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Phylogenetic analysis of *Monascus* and new species from honey, pollen and nests of stingless bees

R.N. Barbosa[1,2], S.L. Leong[3], O. Vinnere-Pettersson[3,4], A.J. Chen[1,5], C.M. Souza-Motta[2], J.C. Frisvad[6], R.A. Samson[1], N.T. Oliveira[2], and J. Houbraken[1*]

[1]*Westerdijk Fungal Biodiversity Institute, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands;* [2]*Departamento de Micologia Prof. Chaves Batista, Universidade Federal de Pernambuco, Av. Prof. Moraes Rego, s/n, Centro de Biociências, Cidade Universitária, CEP: 50670-901 Recife, PE, Brazil;* [3]*Swedish University of Agricultural Sciences, Department of Molecular Sciences, Box 7015, SE-750 07 Uppsala, Sweden;* [4]*National Genomics Infrastructure-Sweden, Science for Life Laboratory, Department of Immunology, Genetics and Pathology, Uppsala University, BMC, Box 815, SE-752 37 Uppsala, Sweden;* [5]*Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100193, PR China;* [6]*Department of Biotechnology and Biomedicine, Technical University of Denmark, 2800 Kongens Lyngby, Denmark*

**Correspondence*: J. Houbraken, j.houbraken@westerdijkinstitute.nl

Abstract: The genus *Monascus* was described by van Tieghem (1884) to accommodate *M. ruber* and *M. mucoroides*, two species with non-ostiolate ascomata. Species delimitation in the genus is still mainly based on phenotypic characters, and taxonomic studies that include sequence data are limited. The genus is of economic importance. Species are used in fermented Asian foods as food colourants (e.g. 'red rice' (ang-kak, angka)) and found as spoilage organisms, and recently *Monascus* was found to be essential in the lifecycle of stingless bees. In this study, a polyphasic approach was applied combining morphological characters, ITS, LSU, β-tubulin, calmodulin and RNA polymerase II second largest subunit sequences and extrolite data, to delimit species and to study phylogenetic relationships in *Monascus*. Furthermore, 30 *Monascus* isolates from honey, pollen and nests of stingless bees in Brazil were included. Based on this polyphasic approach, the genus *Monascus* is resolved in nine species, including three new species associated with stingless bees (*M. flavipigmentosus* sp. nov., *M. mellicola* sp. nov., *M. recifensis* sp. nov., *M. argentinensis*, *M. floridanus*, *M. lunisporas*, *M. pallens*, *M. purpureus*, *M. ruber*), and split in two new sections (section *Floridani* sect. nov., section *Rubri* sect. nov.). Phylogenetic analysis showed that the xerophile *Monascus eremophilus* does not belong in *Monascus* and monophyly in *Monascus* is restored with the transfer of *M. eremophilus* to *Penicillium* (*P. eremophilum* comb. nov.). A list of accepted and excluded *Monascus* and *Basipetospora* species is given, together with information on (ex-)types cultures and barcode sequence data.

Key words: Aspergillaceae, Extrolites, Fungal ecology, Phylogeny, Taxonomy.
Taxonomic novelties: New sections: *Monascus* section *Floridani* R.N. Barbosa & Houbraken, *Monascus* section *Rubri* R.N. Barbosa & Houbraken; **New species:** *Monascus flavipigmentosus* R.N. Barbosa, Souza-Motta, N.T. Oliveira & Houbraken, *Monascus mellicola* R.N. Barbosa, Souza-Motta, N.T. Oliveira & Houbraken, *Monascus recifensis* R.N. Barbosa, Souza-Motta, N.T. Oliveira & Houbraken; **New combination:** *Penicillium eremophilum* (A.D. Hocking & Pitt) Houbraken, Leong & Vinnere-Pettersson.

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INTRODUCTION

Van Tieghem (1884) introduced the genus *Monascus* for species that produce non-ostiolate ascomata and introduced two species, *M. ruber* and *M. mucoroides*. The position of *Monascus* (and the *Monascaceae*) has been the subject of discussion in various papers and it was often placed outside the order *Eurotiales* (Benny & Kimbrough 1980, von Arx 1987, Stchigel & Guarro 2007), but phylogenetic analyses confidentially places this genus in *Aspergillaceae* (*Eurotiales*) (Berbee *et al.* 1995, Ogawa *et al.* 1997, Ogawa & Sugiyama 2000, Peterson 2008, Houbraken & Samson 2011, Vinnere-Pettersson *et al.* 2011). The genus *Basipetospora* was found to be the anamorph of *Monascus* and is characterized by the production of aleurioconidia in a basipetal manner from undifferentiated conidiogenous cells that progressively shorten (retrogression, Cole & Samson 1979). The conidia have a truncated base and resemble chlamydospores. These features set this genus apart from the phylogenetically related genera *Aspergillus* and *Penicillium*.

After the description of the genus, more than 20 species have been introduced and many of them are considered to be synonyms (Shao *et al.* 2011). Classification of *Monascus* has primary been based on macro- and microscopic features, such as the pigmentation of the cleistothecial walls and conidia and growth rates on agar media. Hawksworth & Pitt (1983) revised the genus based on physiological and morphological characteristics and reduced the number of accepted species to three: *M. pilosus*, *M. ruber* and *M. purpureus*. Since that study, ten new species were introduced: *M. albidulus*, *M. argentinensis*, *M. aurantiacus*, *M. eremophilus*, *M. floridanus*, *M. fumeus*, *M. lunisporas*, *M. pallens*, *M. rutilus* and *M. sanguineus* (Barnard & Cannon 1987, Hocking & Pitt 1988, Cannon *et al.* 1995, Udagawa & Baba 1998, Stchigel *et al.* 2004, Li & Guo 2004). With the description of those species, the genus became morphologically and physiologically more diverse, suggesting a large genetic diversity. For example, *Monascus ruber* grows rapidly on agar media, *M. lunisporas* and *M. pallens* grow restrictedly and *M. eremophilus* is a strict xerophile and only grows on low water activity media. The phenotype-based identification schemes in *Monascus* were difficult to match with the results obtained by ITS, partial LSU and/or β-tubulin gene sequencing (Park & Jong 2003, Park *et al.* 2004). Nowadays, species can be delimited on the genotype, for example based on the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) concept. The

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application of this concept in *Monascus* has yet not been performed and the results of such an analysis will give insight on the species boundaries.

The genus *Monascus* has economic importance in several areas, and several species have been widely used for over years in the production of yellow and red food colourants and Asian fermented foods, particularly red rice (ang-kak, angka, 'red kojic rice'). Red rice is of particular interest because of its health promoting effects (Lee & Pan 2011, 2012, Hsu & Pan 2012, Shi & Pan 2012) and indeed, production of compounds with antibacterial properties and cholesterol-lowering statins of the monacolin K-type (= mevinolin = lovastatin) are reported in the species *M. pilosus*, *M. pubigerus*, *M. purpureus*, *M. ruber* and *M. vitreus* (Negishi *et al.* 1986, Jůzlova *et al.* 1996, Vendruscolo *et al.* 2014). However, *Monascus* species such as *M. anka*, *M. aurantiacus*, *M. kaoliang*, *M. pilosus*, *M. purpureus*, *M. ruber* and *M. sanguineus* have been reported to produce the mycotoxin citrinin (Blanc *et al.* 1995, Dietrich *et al.* 1999, Wang *et al.* 2003, Wang *et al.* 2005, Pisareva *et al.* 2005, Shimizu *et al.* 2005, Huang *et al.* 2007, Pattangul *et al.* 2008, Kim *et al.* 2010, Li *et al.* 2012, Li *et al.* 2015), and the presence of this mycotoxin in food, including red rice, should be avoided. Among these reports on citrinin production by *Monascus* species, Wang *et al.* (2005) also reported on citrinin production by *M. floridanus*, *M. lunisporas* and *M. pallens*, but this has not been confirmed by any other authors working on citrinin and *Monascus*. Besides their beneficial properties for human, *Monascus* species can also cause spoilage, for example of silage, bakery (tortillas), pasteurized products (olives) and dried prunes (*M. eremophilus*). Species are also rarely associated with human infections, and an invasive gastric infection case was linked to the consumption of *Monascus* contaminated dried and salted fish (Moreau 1971, Iriart *et al.* 2010, Samson *et al.* 2010).

