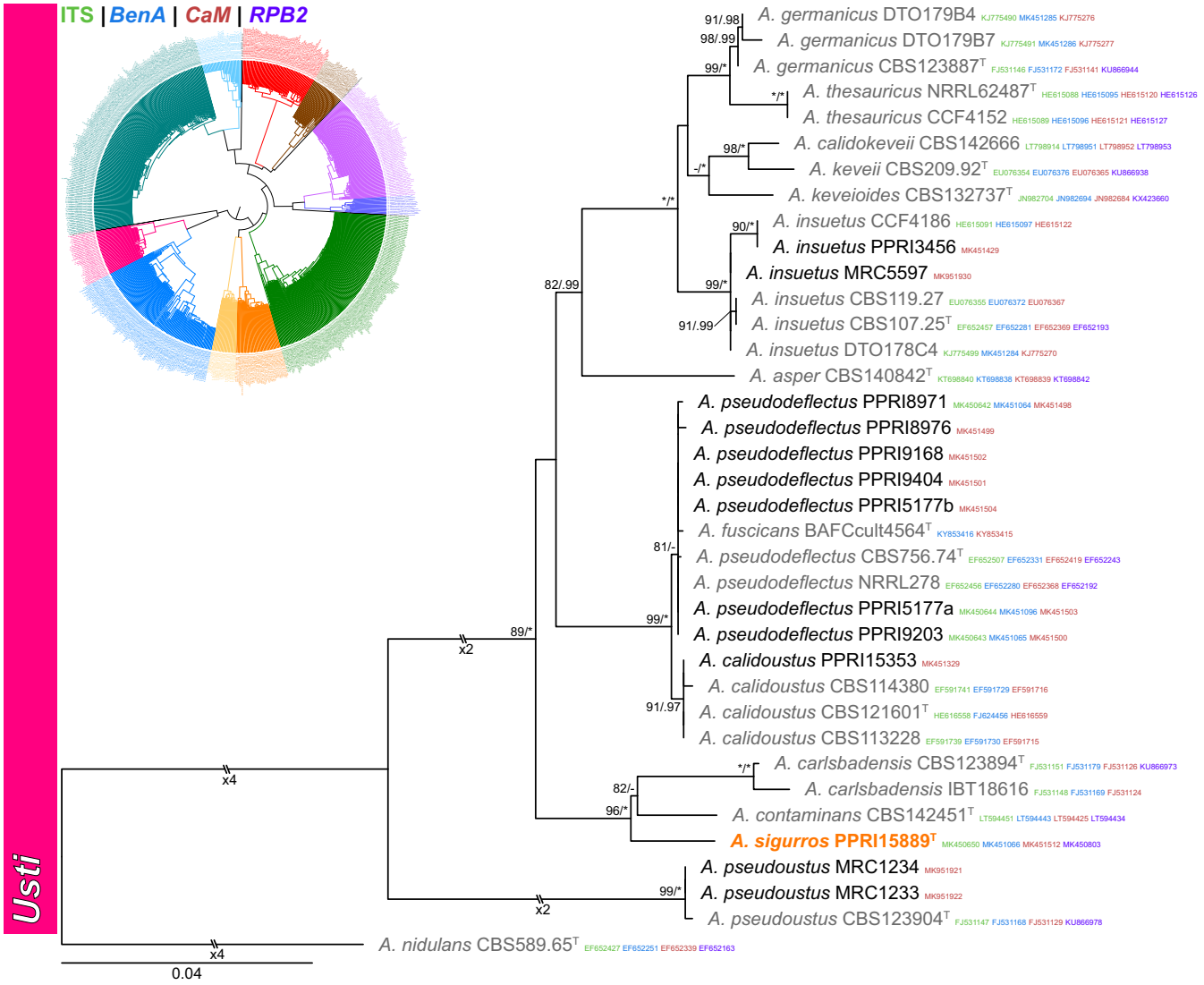

Fig. 7. Multigene phylogeny of *Aspergillus* sect *Usti* based on a combined ITS, *BenA*, *CaM* and *RPB2* dataset. Strains from new species are shown in orange text, strains identified during this study in black text and reference strains in grey text. GenBank accession numbers for ITS (green), *BenA* (blue), *CaM* (maroon) and *RPB2* (purple) are given behind strain numbers. Branch support in nodes higher than 80 % bs and/or 0.95 pp are indicated above thickened branches (^{T} = ex-type; * = 100 % bs or 1.00 pp; - = support lower than 80 % bs and/or 0.95 pp).

brunneoviolaceus, A. japonicus, A. neoniger, A. niger, A. piperis, A. tubingensis and *A. welwitschiae.*

Morphology

We introduce seven new species in the Taxonomy section below. These species belong to sections *Clavati*, *Flavi, Fumigati, Nidulantes, Terrei* and *Usti* based on the phylogenetic analyses. Strains conformed to the general morphological characters previously observed for species accepted in these sections. All of the new species were compared with respective close relatives, with notes provided on distinguishing characters after each species description in the Taxonomy section.

TAXONOMY

Aspergillus elsenburgensis Visagie, S.M. Romero & Houbraken, ***sp. nov.*** MycoBank MB834199. Fig. 14.

Etymology: Latin, *elsenburgensis*, named after Elsenburg, the town the ex-type was collected from.

Classification: *Eurotiomycetes*, *Eurotiales*, *Aspergillaceae*, *Aspergillus* section *Fumigati.*

Diagnosis: Colonies showing faster growth at 37 °C than at 25 °C, white and floccose, ascomata produced in aerial hyphae after prolonged incubation, white to cream colored, sporulation very sparse, conidiophores with short stipes (10–70 µm) and small globose conidia (1.5–2 µm).

Typus: **South Africa**, Western Cape, Elsenburg, soil, June 1986, (**holotype** PREM 62313, culture ex-type PPRI 2994 = CMV 011G4 = CSIR1013).

ITS Barcode: MK450651 (alternative identification markers: *BenA* = MK451215; *CaM* = MK451513; *RPB2* = MK450804).

Colony diam (7 d, in mm): CYA 35–40; CYA 30 °C 50–53; CYA 37 °C 50–60; CYAS 8–12; MEAbl 55–60; MEA 40–45; DG18 20–25; YES 45–50; OA 45–50; CREA 35–36.

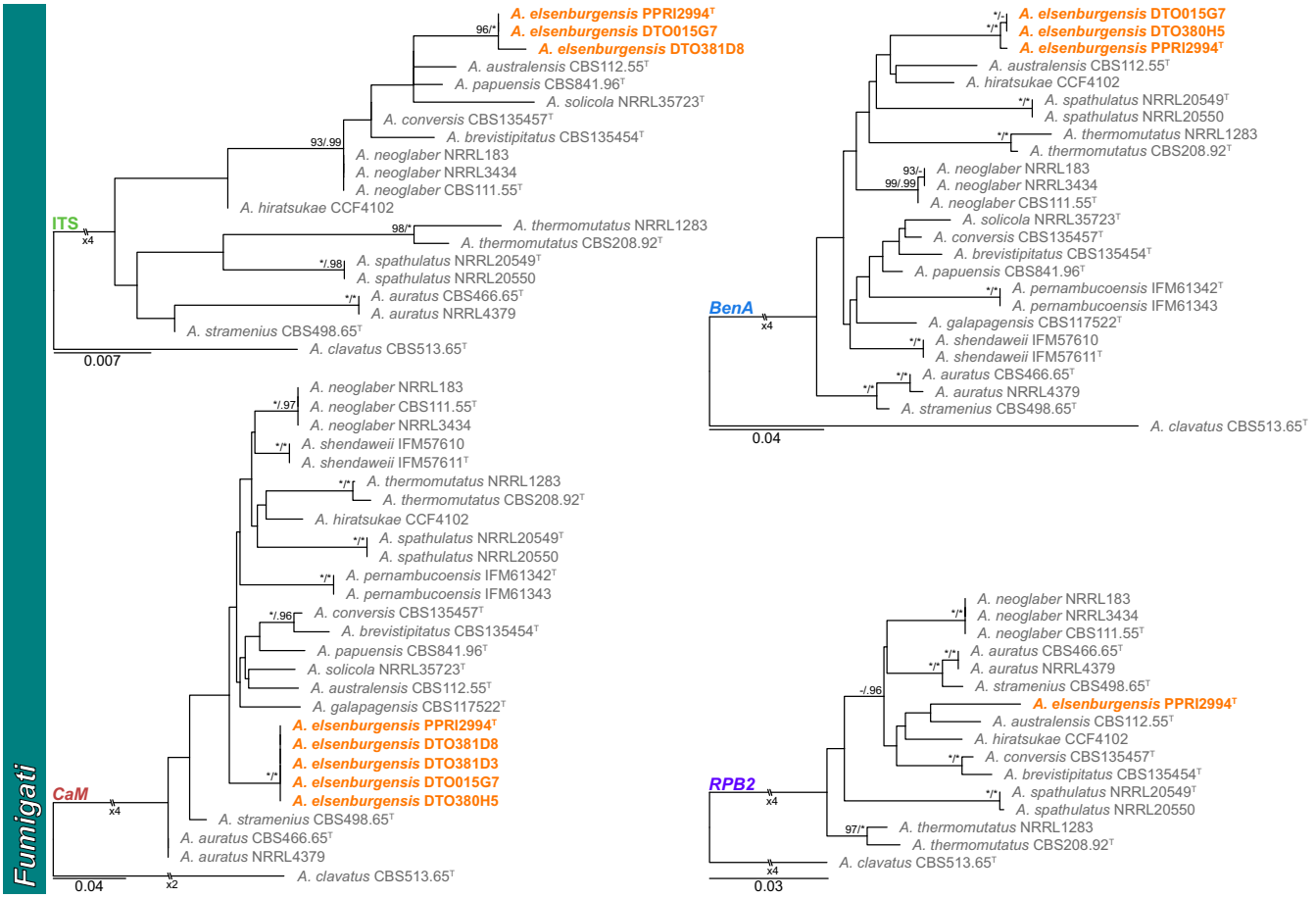

Fig. 8. Single gene phylogenies of *Aspergillus* sect *Fumigati* based on ITS, *BenA*, *CaM* and *RPB2*. Strains from new species are shown in orange text and reference strains in grey text. Branch support in nodes higher than 80 % bs and/or 0.95 pp are indicated above thickened branches (^{T} = ex-type; * = 100 % bs or 1.00 pp; - = support lower than 80 % bs and/or 0.95 pp).