Specific fungi and other micro-organisms live in close association with social and solitary bees. This association is mandatory, and investigations on the biology, ecology and evolution have been undertaken (Wynns 2015). Recently, a study described a symbiosis between *Scaptotrigona postica* bees and a fungus (Menezes *et al.* 2015). The fungus was identified by morphology and ITS sequencing as being closely related to *M. ruber* and *M. pilosus*. The study showed that the *Monascus* biomass on the food inside the brood cells is essential for the larvae of the *S. postica* bees, and without the consumption of this biomass, only a few larvae can continue their life cycle.

Monascus was one of the predominant genera during the study of fungi associated with honey, pollen and nests of *Melipona scutellaris* bees living in the Atlantic Forest in Pernambuco, Brazil. The phylogenetic relationship of those strains with other species of the genus was determined by the analysis of ITS, LSU, β-tubulin (*BenA*), calmodulin (*CaM*) and RNA polymerase II second largest subunit (*RPB2*) sequences. Furthermore, three new species from honey, pollen and the inside of the nest are described based on a polyphasic approach combining sequence data, macro- and microscopic characters and extrolites.

MATERIALS AND METHODS

Fungal isolation

Samples were collected from honey, pollen and inside nests of *Melipona scutellaris* bees in the Brazilian Tropical Forest in Pernambuco state (8°7′30″S, 34°52′30″W and 8°4′36″S, 34°57′34″W) between January and June 2014. For the honey and pollen samples, 25 g of each specimen was suspended in 225 mL peptone water (0.1 %) and decimal dilutions were made until 10^{-3}. Subsequently, 0.1 mL of each dilution was spread plated on the agar media dichloran 18 % glycerol agar (DG18) and malt extract agar supplemented with chloramphenicol. The plates were incubated at 25 °C for 7–14 d in darkness. For collection of the samples inside nests, a sterile cotton swab was used to sample the surface of the pollen and honey pots, and brood cells. The swab was soaked in 3 mL peptone water (0.1 %) and vortexed vigorously. The samples were subsequently analysed as described above. All fungal colonies were isolated and purified prior identification.

Cultivation and morphological analyses

Thirty *Monascus* strains were obtained from honey, pollen and inside nests of *Melipona scutellaris* bees (Table 1). The colony characteristics of these strains were compared with representative and type cultures of currently accepted *Monascus* species. For this purpose, the strains were cultivated in three points in creatine agar (CREA), cornmeal agar (CMA), Czapek yeast extract agar (CYA), CYA supplemented with 5 % NaCl (CYAS), dichloran 18 % glycerol agar (DG18), malt extract agar (MEA, Oxoid), oatmeal agar (OA), potato dextrose agar (PDA) and yeast extract sucrose agar (YES) incubated at 25 °C for 7 d. Additional CYA and MEA plates were incubated at 30 and 37 °C. *Monascus eremophilus* was inoculated on the malt agar 20 % sucrose (MA20S) and malt yeast extract 50 % glucose agar (MY50G). All media were prepared according to Samson *et al.* (2010). Colony diameters were measured after 7 d of incubation and colony characteristics (e.g. presence of soluble pigments, exudates, obverse and reverse colony colours, colour of mycelium) were recorded. Microscopic observations of the asexual stage were made from colonies grown on MEA. The presence of a sexual stage was determined on MEA, CMA, PDA and OA, and PDA was used for illustrations and measurements. Lactic acid (60 %) was used as mounting fluid and 96 % ethanol was used to remove the excess of conidia. The size, shape and pigmentation of conidia, conidiophores, ascomata, asci and ascospores were recorded. A Zeiss Stereo Discovery V20 dissecting microscope and Zeiss AX10 Imager A2 light microscope equipped with Nikon DS-Ri2 cameras and software NIS-Elements D v4.50 were used to capture digital images. New species names and associated information were deposited in MycoBank. All strains were deposited in the culture collection of Micoteca URM (Federal University of Pernambuco, Recife, Brazil) and the ex-type strains were also deposited in the CBS culture collection housed at the Westerdijk Fungal Biodiversity Institute (formerly known as Centraalbureau voor Schimmelcultures), Utrecht, The Netherlands (under Material Transfer Agreement – MTA No. 01/2016/Micoteca URM).

Molecular characterization

Genomic DNA of 7 d old cultures was extracted using the UltraClean Microbial DNA kit (MoBio Laboratories, Solana Beach, CA, USA) and processed according to the manufacturer's instructions. Polymerase chain reaction (PCR) amplification of the ITS region (ITS1, 5.8S rDNA and ITS2) was performed using the primers

Table 1. Strains and sequences used in the morphological and molecular study.

Species	Strain numbers	Substrate; location	GenBank accession no.				
			ITS	*BenA*	LSU	*CaM*	*RPB2*
Leiothecium ellipsoideum	CBS 607.74^{T} = ATCC 32453	Soil, between rocks; Pelopennesos, Greece	KF732839	KY709178	FJ358285	KY611939	JN121541
Monascus argentinensis	CBS 109402^{T} = DTO 138-C5 = FMR 7393	Soil sample; Tucumán province, Argentina	JF922046	KY709174	KY645974	KY611935	JN121423
M. eremophilus	CBS 123361^{T} = DTO 122-C7 = FRR 3338	Mouldy prunes; New South Wales, Australia	GU733347	KY709170	KY645973	KY611931	KY611970
M. flavipigmentosus	URM 7536^{T} = CBS 142366	Inside nest of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511751	KY709168	KY511781	KY611929	KY611968
M. flavipigmentosus	URM 7535	Inside nest of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511752	KY709169	KY511782	KY611930	KY611969
M. flavipigmentosus	URM 7534	Pollen of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511750	KY709167	KY511780	KY611928	KY611967
M. floridanus	CBS 142228^{T} = DTO 360-E7 = CGMCC 3.5843 = IMI 282587 = UAMH 4180	Sand pine roots; USA	KY635848	KY709172	KY635856	KY611933	KY611972
M. lunisporas	CBS 142230^{T} = DTO 360-E9 = CGMCC 3.7951 = ATCC 204397	Mouldy feed for race horses; Japan	KY635847	KY709171	KY635855	KY611932	KY611971
M. mellicola	URM 7510^{T} = CBS 142364	Honey of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511726	KY709143	KY511756	KY611904	KY611943
M. mellicola	URM 7507	Honey of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511723	KY709140	KY511753	KY611901	KY611940
M. mellicola	URM 7508	Honey of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511724	KY709141	KY511754	KY611902	KY611941
M. mellicola	URM 7509	Honey of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511725	KY709142	KY511755	KY611903	KY611942
M. mellicola	URM 7511	Honey of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511727	KY709144	KY511757	KY611905	KY611944
M. mellicola	URM 7512	Honey of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511728	KY709145	KY511758	KY611906	KY611945
M. mellicola	URM 7513	Honey of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511729	KY709146	KY511759	KY611907	KY611946
M. mellicola	URM 7514	Inside nest of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511730	KY709147	KY511760	KY611908	KY611947
M. mellicola	URM 7515	Inside nest of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511731	KY709148	KY511761	KY611909	KY611948
M. mellicola	URM 7516	Inside nest of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511732	KY709149	KY511762	KY611910	KY611949
M. mellicola	URM 7517	Inside nest of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511733	KY709150	KY511763	KY611911	KY611950
M. mellicola	URM 7518	Honey of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511734	KY709151	KY511764	KY611912	KY611951
M. mellicola	URM 7519	Honey of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511735	KY709152	KY511765	KY611913	KY611952
M. mellicola	URM 7520	Pollen; Recife, Pernambuco, Brazil	KY511736	KY709153	KY511766	KY611914	KY611953
M. mellicola	URM 7521	Honey of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511737	KY709154	KY511767	KY611915	KY611954
M. mellicola	URM 7522	Inside nest of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511738	KY709155	KY511768	KY611916	KY611955

(*continued on next page*)

Table 1. (Continued).

Species	Strain numbers	Substrate; location	GenBank accession no.				
			ITS	*BenA*	LSU	*CaM*	*RPB2*
M. pallens	CBS 142229^{T} = DTO 360-E8 = CGMCC 3.5844 = ATCC 200612 = IMI 356820	River sediment; Iraq	KY635849	KY709173	KY635857	KY611934	KY611973
M. pilosus	CBS 286.34^{T} = DTO 165-B1 = ATCC 16363 = FRR 2194 = IFO 4480	Fermented grain, *Sorghum vulgare*; Japan	KY635852	JF922085	KY635860	KY849968	KY849967
M. purpureus	CBS 109.07^{T} = DTO 364-D8 = ATCC 16365 = IFO 4513 = IMI 210765 = NRRL 1596	Fermented rice grain ('ang-quac'); Java, Indonesia	KY635851	KY709176	KY635859	KY611937	JN121422
M. recifensis	URM 7524^{T} = CBS 142365	Pollen of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511740	KY709157	KY511770	KY611918	KY611957
M. recifensis	URM 7523	Pollen of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511739	KY709156	KY511769	KY611917	KY611956
M. ruber	URM 7525	Inside nest of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511741	KY709158	KY511771	KY611919	KY611958
M. ruber	URM 7526	Inside nest of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511742	KY709159	KY511772	KY611920	KY611959
M. ruber	URM 7527	Inside nest of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511743	KY709160	KY511773	KY611921	KY611960
M. ruber	URM 7528	Inside nest of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511744	KY709161	KY511774	KY611922	KY611961
M. ruber	URM 7529	Inside nest of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511745	KY709162	KY511775	KY611923	KY611962
M. ruber	URM 7530	Inside nest of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511746	KY709163	KY511776	KY611924	KY611963
M. ruber	URM 7531	Inside nest of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511747	KY709164	KY511777	KY611925	KY611964
M. ruber	URM 7532	Inside nest of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511748	KY709165	KY511778	KY611926	KY611965
M. ruber	URM 7533	Inside nest of *Melipona scutellaris*; Recife, Pernambuco, Brazil	KY511749	KY709166	KY511779	KY611927	KY611966
M. ruber	CBS 135.60^{NT} = DTO 359-E8 = ATCC 15670 = IFO 8451 = IMI 081596	Soil; India	KY635850	KY709175	KY635858	KY611936	KY611974
M. sanguineus	IMI 356821^{T} = ATCC 200613	River sediment; Iraq	JF922055	JF922088	AF364968	KY611938	n/a
Penicillium polonicum	CBS 222.28^{T} = IBT 12821 = IMI 291194 = NRRL 995	Soil, Poland	AF033475	AF001206	JN939272	KU896848	JN985417
P. verrucosum	CBS 603.74^{NT} = IMI 200310 = ATCC 48957 = FRR 965 = IBT 4733 = NRRL 965	Unknown source, Belgium	AB479317	AF001205	AB479285	DQ911138	JN121539
Talaromyces purpurogenus	CBS 286.36 = IMI 091926	Unknown source; Japan	JX315671	JX315639	KY635863	KF741947	JX315709
T. ruber	CBS 132704 = IBT 10703	Aircraft fuel tank; UK	NR111780	JX315629	KY635864	KF741938	JX315700
Xerochrysium dermatitidis	CBS 132.31^{T} = IMI 096729 = UAMH 802	Skin, man; Italy	KY635853	n/a	KY635861	n/a	JN121443
Xeromyces bisporus	CBS 236.71^{T} = IMI 063718	Mouldy stick of liquorice; New South Wales, Australia	KY635854	JF922089	KY635862	741987712[1]	JN121612

Abbreviations: T = type strain; NT = neotype strain; URM, URM Culture Collection (www.ufpe.br/micoteca), Brazil; CBS, Culture collection of the Westerdijk Fungal Biodiversity Institute (formerly known as Centraalbureau voor Schimmelcultures), The Netherlands; DTO, Internal culture collection at Westerdijk Fungal Biodiversity Institute.

[1] Sequence from genome sequenced strain; n/a: no sequence available.

V9G and LS266 and a part of the Large SubUnit (LSU) rDNA was amplified using the primers LR0R and LR5. Partial β-tubulin fragments were generated using the primer combination Bt2a and Bt2b, for calmodulin the primers Cmd5 and Cmd6 were used and for *RPB2* the primers RPB2-5F and RPB2-7CR. Details on the primer sequences, PCR mixtures and conditions are previously described (Samson *et al.* 2010, Houbraken *et al.* 2012).

The PCR products were sequenced in both directions with the same primers using the BigDye® Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems Life Technologies, Carlsbad, CA, USA) and were purified with Sephadex, according to the manufacturers' recommendations. Contigs were assembled using the forward and reverse sequence with the SeqMan v. 10.0.1 program. Newly generated sequences were deposited in GenBank. Sequence datasets were generated by combining the newly generated sequences with sequences from GenBank (Table 1). The sequences were aligned using MAFFT (Katoh *et al.* 2005) and were manually optimized using MEGA 5 (Tamura *et al.* 2011). The most suitable substitution model was determined using FindModel (Posada & Crandall 1998). Phylogenetic trees were constructed using maximum likelihood (ML) analysis in RAxML-VI-HPC v. 7.0.3 (Stamatakis 2006) using the GTRGAMMA substitution model and 1 000 bootstrap replicates. Bayesian inference (BI) in MrBayes v.3.2.1 (Ronquist *et al.* 2012) was performed using Markov Chain Monte Carlo (MCMC) algorithm and the best scoring substitution model is indicated in the results section. Trees were visualized in FigTree v. 1.1.2 (Rambaut 2009) and edited in Adobe Illustrator v.CS5.1. Individual alignments were concatenated by using Mesquite v3.04 (Maddison & Maddison 2016). The quality of final alignment was evaluated using Transitive Consistence Score (TCS) by the T-Coffee web server (Chang *et al.* 2015).

Extrolite analysis

Extrolites were extracted from fungal strains grown on CYA, YES, MEA, OA at 25 °C for 14 d and PDA and DG18 at 25 °C for 20 d. Three agar plugs of each culture were extracted as previously described (Smedsgaard 1997, Houbraken *et al.* 2012). After extraction, the liquid was transferred to a clean screw-cap vial and evaporated to dryness. Prior analysis, the dried extracts were re-dissolved in methanol by ultrasonication and filtered through a 0.45 µm filter. The extracts were analysed by ultra-high performance liquid chromatography with diode-array detection (UHPLC-DAD) (Houbraken *et al.* 2012). The detected eluted compounds were identified by comparing the retention time, retention index and UV spectra measured at 200–600 nm. The UV spectra were compared to a database of UV spectra and data from literature (Nielsen *et al.* 2011, Klitgaard *et al.* 2014).

RESULTS

Phylogeny and GCPSR

The phylogenetic relationship of the commonly accepted *Monascus* species and the isolates obtained from honey, pollen and nests of *Melipona scutellaris* bees were studied using concatenated five-gene data set (ITS, *BenA*, *CaM*, LSU and *RPB2*). The Transitive Consistence Score (TCS) evaluated the robustness of the five-gene with the high score of 929. The total length of the aligned data set was 2930 characters (ITS, 583 bp; *BenA*, 505 bp; *CaM*, 490 bp; *RPB2*, 784 bp; LSU, 568 bp) including alignment gaps. The GTR+G model was the most optimal and selected for the ITS and LSU data sets, the HKY+G model for *BenA* and K80+G for the *CaM* and *RPB2* data sets. A similar topology was observed in the five single gene phylogenies and no significant incongruence were found (Fig. 2A–E). A total of 1692 trees were generated during the Bayesian inference from which 422 trees were discarded after 25 per cent of the generations in 'burn-in phase' and posterior probabilities were calculated from the remaining 1 270 trees. The results of the Bayesian analysis were similar to the results of the ML analysis, and differences were only in the degree of support for some branches. The Bayesian consensus tree is presented here with the relevant bootstrap percentages (>70 %) and posterior probability values (>0.95) (Fig. 1).

Monascus eremophilus is positioned outside the main *Monascus* clade and proved to be related to *Penicillium* species (100 % bs, 1.00 pp) (Fig. 1). Our analysis revealed two well-supported groups in *Monascus*, referred here to as the *M. floridanus*- and *M. ruber*-clades. Seven well-supported lineages are present in the *M. floridanus*-clade and these lineages are treated as separate species. Four are known species (*M. lunisporas*, *M. argentinensis*, *M. floridanus*, *M. pallens*), and three are proposed as newly described below (*Monascus mellicola*, *M. recifensis* and *M. flavipigmentosus*). *Monascus mellicola* is phylogenetically distinct and is with moderate bootstrap and posterior probability support (82 % bs, 0.96 pp) related to *M. argentinensis*, *M. lunisporas*, *M. recifensis* and *M. flavipigmentosus*. The latter three species are resolved as close relatives in a distinct, well-supported clade. In our concatenate phylogenetic analysis these species are separated in three well-supported groups, with *M. lunisporas* and *M. flavipigmentosus* being sister species and *M. recifensis* taking a basal position. Similar clustering was obtained in the single gene analyses; however, the species were unresolved in the LSU phylogram (Fig. 2).