Colony characters (25 °C, 7 d): *CYA* colonies surface floccose, mycelial areas white, ascomata present after prolonged incubation, produced in aerial hyphae, sparse sporulation present after >2 wk incubation, greenish, soluble pigment absent, exudate clear, reverse pigmentation yellowish white to pale yellow (3A2–3A3). *MEAbl* colonies surface floccose, mycelial areas white, ascomata present after prolonged incubation, produced in aerial hyphae, sporulation absent, sparse sporulation present after >2 wk incubation, greenish, soluble pigment absent, exudate clear, reverse pigmentation yellowish white to pale yellow (3A2–3A3). *YES* colonies surface floccose, mycelial areas white, sporulation absent, soluble pigment absent, exudate clear, reverse pigmentation yellowish white to pale yellow (3A2–3A3). *DG18* colonies surface floccose, mycelial areas white, sporulation sparse, white but becomes greenish with age, soluble pigment absent, exudate clear, reverse pigmentation yellowish white to pale yellow (3A2–3A3). *CREA* colonies weak growth, acid not produced.

Micromorphology: Conidial heads radiate. *Conidiophores* uniseriate. *Stipes* hyaline, smooth, 10–70 × 2.5–4(–4.5) µm. *Vesicles* subclavate, phialides cover 50 % of head, 5–8 µm wide. *Phialides* ampulliform, 4.5–6 × 2–3 µm. *Conidia* globose, smooth, 1.5–2.5 × 1.5–2.5 µm, (2.06 ± 0.15 × 2 ± 0.16, n = 56) µm, length/width 1.03 ± 0.05. *Ascomata* neosartorya-like, white to cream, abundant on OA, 70–270 µm. *Asci* 8-spored, 9–15 µm. *Ascospores* smooth, with 2 prominent equatorial furrow, globose to subglobose from the top, 4–5 × 3.5–5 µm (4.5 ± 0.2 × 4.2 ± 0.3, n = 43) µm, length/width 1.08 ± 0.07.

Notes: The multigene phylogeny resolves *A. elsenburgensis* as a close relative of *A. australensis* and *A. galapagensis* in section *Fumigati* (Figs 4, 8). The new species grows faster on MEAbl and have somewhat longer stipes than *A. australensis* (55–60 *vs* 40–45 mm; up to 70 µm *vs* up to 30 µm (Samson *et al.* 2007)). Compared to *A. galapagensis*, *A. elsenburgensis* shows slightly faster growth on most media, while it also produces smaller conidia (1.5–2 *vs* 2.5–3 µm (Samson *et al.* 2007)).

Aspergillus heldtiae Visagie, ***sp. nov.*** MycoBank MB834200. Fig. 15.

Etymology: Latin, *heltdiae*, named after Margaret Vinci Heldt, the creator of the beehive hairstyle that was popular during the 1960s and famously Marge Simpson's choice of hairstyle. This species resembles the beehive when observed through a dissection microscope.

Classification: *Eurotiomycetes*, *Eurotiales*, *Aspergillaceae*, *Aspergillus* section *Terrei*.

Diagnosis: Colonies showing rapid growth, bright yellow mycelial areas, cinnamon sporulation, conidiophores biseriate, vesicle

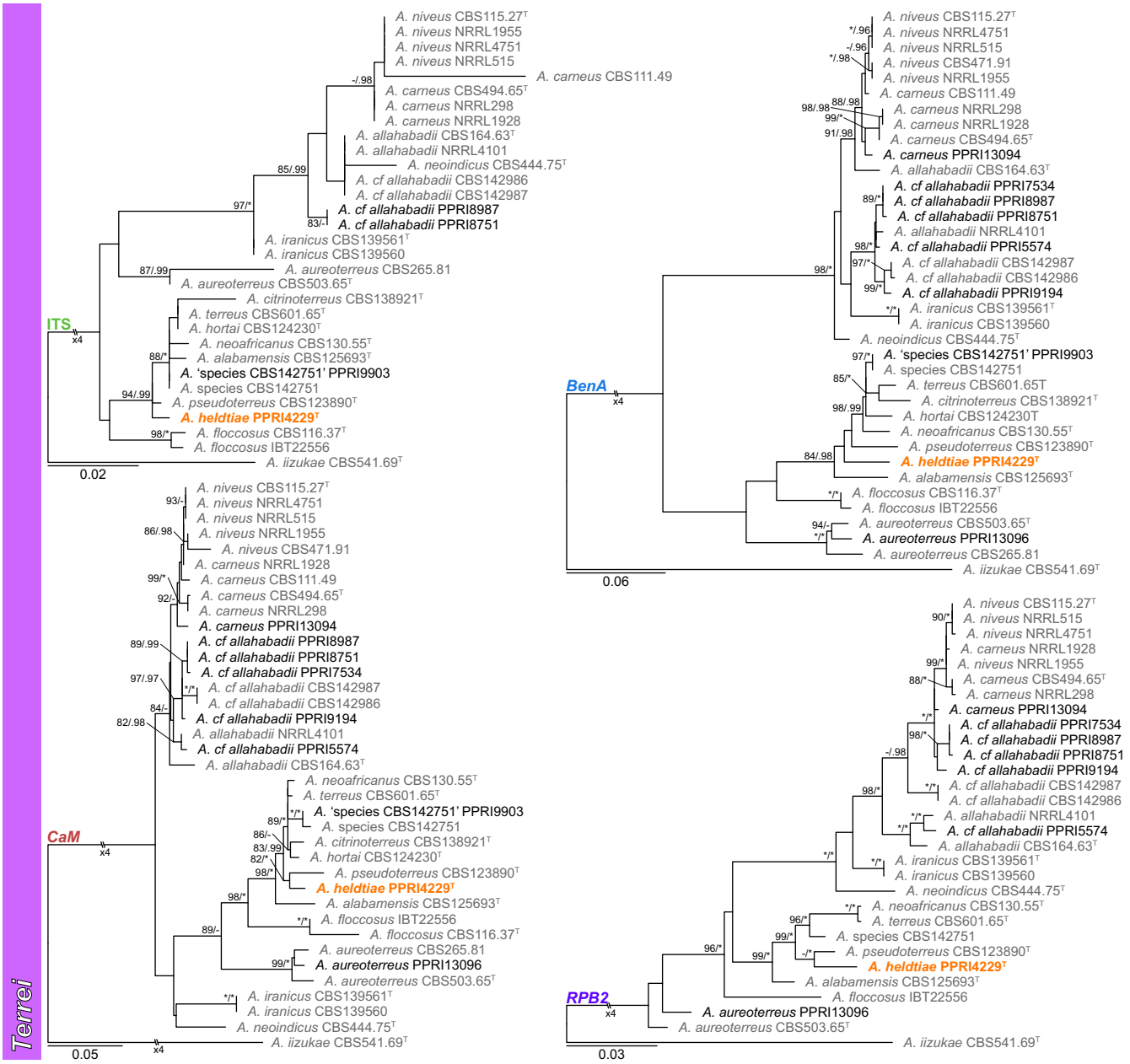

Fig. 9. Single gene phylogenies of *Aspergillus* sect *Terrei* based on ITS, *BenA*, *CaM* and *RPB2*. Strains from new species are shown in orange text, strains identified during this study in black text and reference strains in grey text. Branch support in nodes higher than 80 % bs and/or 0.95 pp are indicated above thickened branches (^{T} = ex-type; * = 100 % bs or 1.00 pp; - = support lower than 80 % bs and/or 0.95 pp).

17–28 µm, stipes hyaline with a small proportion darkened, conidia smooth, globose to subglobose, 2–2.5 µm.

Typus: **South Africa**, unknown, Millet seed, June 1991, (**holotype** PREM 50864, culture ex-type PPRI 4229 = CMV 004A2).

ITS Barcode: MK450656 (alternative identification markers: *BenA* = MK450981; *CaM* = MK451518; *RPB2* = MK450809).

Colony diam (7 d, in mm): CYA 54–58; CYA 30 °C 53–56; CYA 37 °C 60–65; CYAS 50–55; MEAbl 55–60; MEA 36–38; DG18 55–65; YES > 70; OA 33–35; CREA 30–32.

Colony characters (25 °C, 7 d): *CYA* colonies surface floccose, mycelial areas greenish yellow (1A8), sporulation sparse, cinnamon colored, soluble pigment absent, exudate absent, reverse pigmentation olive brown (4B8), light yellow (3A5). *MEAbl* colonies surface floccose, mycelial areas yellow (2A7), sporulation sparse, cinnamon colored, soluble pigment absent, exudate absent, reverse pigmentation olive brown (4B8), light yellow (3A5). *YES* colonies surface floccose, mycelial areas greenish yellow (1A8), sporulation sparse, cinnamon colored, soluble pigment absent, exudate absent, reverse pigmentation olive brown (4B8), light yellow (3A5). *DG18* colonies surface floccose, mycelial areas greenish yellow (1A8), sporulation sparse, cinnamon colored, soluble pigment absent, exudate absent, reverse pigmentation olive brown (4B8), light yellow (3A5). *CREA* colonies strong growth, weak acid production.

Micromorphology: Conidial heads columnar. *Conidiophores* biseriate. *Stipes* hyaline, small proportion darkened, smooth, 140–330 × 5–8 µm. *Vesicles* globose, metulae cover 100 % of head, 17–28 µm wide. *Metulae* 6.5–8.5 × 3–4 µm. *Phialides* ampulliform, 5.5–7.5 × 2–2.5 µm. *Conidia* globose to subglobose, smooth, 2–2.5 × 2–2.5 µm, (2.4 ± 0.1 × 2.1 ± 0.1, n = 52) µm, length/width 1.15 ± 0.07. *Ascomata* not observed.