The (neo)type strains of *M. pilosus* (CBS 286.34^{T}), *M. purpureus* (CBS 109.07^{T}), *M. ruber* (CBS 135.60^{NT}) and *M. sanguineus* (ATCC 200613^{T}) are located in the *M. ruber*-clade. Two lineages are present within the *M. ruber*-clade (Fig. 1). The (neo)type strains of *M. pilosus* (CBS 286.34^{T}) and *M. ruber* (CBS 135.60^{NT}) are together on a well-supported branch (97 % bs; 1.00 pp), and the branch containing the types of *M. purpureus* (CBS 109.07^{T}) and *M. sanguineus* (ATCC 200613^{T}) has weak statistical support (78 % bs; <0.95 pp). In our single gene analyses, *M. pilosus* (CBS 286.34^{T}) and *M. ruber* (CBS 135.60^{NT}) always cluster together with high (ITS: 99 % bs, 1.00 pp; LSU: 98 % bs, 1.00 pp; *CaM*: 88 % bs, 0.98 pp) or moderate (*RPB2*: 96 % bs, <0.95 pp, *BenA* 89 % bs, <0.95 pp) statistical support. The branch with *M. purpureus* (CBS 109.07^{T}) and *M. sanguineus* (ATCC 200613^{T}) is well supported in the ITS phylogram (87 % bs, 0.99 pp), and no support was found in the *BenA*, *CaM* and LSU analyses (<70 %, <0.95 pp). Following the GCPSR concept, we keep two lineages in the *M. ruber*-clade. *Monascus pilosus* and *M. sanguineus* are treated here as synonym of *M. ruber* and *M. purpureus*, respectively.

Morphology

Monascus can also be split into two groups based on morphological characters. The majority of the species belonging to the

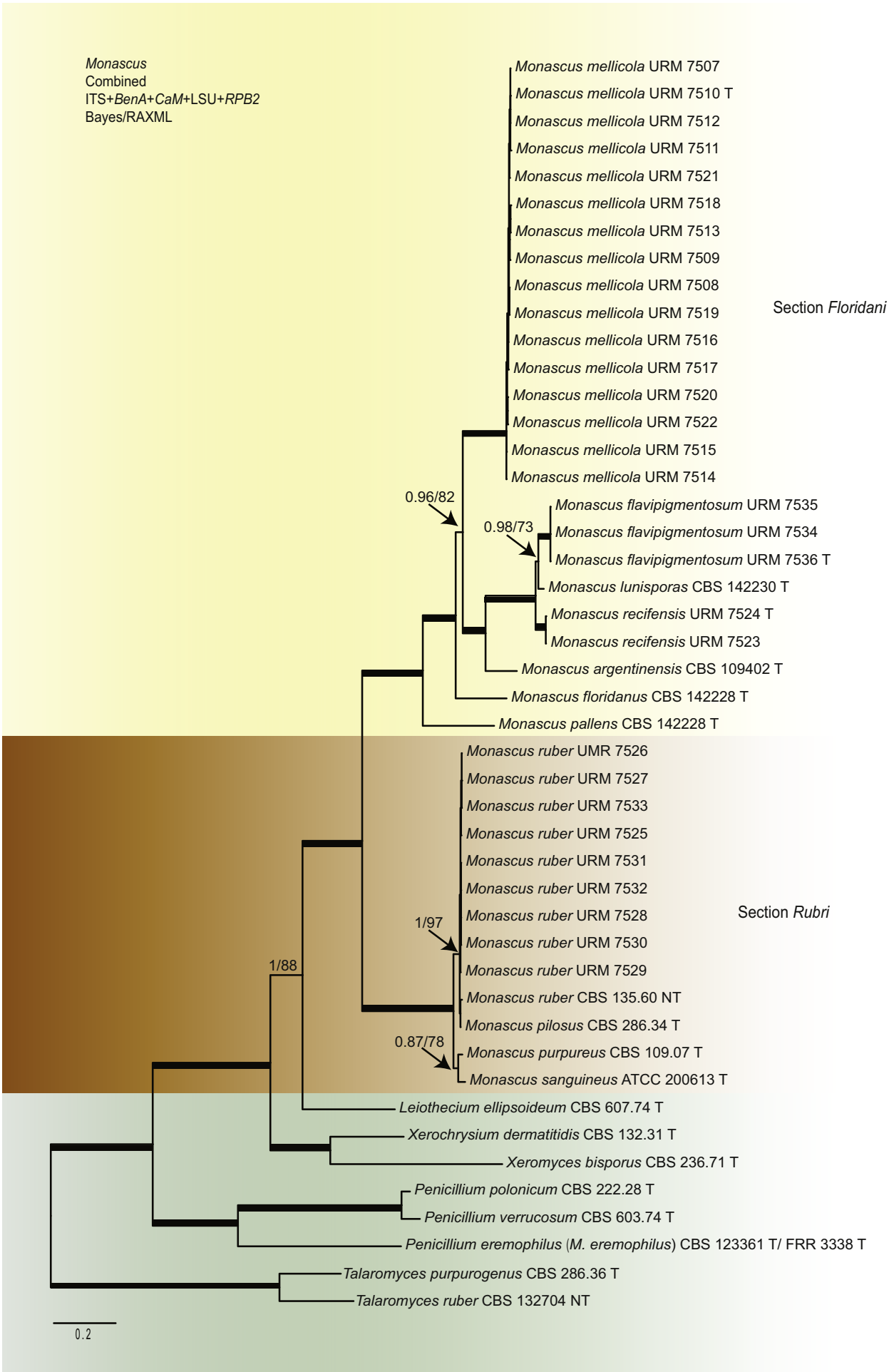

Fig. 1. Concatenated phylogeny of the ITS, *BenA*, *CaM*, LSU and *RPB2* gene regions showing the relationship in *Monascus*. Branches with posterior probability values of 1.00 and >95 % are thickened.