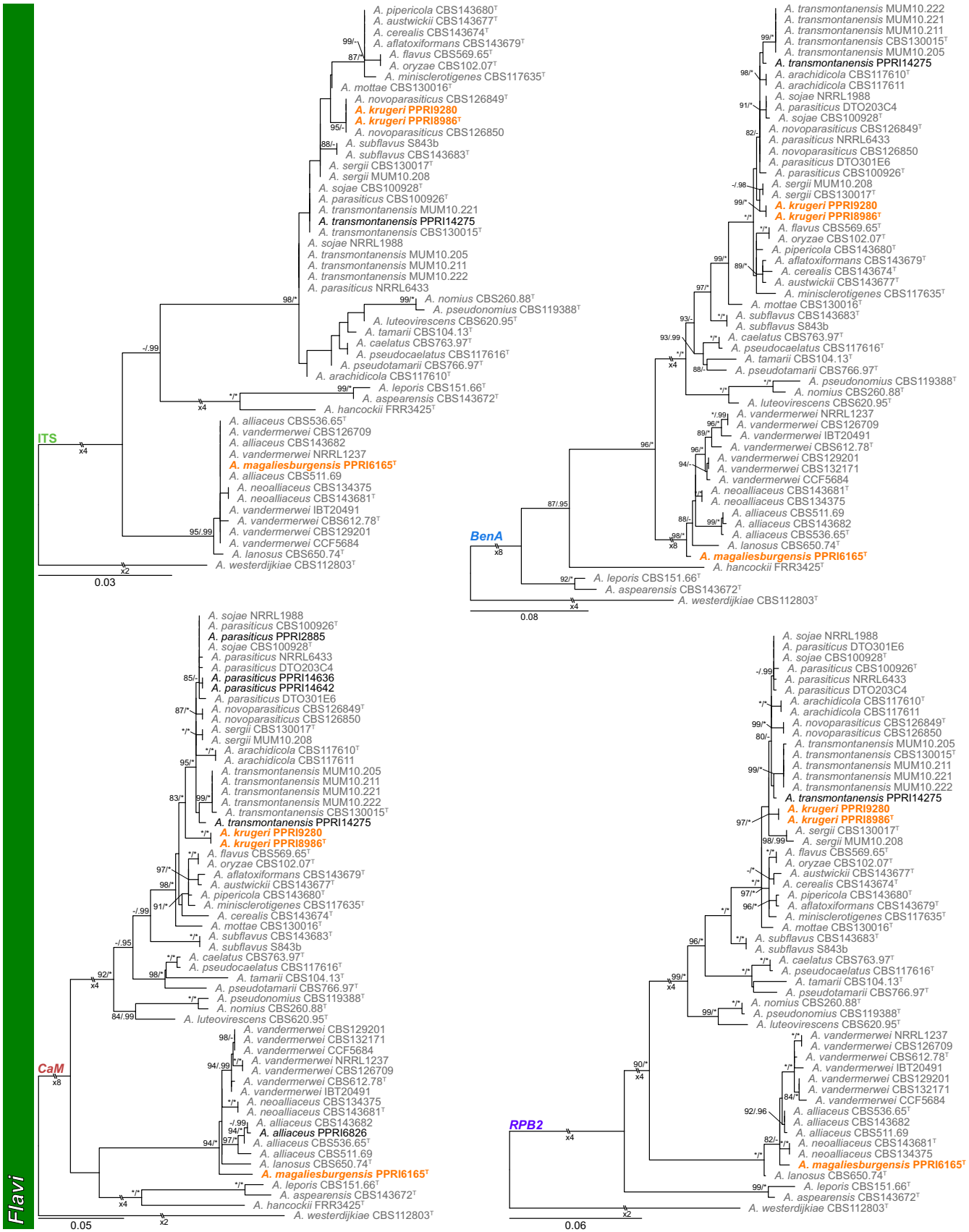

Fig. 10. Phylogenies of *Aspergillus* sect *Flavi* based on ITS, *BenA*, *CaM* and *RPB2*. Strains from new species are shown in orange text, strains identified during this study in black text and reference strains in grey text. Branch support in nodes higher than 80 % bs and/or 0.95 pp are indicated above thickened branches (T = ex-type; * = 100 % bs or 1.00 pp; - = support lower than 80 % bs and/or 0.95 pp).

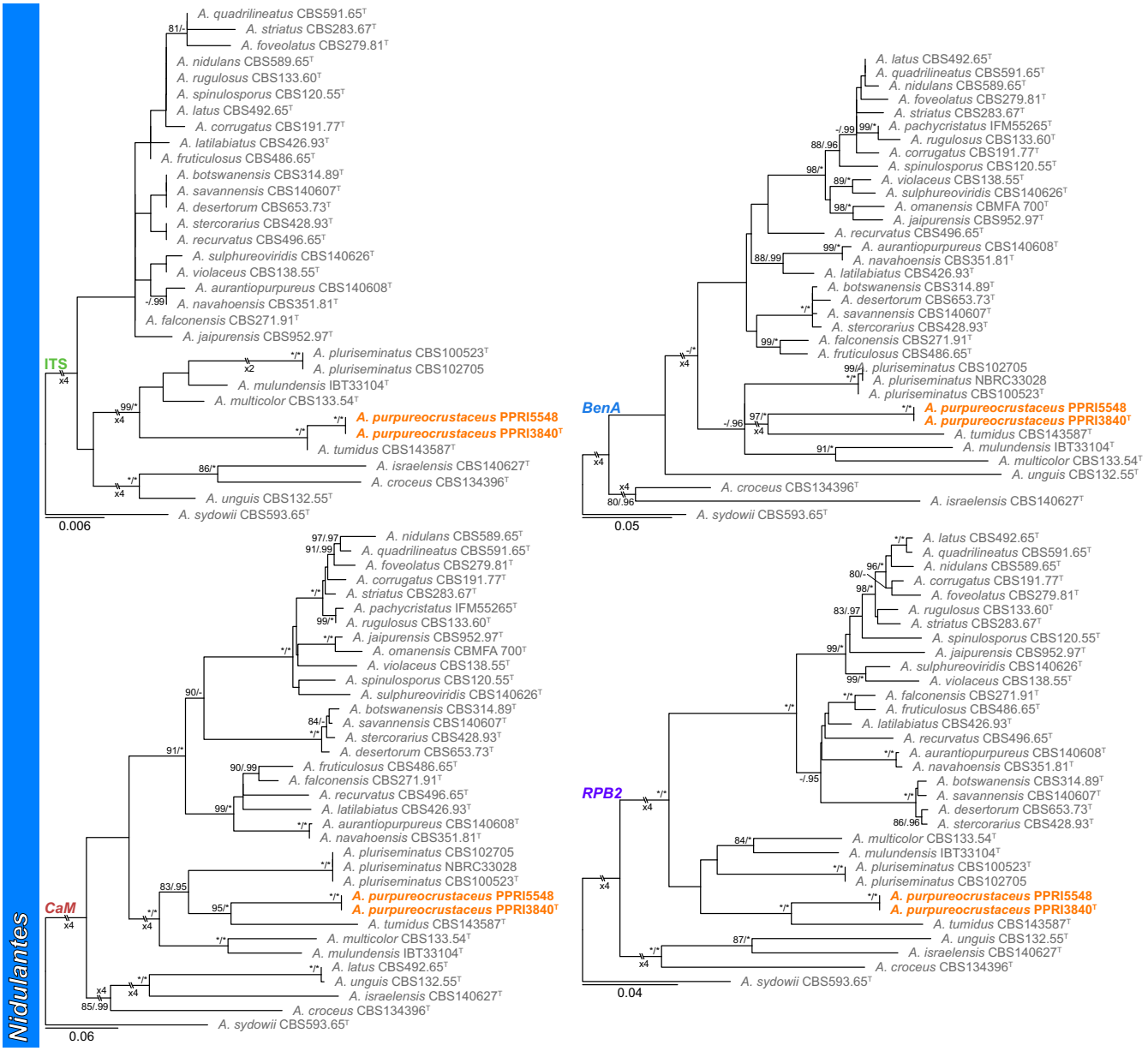

Fig. 11. Single gene phylogenies of *Aspergillus* sect *Nidulantes* based on ITS, *BenA*, *CaM* and *RPB2*. Strains from new species are shown in orange text and reference strains in grey text. Branch support in nodes higher than 80 % bs and/or 0.95 pp are indicated above thickened branches (^{T} = ex-type; * = 100 % bs or 1.00 pp; - = support lower than 80 % bs and/or 0.95 pp).

Notes: Phylogenies resolve *A. heldtiae* as a close relative of *A. pseudoterreus* in section *Terrei* (Figs 6, 9). Both species produce bright yellow colonies with cinnamon colored sporulation. However, *A. pseudoterreus* produce conidiophores in distinctive loosely bundled synnema (Samson *et al.* 2011a), which is absent in the new species. *Aspergillus heldtiae* produces a minor proportion of darkened stipes, which are not reported for *A. pseudoterreus*.

Aspergillus krugeri Visagie, ***sp. nov.*** MycoBank MB834203. Fig. 16.

Etymology: Latin, *krugeri*, named after the Kruger National Park, the National Park where the ex-type was collected from.

Classification: *Eurotiomycetes*, *Eurotiales*, *Aspergillaceae*, *Aspergillus* section *Flavi.*

Diagnosis: Colonies on CYA showing rapid growth at 25 °C and moderate growth at 37 °C, dense sporulation, greyish to dark green, dark brown sclerotia abundant, conidial heads radiate, splitting into 3 or more columns, conidiophores uni- to biseriate, stipes rough, vesicle 40–80 µm wide, conidia broadly ellipsoid, rough, 4–7 × 3.5–6.5 µm.

Typus: **South Africa**, Kruger National Park, Mopane tree debris (*Colophospermum mopane*), October 2005, collected by *E.J. vd Linde* (**holotype** PREM 62309, culture ex-type PPRI 8986 = CMV 006G4).

ITS Barcode. MK450655 (alternative identification markers: *BenA* = MK451098; *CaM* = MK451517; *RPB2* = MK450808).

Colony diam (7 d, in mm): CYA 60–70; CYA 30 °C 65–70; CYA 37 °C 40–47; CYAS 55–58; MEAbl > 70; MEA > 70; DG18 > 70; YES > 70; OA 52–56; CREA 33–36.

Colony characters (25 °C, 7 d): *CYA* colonies surface granular and velutinous, mycelial areas white, sporulation moderately dense to dense, greyish green to dark green (29E7–F7) colored, sclerotia abundant, white when young becoming brown to almost purplish, soluble pigment absent, exudate

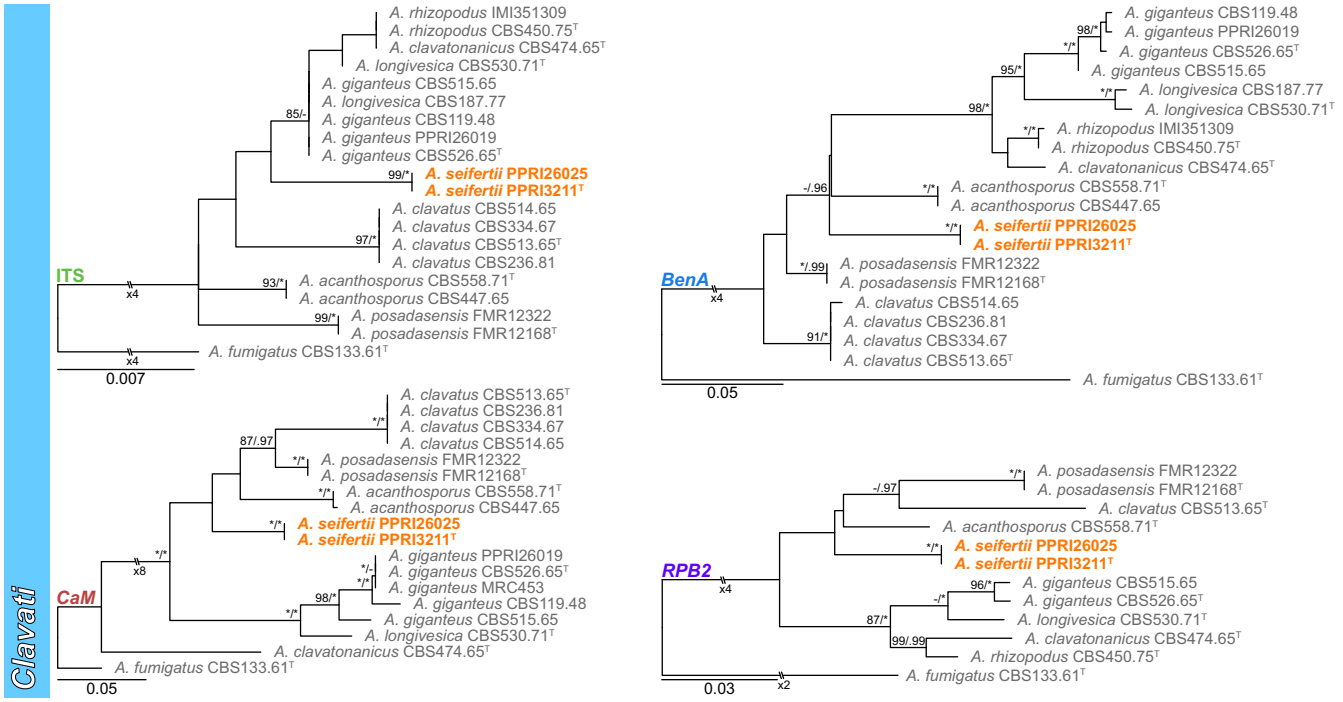

Fig. 12. Single gene phylogenies of *Aspergillus* sect *Terrei* based on ITS, *BenA*, *CaM* and *RPB2*. Strains from new species are shown in orange text, strains identified during this study in black text and reference strains in grey text. Branch support in nodes higher than 80 % bs and/or 0.95 pp are indicated above thickened branches (T = ex-type; * = 100 % bs or 1.00 pp; - = support lower than 80 % bs and/or 0.95 pp).