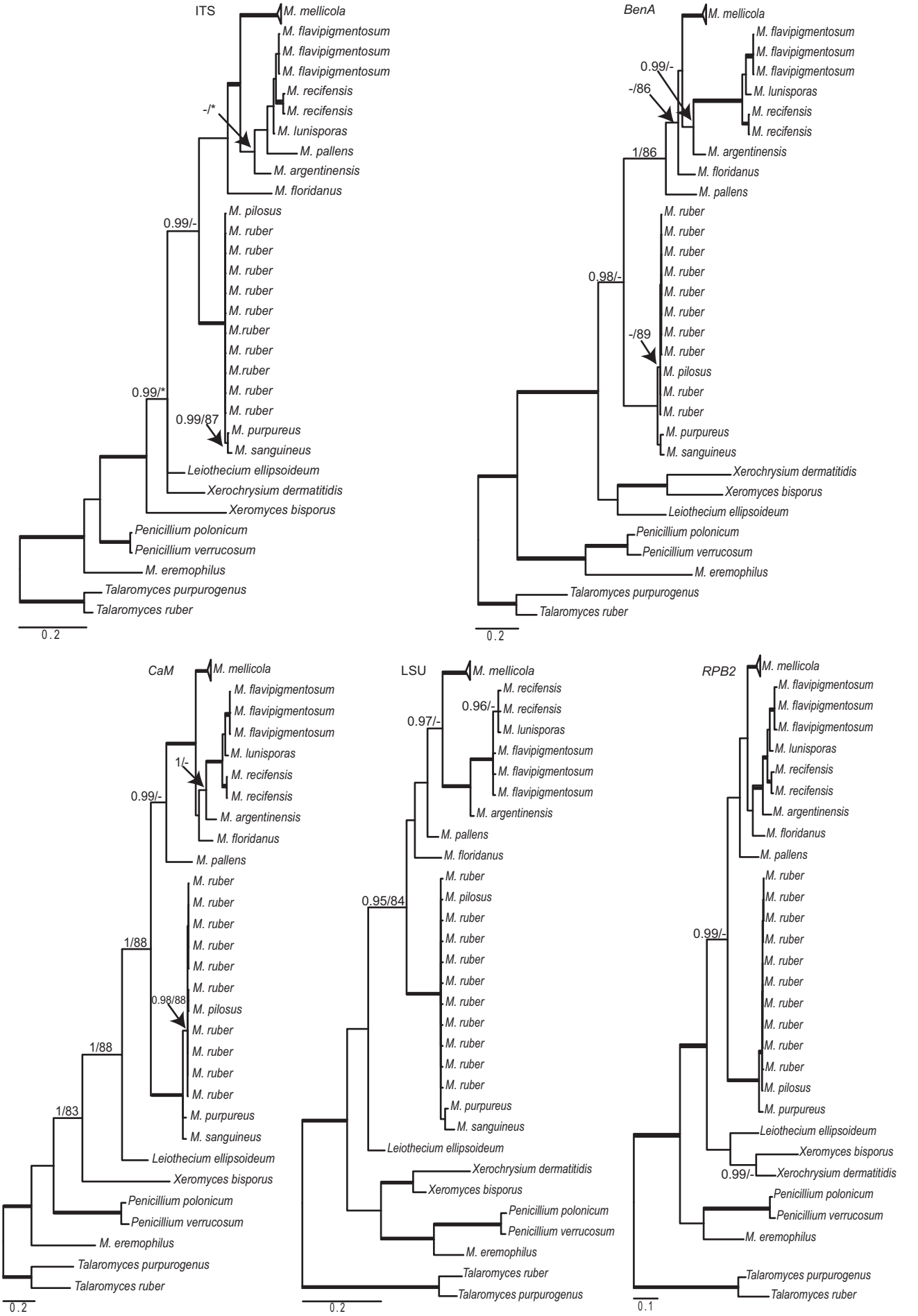

Fig. 2. Single gene phylogenetic trees of the ITS, *BenA*, *CaM*, LSU and *RPB2* gene regions of species from *Monascus*. Branches with posterior probability values of 1.00 and >95 % are thickened.

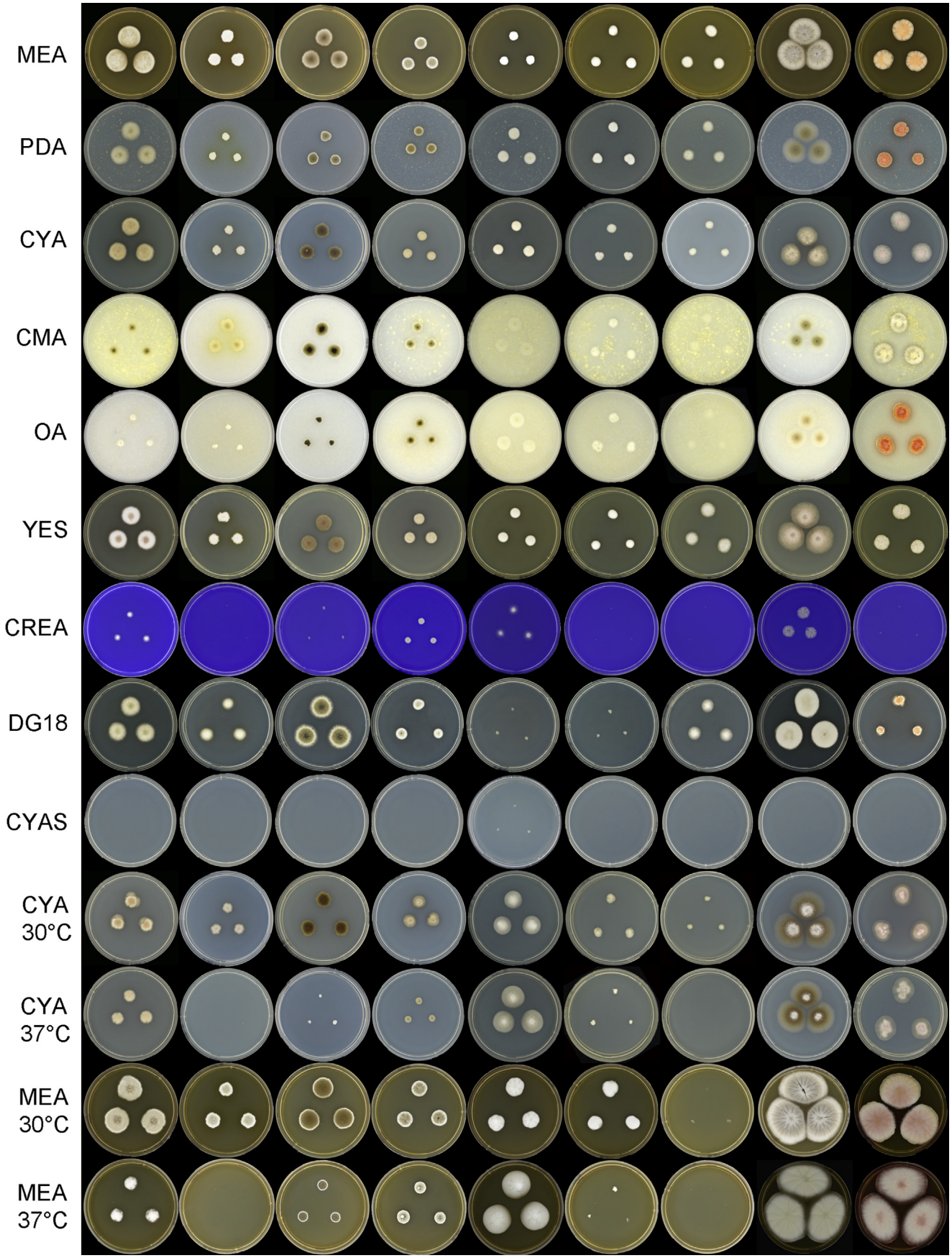

Fig. 3. Cultural characters of *Monascus* species on different agar media and incubation conditions. Left to right: *M. lunisporas*, *M. flavipigmentosus*, *M. recifensis*, *M. mellicola*, *M. pallens*, *M. floridanus*, *M. argentinensis*, *M. ruber* and *M. purpureus*.

M. floridanus-clade grow restricted on MEA, PDA, CYA, CMA, OA and YES and have no or restricted growth on CYA incubated at 37 °C. Furthermore, the colonies are in shades of brown, the conidia are brown pigmented and the mycelium is white to olivaceous-brown (Fig. 3). The isolates belonging to the *M. ruber*-clade can be differentiated from the *M. floridanus*-clade species by their faster growth on MEA, PDA, CYA, CMA, OA and YES at 25 °C and CYA incubated at 37 °C. The colonies of

M. ruber-clade species are in shades of white when young, and turn to shades of brownish-red, orange to red after 7–10 d incubation (Fig. 3). The species can be differentiated using phenotypic characters; however, for most species only the type strain or a representative was available and examined. The most important macro- and microscopic characters are given in Tables 2, 3 and Fig. 4.

Extrolites

Monascus species are good producers of known and uncharacterized extrolites and an overview is given in Table 4. The majority of the species produced a species specific profile of extrolites. *Monascus recifensis* produced asterric acid, methylasterrate, secalonic acid, (−)-bisdechlorogeodin, questin, secalonic acid D and a compound with an orthosporin-chromophore (orthosporin-like), *M. argentinensis* produced rubratoxin A and B or similar nonadrides (rubratoxin-like extrolites) and *M. flavipigmentosus* produced a series of extrolites that are to our knowledge never detected in any other filamentous fungus until now. *Monascus floridanus* produced an orthosporin and other extrolites only found in this species. The extrolite called "GULLA" is produced by *M. mellicola* and *M. recifensis*, while *M. pallens* produces curvularin and dehydrocurvularin (Tables 4 and 5). It is interesting that a series of extrolites with characteristic chromophores (metabolite families M, N, O, Y) (see Supplementary material for UV spectra) have only been found in *Monascus* species so far. In the examination of thousands of extrolite extracts from species in *Penicillium*, *Aspergillus*, *Paecilomyces*, *Talaromyces*, *Fusarium*, *Trichoderma*, *Alternaria*, *Curvularia*, *Chaetomium* and other genera, those compounds have never been detected (JC Frisvad, personal observations). These extrolites are unique for *Monascus* species may have ecological roles in the interaction with bees.

Identification of *Monascus* isolates associated with *Melipona scutellaris*

Thirty isolates were obtained from honey, pollen and the inside of the nest of *Melipona scutellaris*, representing three new (*M. flavipigmentosus*, *M. mellicola* and *M. recifensis*) and one described species (*M. ruber*). *Monascus mellicola* was predominantly present (16 isolates), followed by *M. ruber* (9), *M. flavipigmentosus* (3) and *M. recifensis* (2). Nine *M. mellicola* isolates were isolated from honey, five from the inside of the nest and two from pollen. Sequence variation is observed among the investigated *M. mellicola* isolates, showing that the isolates don't have a clonal distribution. The *M. ruber* isolates were all from the inside of the nest and their identity was in agreement with the results of the morphological examination (Fig. 5). *Monascus flavipigmentosus* was isolated from inside nests (2 isolates) and pollen (1 isolate) and both *M. recifensis* isolates were from pollen.