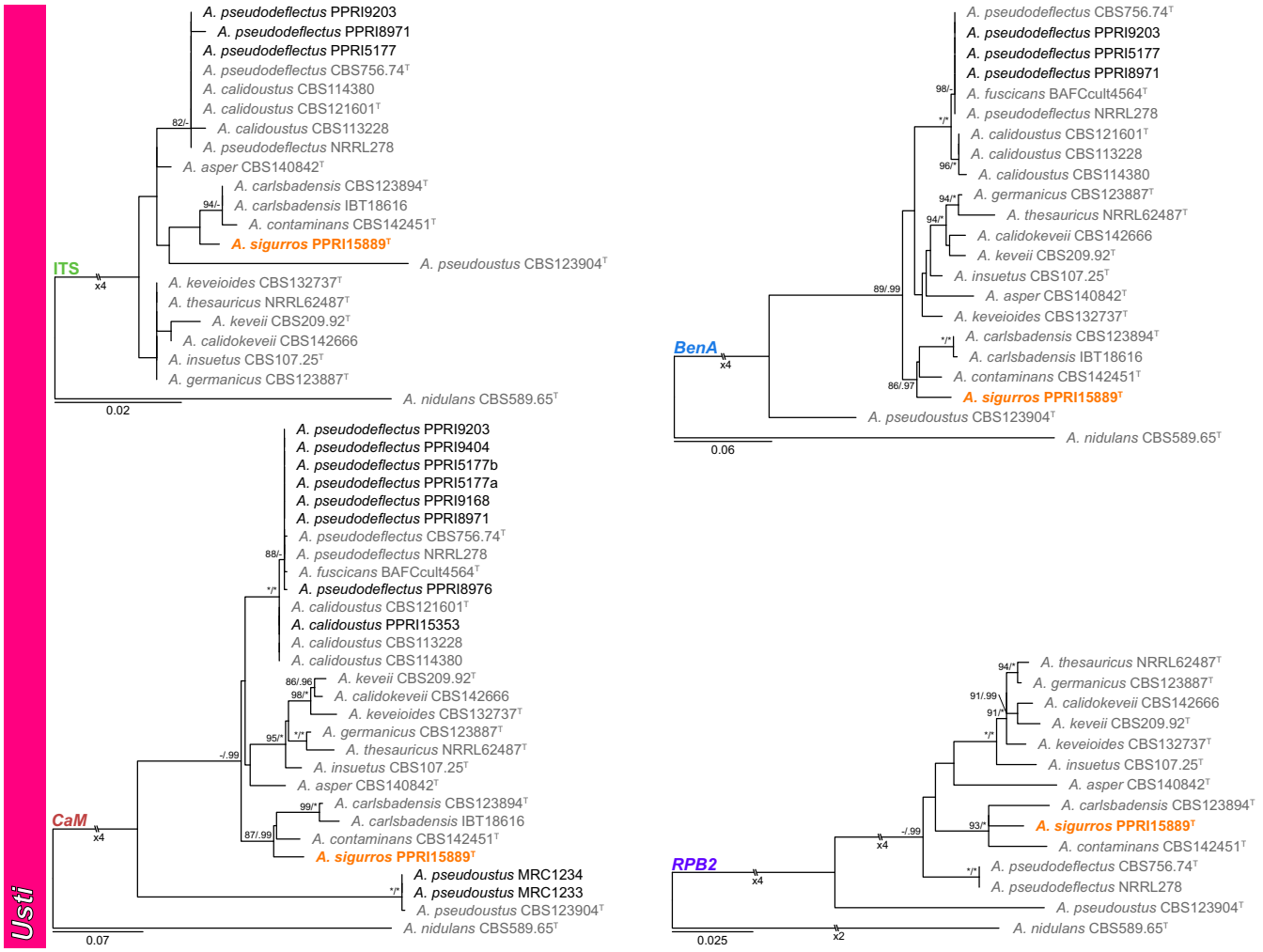

Fig. 13. Single gene phylogenies of *Aspergillus* sect *Usti* based on ITS, *BenA*, *CaM* and *RPB2*. Strains from new species are shown in orange text, strains identified during this study in black text and reference strains in grey text. Branch support in nodes higher than 80 % bs and/or 0.95 pp are indicated above thickened branches (T = ex-type; * = 100 % bs or 1.00 pp; - = support lower than 80 % bs and/or 0.95 pp).

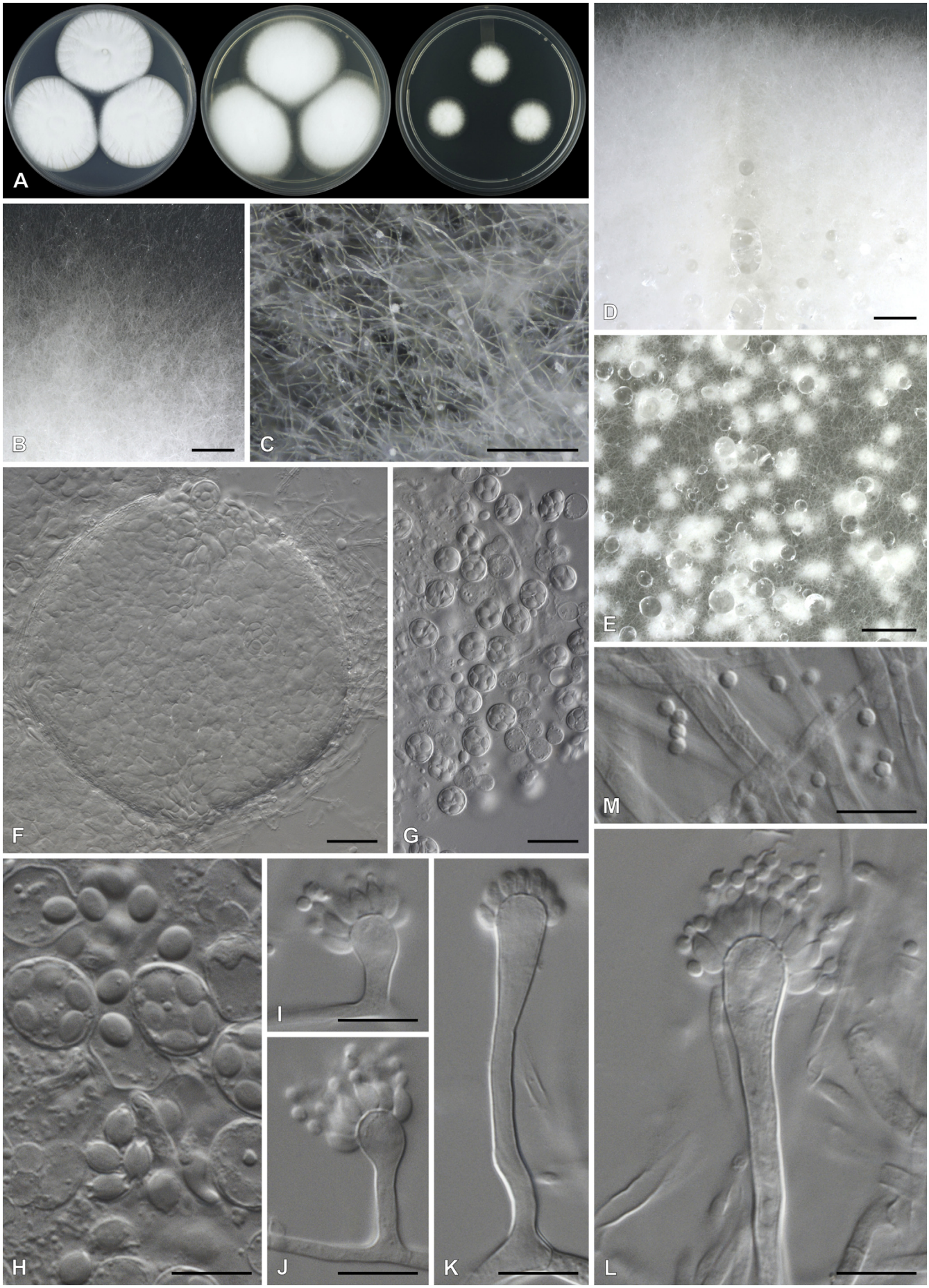

Fig. 14. *Aspergillus elsenburgensis*. **A**. Colonies, from left to right, CYA, MEAbl, DG18. **B–E**. Close-up of colonies on DG18 (**B, C**), CYA (**D**) and OA (**E**). **F**. Ascoma. **G, H**. Asci and ascospores. **I–L**. Conidiophores. **M**. Conidia. Scale bars: B, D, E = 1 mm; C = 0.2 mm; F, G = 20 µm; H–M = 10 µm.

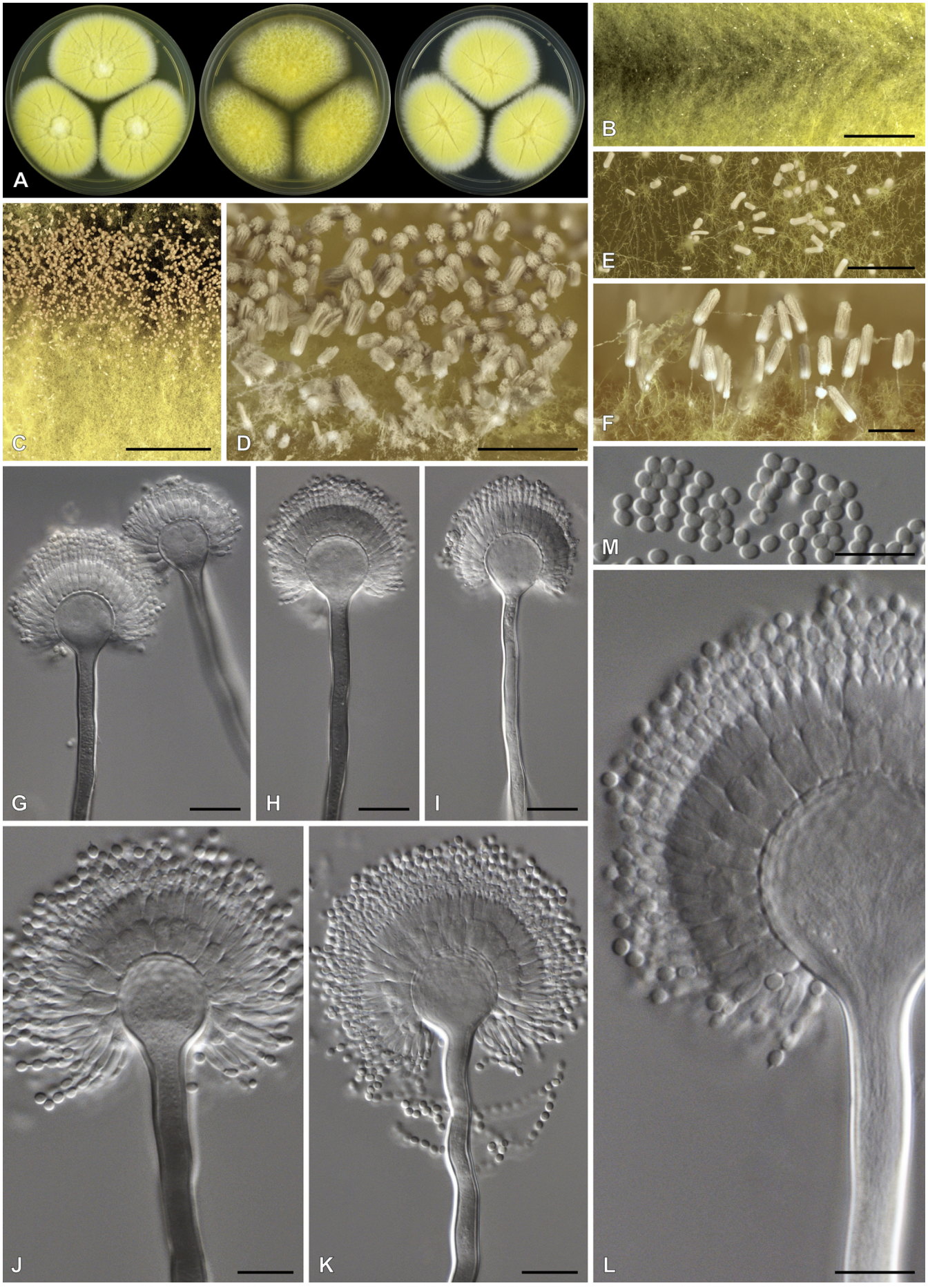

Fig. 15. *Aspergillus heldtiae* **A**. Colonies, from left to right, CYA, MEAbl, DG18. **B–**F. Close-up of colonies on CYA (**B**), DG18 (**C, D**) and MEAbl (**E, F**). **G–L**. Conidiophores. **M**. Conidia. Scale bars: B–C = 2 mm; D, E = 0.5 mm; F = 0.2 mm; G–I = 20 µm; J–M = 10 µm.

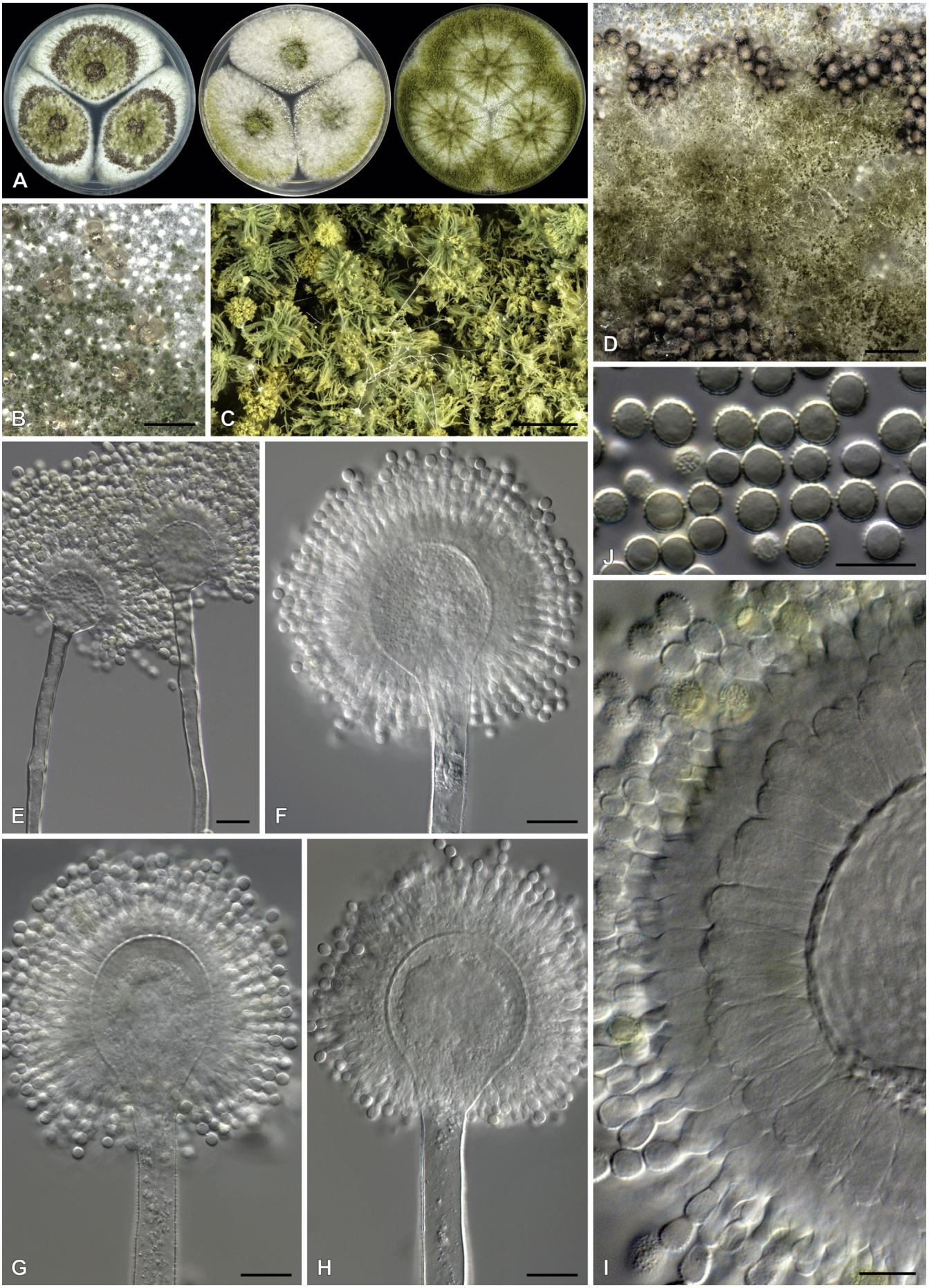

Fig. 16. *Aspergillus krugeri*. **A**. Colonies, from left to right, CYA, MEAbl, DG18. **B–D**. Close-up of colonies on MEAbl (**B**), DG18 (**C**) and CYA (**D**). **E–I**. Conidiophores. **J**. Conidia. Scale bars: B, D = 2 mm; C = 0.5 mm; F, G = 20 µm; H–M = 10 µm.

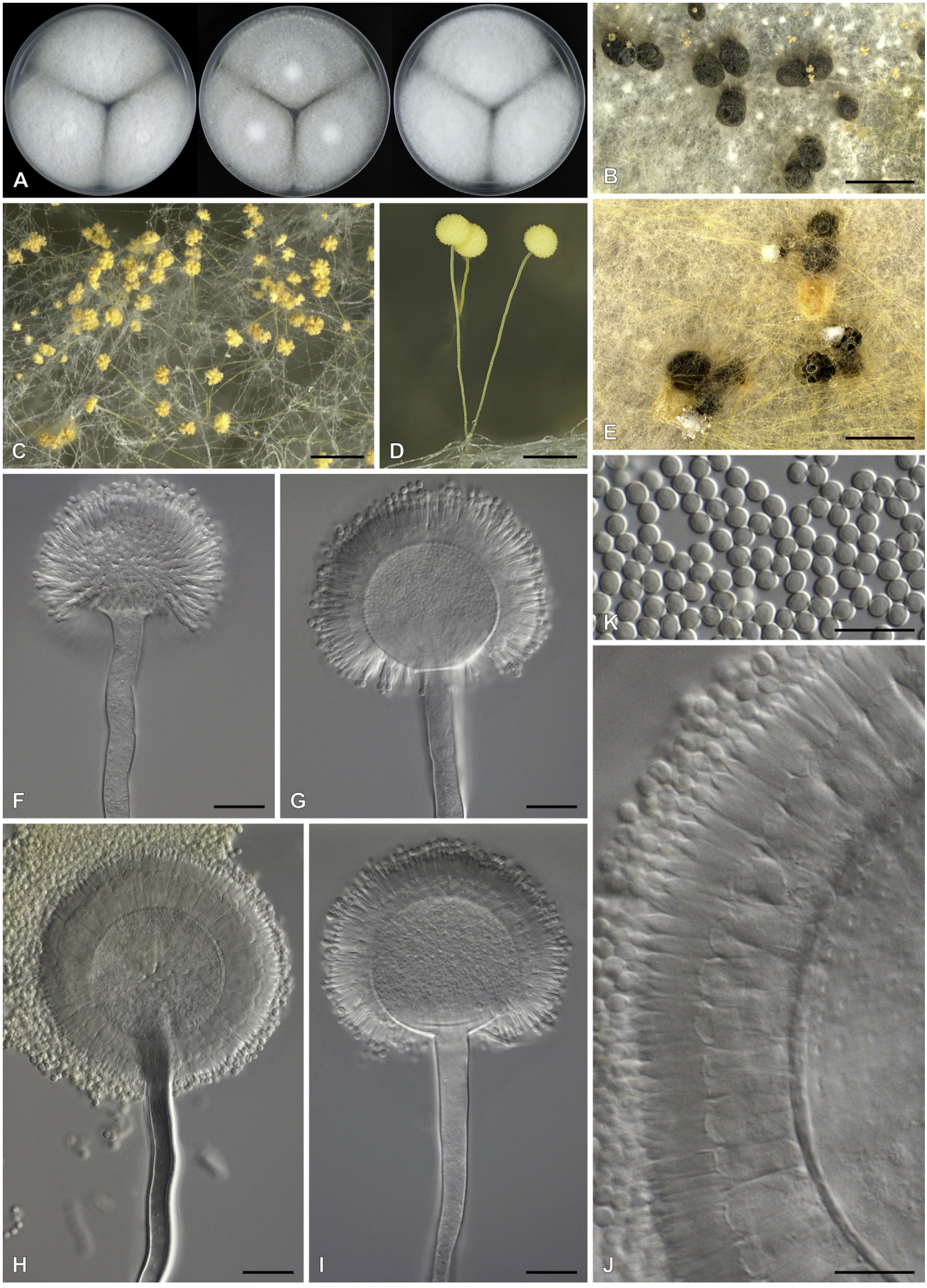

Fig. 17. *Aspergillus magaliesburgensis*. **A**. Colonies, from left to right, CYA, MEAbl, DG18. **B–E**. Close-up of colonies after prolonged incubation on CYA (**B–D**) and MEAbl (**E**). **F–J**. Conidiophores. **K**. Conidia. Scale bars: B, E = 2 mm; C = 0.2 mm; D = 0.5 mm; F–L = 20 µm; J, K = 10 µm.