DISCUSSION

Monascus belongs to the order Eurotiales, and this genus is characterized by the production of stalked cleistothecial ascomata that are non-ostiolate and have hyaline to brown walls. The ascomatal cavity is filled with unicellular ascospores. Asexual reproduction takes place on basipetospora-type

Table 2. Growth rate comparison of *Monascus* species after 7 d (in mm) and most important colony characters.

Species	CYA	MEA	DG18	CYAS	OA	CREA	YES	CMA	PDA	MEA 30 °C	CYA 30 °C	MEA 37 °C	CYA 37 °C	Colour mycelium on MEA	Soluble pigments
Monascus argentinensis	8–9	11–13	13–15	ng	10–11	ng	14–15	9–10	10–11	ng	5–6	ng	ng	White	Absent
M. flavipigmentosus	7–10	10–12	8–10	ng	4–5	ng	10–11	10–12	6–8	10–11	9–10	0–2	0–3	White	Yellow
M. floridanus	9–10	9–10	3–5	ng	10–11	ng	9–10	9–10	10–11	10–11	8–10	2–4	3–4	White	Absent
M. lunisporas	15–17	24–25	20–22	ng	14–15	3–5	20–23	19–20	18–20	24–25	15–17	9–10	12–13	Brownish	Absent
M. mellicola	8–10	11–12	7–10	ng	9–10	5–7	10–11	9–10	9–10	21–22	11–12	11–12	5–7	White	Absent
M. pallens	10–11	8–10	3–4	3–4	14–15	9–10	10–11	9–11	12–13	11–15	17–18	26–30	21–22	White	Absent
M. purpureus	19–20	20–22	3–5	ng	16–20	ng	13–18	18–20	11–15	39–40	20–21	52–55	20–21	Red to orange	Orange
M. recifensis	12–14	16–18	20–21	ng	3–5	1–2	14–15	10–12	10–11	19–20	10–11	9–10	3–4	White to brownish	Absent
M. ruber	17–19	25–26	18–20	ng	18–20	8–10	17–28	15–20	26–30	47–48	35–37	49–50	35–40	White	Absent

Table 3. Most important micromorphological characters for species recognition.

Species	Colour and size (µm) ascomata on PDA	Shape ascospores on PDA	Size ascospores (µm)	Shape and colour conidia	Size of conidia (µm)	Number of conidia per phialide
*Monascus argentinensis**	Dark olivaceous-brown, 20–75	Ellipsoidal to subglobose	3–4 × 2.5–3	Globose to obovoid or obpyriform	Globose, 5–15; obpyriform, 7–15 × 5–9	Single or formed in short chains
M. flavipigmentosus	Hyaline to brown, 40–60	Lunate	4–5 × 1.7–2.5	Globose to subglobose, hyaline to brown	5.5–7.5	Single or formed in short chains
*M. floridanus**	Dark brown, 22–58	Ellipsoidal	3.5–4.5 × 2–3	Globose to obovoid or obpyriform, pale brown	4–9 × 3.5–9	Single or formed in short chains (up to 6?)
*M. lunisporas**	Brown, 25–60	Lunate	6–7 × 2–2.5	Globose to obpyriform, hyaline to brown	Globose, 6–11; obpyriform, 5–7 × 7–10	Single or formed in short chains
M. mellicola	–	–	–	Globose to subglobose, hyaline to brown	2.5–5.0 × 3.5–5.0	Single or up to 17 conidia
*M. pallens**	Hyaline, 23–38	Ellipsoidal	3.5–4 × 2.5–3	Usually pyriform, hyaline	3.5–10 (–13) × 2.5–8	Short terminal or intercalary basipetal
*M. purpureus***	Hyaline, (25–) 45 × 60 (–70)	Ellipsoidal	(5.5–) 6–7 × 4–5	Globose to obpyriform	8–11 × 8–10	Single or in short chains
M. recifensis	–	–	–	Globose to subglobose, hyaline to brown	4.0–7.0	Single or in short chains
*M. ruber***	Brown, 30–50 (–60)	Ellipsoidal	5–6 (–7.5) × (3.5–) 4–5	Globose to obpyriform	10–18 × 8–14	Single or up to 10 conidia

Abbreviations: *Data from original description; **Data from Hawksworth & Pitt (1983); –: not observed.

conidiophores. These conidiophores are erect, variable in length, and the conidia are hyaline to brown and produced singly or in short basipetal chains (up to 15–20 conidia). Phenotypic identification of *Monascus* species largely depends on shape, size and pigmentation of the cleistothecia and ascospores (Hawksworth & Pitt 1983). No cleistothecia and only the basipetospora-state was observed in the two newly described species *M. mellicola* and *M. recifensis*; however, these species do phylogenetically belong to the *Monascus* clade. They produce a basipetospora-state, which is the characteristic asexual stage of this genus. Following the latest International Code of Nomenclature for algae, fungi and plants (McNeill *et al.* 2012), in respect to the principle of priority, and that nomenclature has economic and social implications, particularly for old, important genera, we give priority to *Monascus* over *Basipetospora*, even when no sexual state is observed in those species. This is in line with the recommendations of Rossman *et al.* (2016), who also recommended giving priority to the name *Monascus* over *Basipetospora*.

In the last years numerous new genera have been proposed primary based on phylogenetic data and sometimes with only a few distinctive morphological features. Phenotypic and phylogenetic analysis revealed two well-supported clades in *Monascus*. Following the guidelines proposed by Vellinga *et al.* (2015), these differences would justify splitting *Monascus* into two separate genera. On the other hand, *Monascus* species do share various characters, such as similar basipetospora-type conidiophores and stalked cleistothecia. The majority of *Monascus* species produce indole alkaloids (possibly gypsetins) and this study shows that various *Monascus* species are also associated with stingless bees, indicating that they are also ecologically related. We therefore give preference to introduce two new sections instead of two small genera. A sectional classification system is commonly applied in genera related to *Monascus*, such as *Penicillium*, *Aspergillus* and *Talaromyces* and this is in line with that approach (Gams *et al.* 1985, Houbraken & Samson 2011, Yilmaz *et al.* 2014). The two sections have few extrolites in common (Table 5). The *Rubri* section contains species that produce mevinolins, citrinin and other yellow and red azaphilone pigments, including the red pigments (rubropunctamine, PP-V, PP-R etc.) that are colouring red rice, while the species in section *Floridani* do not produce any of these bioactive extrolites at all. Isolates in each species in section *Floridani* produce species specific combinations of extrolites, and few are in common between those species. One example is the red compound "GULLA", which was detected in both *M. mellicola* and *M. recifensis*, but the latter species produce several extrolites that are not produced by *M. mellicola*, including secalonic acid D, asterric acid, questin, (−)-bisdechlorogeodin and some red anthraquinone extrolites not related to the azaphilones produced by *M. purpureus* and *M. ruber*. Strains of *M. flavipigmentosus* produce a high number of unique as yet not structure elucidated extrolites, including some yellow coloured extrolites (Y1 and Y2) and an anthraquinone (Table 5). The red extrolite "GULLA" has previously been found in *Penicillium* species, including *Penicillium oxalicum* and *P. mononematosum* (Frisvad, personal communication).

Several morphological features are shared between *Monascus* species; however, there are also various characters that can be used for identification (Tables 2 and 3). For example, the conidial size can differ between species. All species except two (*M. mellicola*, *M. recifensis*) produce a sexual state and the size and shape of the ascospores can differ among species. The species also differ in their growth rates, and for example *M. flavipigmentosus*, *M. pallens* and *M. floridanus* grow more restrictedly on agar media than *M. ruber* and *M. purpureus*. Most species do not produce soluble pigments; however, the production of red (soluble) pigments is a character of *M. purpureus* and *M. ruber* (Hawksworth & Pitt 1983) and *M. flavipigmentosus* produces yellow pigments on CMA and PDA (and old cultures on