clear, reverse pigmentation pale yellow to dull yellow (3A3–B3), olive brown (4D4) below sclerotia. *MEAbl* colonies surface granular and velutinous, mycelial areas white, sporulation moderately dense to dense, greyish green to dark green (29E7–F7) colored, sclerotia abundant, white when young becoming brown to almost purplish, soluble pigment absent, exudate clear, reverse pigmentation pale yellow to dull yellow (3A3–B3). *YES* colonies surface velutinous, granular and floccose, mycelial areas white, sporulation dense, greyish green (29E7–30E7) colored, covering white to brown to almost purplish sclerotia, soluble pigment absent, exudate clear, reverse pigmentation greyish yellow (4B5), pale yellow to light yellow (4A3–5). *DG18* colonies surface velutinous, mycelial areas white, sporulation dense, greyish green (29E7–30E7) colored, covering white to brown sclerotia, soluble pigment absent, exudate absent, reverse pigmentation pale yellow to dull yellow (3A3–B3). *CREA* colonies weak growth, weak acid production.

Micromorphology: Conidial heads radiate, splitting into 3 or more columns. *Conidiophores* uniseriate to biseriate with an equal ratio. *Stipes* hyaline, rough, 350–1000(–1300) × 10–18(–21) µm. *Vesicles* globose to spathulate, metulae/phialides cover 100 % of head, 40–80 µm wide. *Metulae* 11–22 × 5–10 µm. *Phialides* ampulliform, 10–15 × 4.5–7 µm. *Conidia* broadly ellipsoid, rough, 4–7 × 3.5–6.5 µm, (5.5 ± 0.7 × 5.1 ± 0.6, n = 72) µm, length/width 1.08 ± 0.04. *Ascomata* not observed. *Sclerotia* white when young, becoming dark brown with age, 370–850 µm.

Notes: *Aspergillus krugeri* belongs to the *A. flavus*-clade (Frisvad *et al.* 2019) and is closely related to *A. arachidicola, A. parasiticus, A. novoparasiticus, A. sergii* and *A. transmontanensis* (Figs 3, 10). These species are morphologically similar, but colony growth rates can distinguish between them. *Aspergillus krugeri* grows faster than *A. parasiticus* (40–60 mm), *A. sergii* (<55 mm) and *A. transmontanensis* (55–57 mm) on CYA (Soares *et al.* 2012). On CYA at 37 °C, *Aspergillus krugeri* grows more restricted than *A. arachidicola* (60–70 mm), *A. novoparasiticus* (58–63 mm), *A. sergii* (<60 mm) and *A. transmontanensis* (55–57 mm) (Pildain *et al.* 2008, Gonçalves *et al.* 2012).

Aspergillus magaliesburgensis Visagie, ***sp. nov.*** MycoBank MB834204. Fig. 17.

Etymology: Latin, *magaliesburgensis*, named after Magaliesburg, the town the ex-type was collected from.

Classification: *Eurotiomycetes*, *Eurotiales*, *Aspergillaceae*, *Aspergillus* section *Flavi.*

Diagnosis: Colonies pale, sparse intense yellow sporulation becoming cinnamon with age. Stipes yellow, conidia smooth, globose to subglobose, 2.5–3.5 × 2.5–3.5 µm. Sclerotia present.

Typus: South Africa, Gauteng, Magaliesburg, from an Antlion (*Myrmeleontidae*), April 1996, collected by *J. Pieterse* (**holotype** PREM 62314, culture ex-type PPRI 6165 = CMV 007A3).

ITS Barcode: MK450649 (alternative identification markers: *BenA* = MK451116; *CaM* = MK451511; *RPB2* = MK450802).

Colony diam (7 d, in mm): CYA 65–70; CYA 30 °C 60–65; CYA 37 °C 50–55; CYAS 65-70; MEAbl > 70; MEA 53–56; DG18 > 70; YES > 70; OA 55–60; CREA 55–60.

Colony characters (25 °C, 7 d): *CYA* colonies surface floccose, mycelial areas white, some yellow aerial mycelia present, sporulation absent after 7 d, intense yellow when present, with age cinnamon, sclerotia present, black when mature, soluble pigment absent, exudate absent, reverse pigmentation yellowish white to pale yellow (2A2–3). *MEAbl* colonies surface floccose, mycelial areas white, sporulation absent after 7 d, intense yellow when present, with age cinnamon, sclerotia present, black when mature, soluble pigment absent, exudate absent, reverse pigmentation pale yellow to dull yellow (3A3–B3). *YES* colonies surface floccose, mycelial areas white, sporulation very sparse, bright yellow, black when mature, soluble pigment absent, exudate absent, reverse pigmentation pale yellow to greyish yellow (4A3–B3). *DG18* colonies surface floccose, mycelial areas white, some yellow aerial mycelia present, sporulation absent, soluble pigment absent, exudate absent, reverse pigmentation yellowish white to pale yellow (2A2–3). *CREA* colonies weak growth, acid not produced.

Micromorphology: Conidial heads radiate. *Conidiophores* biseriate. *Stipes* yellow, smooth, (350–)900–1150 × (6–)8–12 µm. *Vesicles* globose, metulae cover 100 % of head, 40–85 µm wide. *Metulae* 8–12(–16) × 3.5–5.5 µm. Phialides ampulliform, 7.5–10 × 2–3 µm. *Conidia* globose to subglobose, smooth, 2.5–3.5 × 2.5–3.5 µm, (3.1 ± 0.2 × 2.9 ± 0.3, n = 52) µm, length/width 1.08 ± 0.12. *Ascomata* not observed. *Sclerotia* black when mature, 550–1500 µm.

Notes: Phylogenies resolve *A. magaliesburgensis* in section *Flavi* in the *A. alliaceus* clade (Frisvad *et al.* 2019), containing *A. alliaceus, A. lanosus, A. neoalliaceus* and *A. vandermerwei* (Fig. 3, 10). *BenA*, *CaM* and *RPB2* can be used to identify the new species. *Aspergillus magaliesburgensis* produces sclerotia, and these structures are absent in *A. vandermerwei*, while *A. lanosus* typically produces bright yellow colonies. The new species is distinct from *A. neoalliaceus* based on the subglobose to ellipsoid conidia of the latter. Morphologically, *A. magaliesburgensis* and *A. alliaceus* could not be distinguished from each other. We do note that "faintly yellow conidiophores" were previously observed for *A. alliaceus* (Raper & Fennell 1965), while *A. magaliesburgensis* produce conidiophores with distinctly yellow stipes.

Aspergillus purpureocrustaceus Visagie, ***sp. nov.*** MycoBank MB834205. Fig. 18.

Etymology: Latin, named *purpureocrustaceus*, meaning purple and crust, in reference to the colonies on CYA and MEAbl that turn purple and crust-like with age.

Classification: *Eurotiomycetes*, *Eurotiales*, *Aspergillaceae*, *Aspergillus* section *Nidulantes.*

Diagnosis: Colonies crust-like and very hard due to abundant Hülle cells produced on surface, having a reddish brown to purple color, sporulation sparse to absent, conidiophores biseriate, stipes 130–310 µm, conidia globose to subglobose, rough, 3.5–4.5(–5) × 3–4.5 µm.

Typus: **South Africa**, Limpopo, plant debris, January 1990, (**holotype** PREM 62264, culture ex-type PPRI 3840 = CMV 008B3).

Additional material examined: **South Africa**, Western Cape, Cape Town, Huntsman spider (*Palystes castaneus*), January 1994, collected by *N. Larsen & H. Robertson* PPRI 5548 = CMV 008B1.

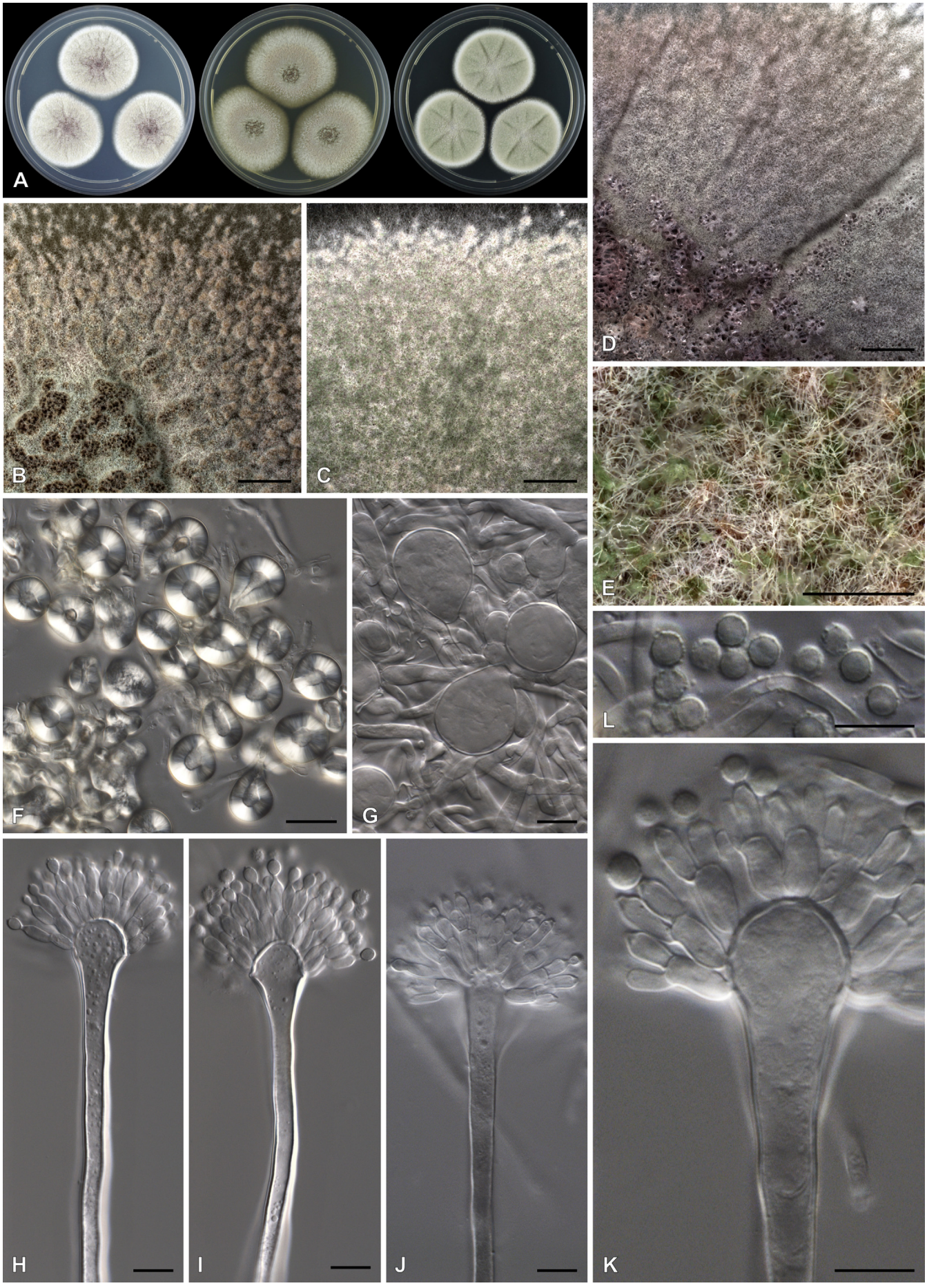

Fig. 18. *Aspergillus purpureocrustaceus*. **A**. Colonies, from left to right, CYA, MEAbl, DG18. **B–E**. Close-up of colonies on MEAbl (**B**), DG18 (**C, E**) and CYA (**D**). **F**. Hülle cells. **G**. Potential immature asci. **H–K**. Conidiophores. **L**. Conidia. Scale bars: B, C, D = 2 mm; E = 0.5 mm; F = 20 µm; G–K = 10 µm.

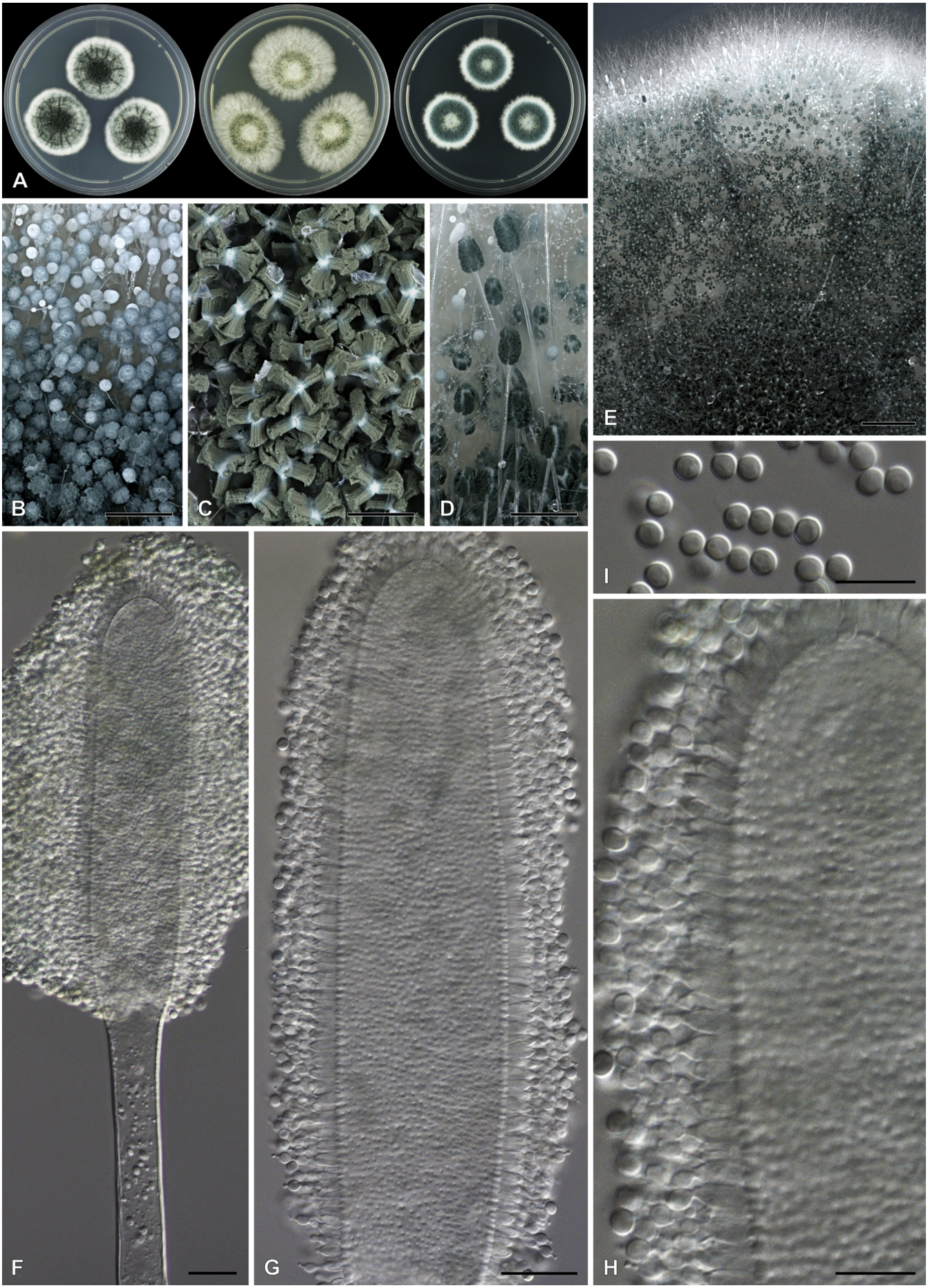

Fig. 19. *Aspergillus seifertii*. **A**. Colonies, from left to right, CYA, MEAbl, DG18. **B–E**. Close-up of colonies on DG18 (**B**) and CYA (**C–E**). **F–H**. I. Conidiophores. **I**. Conidia. Scale bars: B–D = 0.5 mm; E = 2 mm; F, G = 20 µm; H–I = 10 µm.

Fig. 20. *Aspergillus sigurros*. **A**. Colonies, from left to right, CYA, MEAbl, DG18. **B–E**. Close-up of colonies on CYA (**B**), DG18 (**C, D**) and MEAbl (**E**). **F**. Hülle cells. **G–K**. I. Conidiophores. **I**. Conidia. Scale bars: B, C, E = 2 mm; D = 0.5 mm; F–H = 20 µm; I–K = 10 µm.

ITS Barcode: MK450653 (alternative identification markers: *BenA* = MK451138; *CaM* = MK451515; *RPB2* = MK450806).

Colony diam (7 d, in mm): CYA 40–41 (25–26); CYA 30 C 10–15; CYA 37 °C no growth; CYAS 25–28; MEAbl 45–47 (33–35); MEA 38–41; DG18 35–40; YES 53–60; OA 25–30; CREA 27–30.

Colony characters (25 °C, 7 d): *CYA* colonies surface floccose, mycelial areas yellow to grey, sporulation absent, Hülle cells abundant, reddish brown, becoming purple and crust-lie with age, soluble pigment absent, exudate reddish brown and clear, reverse pigmentation dark brown (6F8), yellowish brown (5D4–5). *MEAbl* colonies surface floccose, mycelial areas yellow to grey, sporulation absent, Hülle cells abundant, reddish brown, becoming purple and crust-lie with age, soluble pigment absent, exudate reddish brown and clear, reverse pigmentation dark brown (6F8), yellowish brown (5D4–5). *YES* colonies surface floccose, mycelial areas yellow to grey, sporulation absent, Hülle cells abundant, reddish brown, soluble pigment absent, exudate reddish brown and clear, reverse pigmentation olive brown (4F8), pale yellow (4A2). *DG18* colonies surface floccose, mycelial areas yellow to grey, sporulation sparse, greyish green (28D6), Hülle cells abundant, reddish brown, soluble pigment absent, exudate reddish brown and clear, reverse pigmentation dark brown (6F8), yellowish brown (5D4–5). *CREA* colonies weak growth, acid not produced.

Micromorphology: Conidial heads radiate. *Conidiophores* biseriate. *Stipes* hyaline, smooth, 130–310 × 5–7.5 µm. *Vesicles* subclavate, metulae cover 75–100 % of head, 10–20 µm wide. *Metulae* 6–11.5 × 3–5.5 µm. *Phialides* ampulliform, 7–10 × 3–4 µm. *Conidia* globose, rough, 3.5–4.5(–5) × 3–4.5 µm, (4.1 ± 0.4 × 3.9 ± 0.4, n = 28) µm, length/width 1.05 ± 0.04. *Hülle cells* globose to subglobose, occurring in hard crusts with reddish purple color, 13–25 µm. *Ascomata* not observed.

Notes: Phylogenies resolve *A. purpureocrustaceus* in a clade of section *Nidulantes* with *A. multicolor*, A. *mulundensis*, *A. pluriseminatus* and *A. tumidus* (Figs 5, 11). This group of species typically produce abundant Hülle cells, often giving the colony a reddish to purple color with age (Roy *et al.* 1987, Stchigel & Guarro 1997, Chen *et al.* 2016, Crous *et al.* 2018). Comparing these species, only *A. multicolor* and *A. mulundensis* are capable of growth on CYA at 37 °C. *Aspergillus pluriseminatus* can be distinguished from the other species in this clade by the presence of a sexual state and absence of asexual state. Compared to the new species, *A. tumidus* grows more restricted on MEA (38–41 *vs* 22–23 mm), grows more rapidly on CYA at 30 °C (10–15 *vs* 32–34 mm), with its colony appearance dominated by good sporulation.

Aspergillus seifertii Visagie & N. Yilmaz, ***sp. nov.*** MycoBank MB834206. Fig. 19.

Etymology: Latin, *seifertii*, named after Dr. Keith A. Seifert, a prominent Canadian mycologist specialised on mycotoxigenic genera and other hyphomycetes.

Classification – *Eurotiomycetes*, *Eurotiales*, *Aspergillaceae*, *Aspergillus* section *Clavati.*

Diagnosis — Colonies greyish to dark green, producing large conidiophores with clavate heads, stipes up to 6 mm long, vesicles 26–60 µm wide, up to 210 µm long.

Typus: **South Africa**, Free State, Golden Gate National Park, Grassroots, January 1988, collected by *R. Anelich* (**holotype** PREM 49066, culture ex-type PPRI 3211 = CMV 006F5).

Additional material examined: **South Africa**, Free State, Golden Gate National Park, Soil, 2018, collected by *R. Jacobs*, PPRI 26025 = CMV 011E3; CMV 011E4.