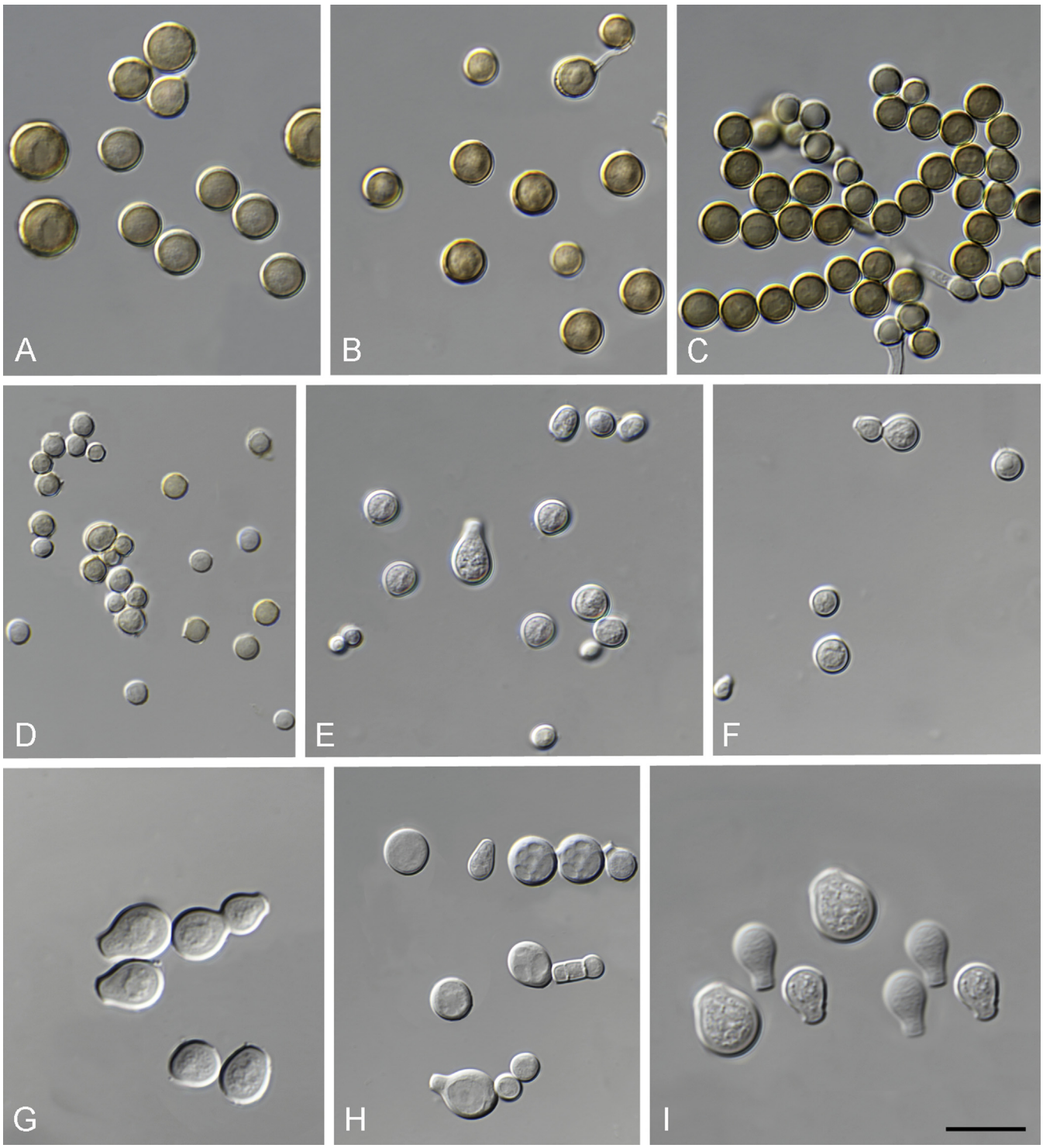

Fig. 4. Conidial shapes and colours of *Monascus* species. **A.** *M. lunisporas*. **B.** *M. flavipigmentosus*. **C.** *M. recifensis*. **D.** *M. mellicola*. **E.** *M. pallens*. **F.** *M. floridanus*. **G.** *M. argentinensis*. **H.** *M. ruber*. **I.** *M. purpureus*. Scale bars = 10 µm.

DG18). Also the growth rate at 37 °C is diagnostic. *M. pallens*, *M. ruber* and *M. purpureus* grow equally or even faster at 37 °C than at 30 °C. On the other hand, *M. floridanus* and *M. mellicola* and *M. recifensis* grow slowly at 37 °C, and *M. argentinensis* and *M. flavipigmentosus* did not grow at this temperature at all.

All species except *M. floridanus* produced a species-specific series of extrolites, consistent with the phenotypic classification and the results obtained in the phylogenetic study. *Monascus lunisporas*, *M. recifensis* and *M. flavipigmentosus* are phylogenetically closely related. Their extrolite profiles are distinct. *Monascus flavipigmentosus* produces metabolites of biosynthetic family M and *M. recifensis* secalonic acid, asterric acid, sulochrin, questin and an anthraquinone with the same UV spectrum as physcion (physcion-like in Table 4). None of these extrolites were found in the closely related species *M. lunisporas* (CBS 142230^{T}). An indole alkaloid (probably gypsetin) was produced by 6 of the 9 species (Table 4) and was the only metabolite found in section *Floridani* and *Rubri*. The metabolites mevinolins and xanthomonasin A were only detected in *M. ruber*-clade species. An important characteristic of *Monascus ruber* is its ability to produce citrinin, a compound with both antibiotic and toxic activity. According literature, this extrolite is also produced by *M. purpureus*, *M. pallens*, *M. lunisporas* and *M. floridanus* (Wang *et al.* 2005). In our study, citrinin was detected only in the

Table 4. Extrolites detected in *Monascus*.

Species	Extrolites
Monascus argentinensis	Anthraquinone Z, indole alkaloid (possibly gypsetin), rubratoxin-like
M. flavipigmentosus	Anthraquinone X (possibly atrochrysone), indole alkaloid (possibly gypsetin), unknown and unique metabolite biosynthetic family M and Y (Y1 and Y2)
M. floridanus	"ENDI", orthosporin-like, "JOPS"
M. lunisporas	Citrinadin-like, indole alkaloid (possibly gypsetin), metabolite N series, metabolite O series, shamixanthone-like
M. mellicola	Indole alkaloid (possibly gypsetin), "GULLA"
M. pallens	Curvularin, dehydrocurvularin, indole alkaloid (possibly gypsetin)
M. purpureus	Citrinin, mevinolins, monascin, PP-V, PP-R, rubropunctamine, rubropunctatin, xanthomonasin A
M. recifensis	Anthraquinone X (= atrochrysone?), asterric acid, (−)-bisdechlorogeodin, "GULLA", orthosporin-like, anthraquinone W (physcion-like), questin, red anthraquinone pigments, secalonic acid D, sulochrin
M. ruber	Indole alkaloid (possibly gypsetin), mevinolins, monascin, PP-V, PP-R, xanthomonasin A, rubropunctamine, rubropunctatin, rubratoxin-like

type of *M. purpureus*. More strains in addition to other culture conditions stimulating citrinin production should be investigated to find out if other species besides *M. purpureus* can produce citrinin.

Based on the results of our study combined with data from previous studies, we accept nine species in *Monascus*: *M. argentinensis*, *M. floridanus*, *M. lunisporas*, *M. mellicola*, *M. pallens*, *M. purpureus*, *M. ruber*, *M. recifensis* and *M. flavipigmentosus* (Hawksworth & Pitt 1983, Park *et al.* 2004). *Monascus pilosus*, *M. sanguineus* are also often mentioned in literature as accepted species in *Monascus*. Phenotypically, *M. pilosus* is very similar to *M. ruber* and according literature, Hawksworth & Pitt (1983) indicated that they can differentiated by the size of ascomata (25–55 *vs* 30–50 (–60) µm), ascospores (5–7 (–8.5) × 3–3.5 (–4) *vs* 5–6.5 (–7.5) × (3.5–) 4–4.5 µm) and the presence of a brownish pigment in the cleistothecial walls and conidia. These sizes and colours are overlapping and during the study of the *M. ruber* isolates associated with bees, we also found considerable variation in pigmentation among the studied strains. Previous studies showed that *M. pilosus* shares ITS and partial LSU and β-tubulin sequences with *M. ruber* (Park & Jong 2003, Park *et al.* 2004), suggesting that these are conspecific. *Monascus pilosus* clusters together with *M. ruber* in all of our single gene phylogenies, confirming these results. Additionally, *M. ruber* and *M. pilosus* are similar also in their metabolite profiles and share the production of mevinolins, rubropunctamine and xanthomonascin. Subsequently, there is no basis to accept *M. pilosus* as a separate species. Based on sequence data, *M. sanguineus* is treated here as a synonym of *M. purpureus*. Analysis of partial β-tubulin sequences (another part of the gene than used in this study) showed that *M. sanguineus* and *M. purpureus* are phylogenetically closely related and distinct from *M. ruber* (Park *et al.* 2004). These results are confirmed in our *BenA*, *CaM*, ITS and LSU phylograms, though statistical support was only found in the ITS phylogram. Based on the GCPSR concept, these species are treated as separate species. Phenotypically, *M. sanguineus* is differentiated from *M. purpureus* by its inability to grow on G25N and colour of ascomata and conidia; however, these characters might not be stable among a larger set of isolates, and this needs further investigation.