ITS Barcode: MK450647 (alternative identification markers: *BenA* = MK451093; *CaM* = MK451509; *RPB2* = MK450800).

Colony diam (7 d, in mm): CYA 33–35; CYA 30 °C 35–38; CYA 37 °C 2–3; CYAS 10–12; MEAbl 40–45; MEA 38–40; DG18 25–28; YES 45–50; OA 28–35; CREA 20–25.

Colony characters (25 °C, 7 d): *CYA* colonies surface floccose, mycelial areas white, sporulation moderately dense, greyish green to dark green (25D5–F5), soluble pigment absent, exudate clear, reverse pigmentation pale green to pale yellow (30A3–1A3–2A3). *MEAbl* colonies surface floccose, mycelial areas white, sporulation sparse, dark green (25F5), soluble pigment absent, exudate clear, reverse pigmentation pale green to pale yellow (30A3–1A3–2A3). *YES* colonies surface floccose, mycelial areas white, sporulation dense, greyish green to dark green (25D5–F5), soluble pigment absent, exudate clear, reverse pigmentation yellowish white to yellow (3A2–6). *DG18* colonies surface floccose, mycelial areas white, sporulation moderately dense, dull green to dark green (25D4–F5), soluble pigment absent, exudate absent, reverse pigmentation pale green to pale yellow to light yellow (30A3–1A3–2A3–3A4). *CREA* colonies weak growth, acid not produced.

Micromorphology: Conidial heads clavate, with age splitting into 3–4 divergent columns. *Conidiophores* uniseriate. *Stipes* hyaline, smooth, up to 6 mm × 17–24 µm. *Vesicles* clavate, phialides cover 100 % of head, 26–60 µm wide, up to 210 µm long. *Phialides* ampulliform, 7–9.5 × 2.5–3.5 µm. *Conidia* globose, smooth, 3–4 × 3–4 µm, 3.4 ± 0.2 × 3.3 ± 0.19, n = 59) µm, length/width 1.03 ± 0.05. *Ascomata* not observed.

Notes: Phylogenies resolves *Aspergillus seifertii* as a unique lineage in section *Clavati* (Figs 4, 12). Generally, species from this section produce blue-green conidia and clavate conidiophores (except for *A. posadasensis* for which only a sexual reproductive state was reported (Marin-Felix *et al.* 2014)). The stipe and vesicle length are generally good characters to distinguish between these species (Varga *et al.* 2007). *Aspergillus clavatus* and *A. seifertii* produce conidiophores with stipes of up to 3 and 6 mm, respectively, while *A. giganteus* and *A. longivesica* can grow several cm in length. The remaining section *Clavati* species have stipes shorter than 1 mm. Vesicle length is also a useful character. *Aspergillus clavati*, *A. seifertii, A. giganteus* and *A. longivesica* have vesicles up to 200, 210, 600 and 3200 µm, respectively.

Aspergillus sigurros Visagie, ***sp. nov.*** MycoBank MB834207. Fig. 20.

Etymology: Latin, *sigurros*, named after Sigurrós, one of Keith A. Seifert's favourite music groups.

Classification: *Eurotiomycetes*, *Eurotiales*, *Aspergillaceae*, *Aspergillus* section *Usti.*

Diagnosis: Colonies with grey to brownish moderately dense sporulation, growth on CYA at 30 °C 10–14 mm; conidiophores with brown stipes, vesicles 11–25 µm wide, conidia spiny, globose 3–4 × 3–4 µm.

Typus: **South Africa**, KwaZulu-Natal, Pinetown, unknown environmental sample, April 2014, collected by *M. Truter* (**holotype** PREM 62308, culture ex-type PPRI 15889 = CMV 005I4 = 2014-M62/147).

ITS Barcode: MK450650 (alternative identification markers: *BenA* = MK451066; *CaM* = MK451512; *RPB2* = MK450803).

Colony diam (7 d, in mm): CYA 34–35; CYA 30 °C 10–15; CYA 37 °C no growth; CYAS 27–29; MEAbl 29–31; MEA 28–30; DG18 28–32; YES 39–42; OA 32–35; CREA 25–26.

Colony characters (25 °C, 7 d): *CYA* colonies surface floccose, mycelial areas white, sporulation moderately dense, brownish grey to brown (5E2–5–6E5–2), soluble pigment absent, exudate clear, minute droplets, reverse pigmentation olive (2D4), yellowish white (3A2). *MEAbl* colonies surface floccose, mycelial areas white, sporulation moderately dense, grey (5E1–6E1) to brown (6E6), soluble pigment absent, exudate absent, reverse pigmentation olive (2D4), yellowish white (3A2). *YES* colonies surface floccose, mycelial areas white, sporulation sparse to moderately dense, greyish brown (5D3–6D3), soluble pigment absent, exudate absent, reverse pigmentation brownish orange (5C5), yellowish white (3A2). *DG18* colonies surface floccose, mycelial areas white, sporulation moderately dense, brownish grey to brown (5E2–5–6E5–2), soluble pigment absent, exudate clear, minute droplets, reverse pigmentation brown (5E5), olive (2D4), yellowish white (3A2). *CREA* colonies strong growth, acid not produced.

Micromorphology: Conidial heads radiate. *Conidiophores* biseriate. *Stipes* brown, smooth, (85–)120–360 × 2.5–6.5 µm. *Vesicles* globose, metulae cover 50–75 % of head, 11–25 µm wide. *Metulae* 6–10 × 3–4 µm. *Phialides* ampulliform, 6–8.5 × 2.5–3.5 µm. *Conidia* globose, spiny to somewhat wart-like, some covered in sheath, 3–4 × 3–4 µm, (3.2 ± 0.2 × 3.2 ± 0.2, n = 53) µm, length/width 1.03 ± 0.04. *Hülle cells* irregularly elongated, in scattered groups, 22–60 × 11–20 µm. *Ascomata* not observed.

Notes: *Aspergillus sigurros* resolves as a close relative of *P. carlsbadensis* and *P. contaminans* in section *Usti* (Figs 7, 13). Compared to *A. carlsbadensis*, the new species produces conidiophores with broader vesicles (11–25 *vs* 10–14 µm), larger conidia (3–4 *vs* 2.5–3 µm) and grows more restricted on CYA at 30 °C (10–15 *vs* 28–32 mm) (Samson *et al.* 2011b). Microscopically *A. contaminans* and *A. sigurros* are very similar. However, the new species grows faster on CYA at 30 °C (4–5 *vs* 10–14 mm) (Crous *et al.* 2017).

DISCUSSION

With this project, we aimed to re-identify strains previously lodged in the PPRI and MRC culture collections as *Aspergillus* or its old associated sexual state genera (*e.g. Eurotium*, *Emericella etc.*). Unfortunately, a large proportion of strains in PPRI were either badly contaminated or not viable (±35 %). As a result, only 250 strains were included in this particular study, with 354 new DNA reference sequences (ITS 24; *BenA* 52; *CaM* 250; *RPB2* 28) generated and published on GenBank. South African *Aspergillus* was found to be relatively diverse with 63 species identified belonging to 11 sections (sections *Aspergillus*, *Candidi*, *Circumdati*, *Clavati*, *Cremei*, *Flavi*, *Flavipedes*, *Fumigati*, *Nidulantes*, *Terrei* and *Usti*). This does not include the 11 *Aspergillus* sect *Nigri* species that will be published elsewhere. Among the 63 species, seven were found to be new and are described in the Taxonomy section above. One problem experienced during this project was that for the new species, very few strains were available, *e.g.* four new species were represented by only one strain, while the remaining three new species had only two strains. This situation is frequent when sequencing smaller collections around the world. For *A. elsenburgensis* we were fortunate that CBS had several additional strains sequenced. Even though not ideal, comparisons based on morphology, multigene phylogenies and single gene trees applying genealogical concordance phylogenetic species recognition (Taylor *et al.* 2000), leaves little doubt about the novelty of the new species introduced here.

Sequence based identifications of PPRI and MRC strains was relatively straight forward thanks to the secondary identification marker *CaM* and associated database (Samson *et al.* 2014). Throughout the genus and between different sections, the primer pairs cmd5&cmd6 performed (Hong *et al.* 2005) well. For only a minor proportion of strains, additional ITS, *BenA* and/or *RPB2* sequences were needed to confirm the *CaM* based identifications. ITS and *BenA* posed no problems in terms of amplification using proposed methods of (Samson *et al.* 2014), but *RPB2* was difficult to amplify using either 5F&7CR (Liu *et al.* 1999) or 5FEur&7CREur (Houbraken *et al.* 2012). Both primer sets provided intermitted hits and misses, with the internal sequencing primers F310, R310, 388F and 527R (Houbraken & Samson 2011) at the end needed to obtain high quality sequences contigs.

Several *Aspergillus* strains belonging to sect *Terrei* were tentatively identified during this study. Both *A. allahabadii* and *A. alabamensis* appears to contain a large degree of infraspecies variation and potentially contain a large number of new species. Even though the multigene phylogeny (Fig. 6) appears to indicate that several new species may exist, we did not feel comfortable introducing new species in a difficult clade without having more data from other regions of the world. Similarly, PPRI 14275 potentially represents a new species closely related to *A. transmontanensis* from section *Flavi*. However, since no consistent morphological differences were observed in this strain, we decided to not introduce a phylogenetic species for this single strain.

One of the big challenges we face in *Aspergillus* is to discover the missing biodiversity. This can either be in the form of new species discovery and/or isolation of additional strains of already known species. Phylogenetic approaches and their incorporation into our species concepts resulted in rather aggressive approaches. It is not ideal to introduce new species based on one or two strains, but as is obvious from this study, often one is left with that as the only option. Monotypic species are frequent in *Aspergillus* with 118 of the 415 accepted species represented by a single strain (the ex-type), while 80 species are represented by

two strains. Within a modern taxonomy like that employed in *Aspergillus*, this creates problems on several levels, but most pressing is infraspecies variation for species that are often not captured. This is true from a morphological and DNA sequence perspective, but especially concerning the latter, it creates difficulties with identifications. It is not uncommon to find strains that show a few nucleotide differences from the ex-type sequence. Trying to identify such a sequence becomes very complicated amongst monotypic species as one will often not know if the strain belongs to a new species or if they found infraspecies variation within a known species. Studies that generate a lot of additional reference sequences are thus of great importance, not only to discover new species but also to discover infraspecies variation which ultimately makes future species delineations and thus identifications easier. For taxonomic revisions it is crucial to have as much data as possible available, as recently illustrated for the *A. viridinutans* species complex, where it was found that *A. parafelis* and *A. pseudofelis* should be considered synonyms of the genetically diverse *A. felis* (Hubka *et al.* 2018). Expanded efforts to isolate and identify fungi should thus remain a priority in important genera such as *Aspergillus*.

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APPENDIX A. SUPPLEMENTARY DATA

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