Many other species are described in *Monascus*: *M. albidulus* (= *M. albidus* nom. inval.), *M. araneosus*, *M. aurantiacus*, *M. fumeus* (= *M. fuliginosus* nom. inval.), *M. kaoliang*, *M. major*, *M. paxii*, *M. pilosus* nom. inval., *M. pubigerus*, *M. rubiginosus*, *M. rutilus* (= *M. anka* nom. inval.), *M. rubropunctatus*, *M. serorubescens*, *M. vitreus*. All these species belong to *M. ruber*-clade (Hawksworth & Pitt 1983, Park & Jong 2003). A detailed study is needed to determine the species diversity within the *M. ruber*-clade and to resolve the placement of the *M. ruber*/*M. purpureus* synonyms. Six *Basipetospora* species (*B. chlamydospora*, *B. denticola*, *B. halophila*, *B. rubra*, *B. variabilis*, *B. vesicarum*) are described and those might compete with the new species that are described here, especially those that lack a sexual state. However, *Basipetospora rubra* was described as the asexual state of *M. ruber* and is in the single name nomenclature system regarded as a synonym of this species. *Basipetospora halophilica* phylogenetically belongs to *Aspergillus* and was recently transferred to this genus (Samson *et al.* 2014, Kocsubé *et al.* 2016). *Basipetospora chlamydospora*, *B. variabilis* and *B. denticola* represented by CBS 228.84 (16S rRNA, AB024045), CBS 995.87 (16S rRNA, AF437892) and CBS 132.78 (ITS, LN850801), respectively, belong to *Microascales*. The first two species might represent a novel genus in this order (J. Woudenberg, pers. comm.) and the latter is a synonym of *Scopulariopsis candida* (Jagielski *et al.* 2016). *Basipetospora vesicarum* can be considered a synonym of *M. ruber*. This species was introduced based on examination of the type specimen of *Sporotrichum vesicarum* and analysis of this specimen revealed the presence of the *Basipetospora* anamorph of *M. ruber* (Stalpers 1984).

When *Monascus eremophilus* was described, Hocking & Pitt (1988) noted the unique features of this species. Based on colony colour and the mode of ascospore production they decided that the species could best be classified in *Monascus*. After its description, *Monascus eremophilum* was included in various phylogenetic studies; however, results concerning its placement inferred from different DNA regions were inconclusive. Park & Jong (2003) evaluated the use of D1/D2 sequences of the LSU rRNA for species differentiation in *Monascus*, and simultaneously performed a phylogenetic analysis. In their study, *M. eremophilus* was found in the clade containing the type of *M. ruber*; however, the bootstrap support of that clade was low (61 %). In 2004, Park *et al.* studied the genus *Monascus* by using ITS and partial beta-tubulin gene sequences. The position of *M. eremophilus* was unresolved in their ITS phylogram, and the species grouped together with *M. lunisporas* and *M. pallens* with less than 50 % bootstrap support. Moreover, when the beta-tubulin sequences were used, *M. eremophilus* was placed outside the ingroup. The authors commented that such an inconclusive placement of *M. eremophilus* might indicate: '… a unique and unpredictable genetic combination for this species. It might reflect enormous and extreme environmental stress and subsequent drastic genetic changes to adapt to extremely dry conditions' (Park *et al.* 2004). More recently, based on D1/D2 sequence data, Vinnere-Pettersson *et al.* (2011) showed that *M. eremophilus* does not

Table 5. Retention index and absorption maxima for extrolites dectected in Monascus (the UV spectra of the unknown compounds are shown in the Supplementary data).

Extrolite	Retention index	Absorption maxima (nm)	Extrolite by section
Anthraquinone X (= atrochrysone?)	1207	220, 261, 282sh, 427	*Floridani*
Anthraquinone Z	948	223, 271, 298, 433	*Floridani*
Asterric acid	921	207, 220sh, 252, 317	*Floridani*
Asterric acid derivative	825	207, 220sh, 252, 317	*Floridani*
(−)-bisdechlorogeodin	868	203, 224sh, 278, 336sh	*Floridani*
Citrinadin-like	783, 821, 830	200, 227sh, 246, 265sh, 325	*Floridani*
Citrinin	907	221, 242sh, 328, 415sh	*Rubri*
Curvularin	881	200, 223, 270, 301	*Floridani*
Dehydrocurvularin	861	202, 225, 283, 334sh	*Floridani*
ENDI	745	End-absorption	*Floridani*
GULLA	1007	202, 258, 286, 328, 369, 428	*Floridani*
Indole alkaloid (= gypsetin-like)	967	224, 278, 288, 295	*Floridani*, *Rubri*
JOPS	1098	208, 248, 275, 353	*Floridani*
Metabolite M series	845, 854, 865, 881, 906, 946	291, 242sh, 283, 318	*Floridani*
Metabolite N series	917, 1048	203, 236, 251sh, 326, 381	*Floridani*
Metabolite O series	905, 982, 993	202, 226sh, 254, 272sh, 335	*Floridani*
Metabolite Y series	1097 (Y1), 1273 (Y2)	200, 228sh, 274, 375	*Floridani*
Methyl asterrate	934	200, 227sh, 246, 265sh, 325	*Floridani*
Mevinolin	1232	230sh, 240, 250sh	*Rubri*
Mevinolin, open acid form	1121	230sh, 240, 250sh	*Rubri*
Monascin	1251	230, 282, 397	*Rubri*
Rubratoxin-like (Nonadrides, provisionally identified as rubratoxins)	1033, 1066	215sh, 263	*Floridani*
Orthosporin-like	721	241sh, 248273, 282, 324	*Floridani*
Physcion-like (anthraquinone W)	1079	221, 250sh, 264, 282, 331, 440	*Floridani*
PP-V	943	250, 296, 420, 524	*Rubri*
PP-R	981	252, 306, 417, 524	*Rubri*
Questin	958	223, 247sh, 280, 428	*Floridani*
Red anthraquinone series	1316, 1326, 1387, 1412, 1422	227, 268, 330, 442	*Floridani*
Rubratoxin-like	1198	202, 251	*Floridani*, *Rubri*
Rubropunctamine	1417	218, 250, 279, 298sh, 447sh, 475, 512sh	*Rubri*
Rubropunctatin	1252	218sh, 235, 279, 394475sh, 521	*Rubri*
Secalonic acid D	1104	200, 215sh, 258, 331, 388sh	*Floridani*
Shamixanthone-like	1121	201, 228, 263, 301, 366	*Floridani*
Sulochrin	873	203, 224sh, 278, 324sh	*Floridani*
Xanthomonascin A	1143	230, 282, 397	*Rubri*

Asterric acid, methyl asterrate and (−)-bisdechlorogeodin are all part of the geodin biosynthetic family; sh: shoulder.

belong to *Monascus*, and appears to be related to *Penicillium*. In order to clarify the difference placements of *M. eremophilus* in literature, we re-analysed the LSU data set of Park & Jong (2003) and Vinnere-Pettersson *et al.* (2011) together with the data set generated in this study (data not shown). These results show that the sequence (AF365023) used in the study of Park *et al.* (2004) does not match with the other sequences generated from *M. eremophilus*, explaining the various phylogenetic placements of this species. Based on a 4-gene phylogeny, Houbraken *et al.* (2014) confirmed its placement in *Penicillium*. They confidently place the species on a branch together with members of section *Charlesia* (*P. charlesii* CBS 304.48^{T}, *P. fellutanum* CBS 229.81), though there is sufficient genetic distance that would warrant placement of this species in a new section. Based on this literature review and additional (sequence) data generated in this study, we propose to transfer *M. eremophilus* in *Penicillium*. The placement of this species in *Penicillium* is unexpected. *Penicillium eremophilum* is, unlike any other *Penicillium* (and *Monascus*) species, an obligate xerophile. The species is not known to produce an asexual state and there were until now no strictly sexually reproducing species within *Penicillium*, though conidiophores can sometimes be sparsely produced in sexually reproducing *Penicillium* species. The formation of two-spored asci is also not shared with other *Penicillium* species. This feature, together with its xerophily, is shared with the phylogenetically distant species *Xeromyces bisporus*.

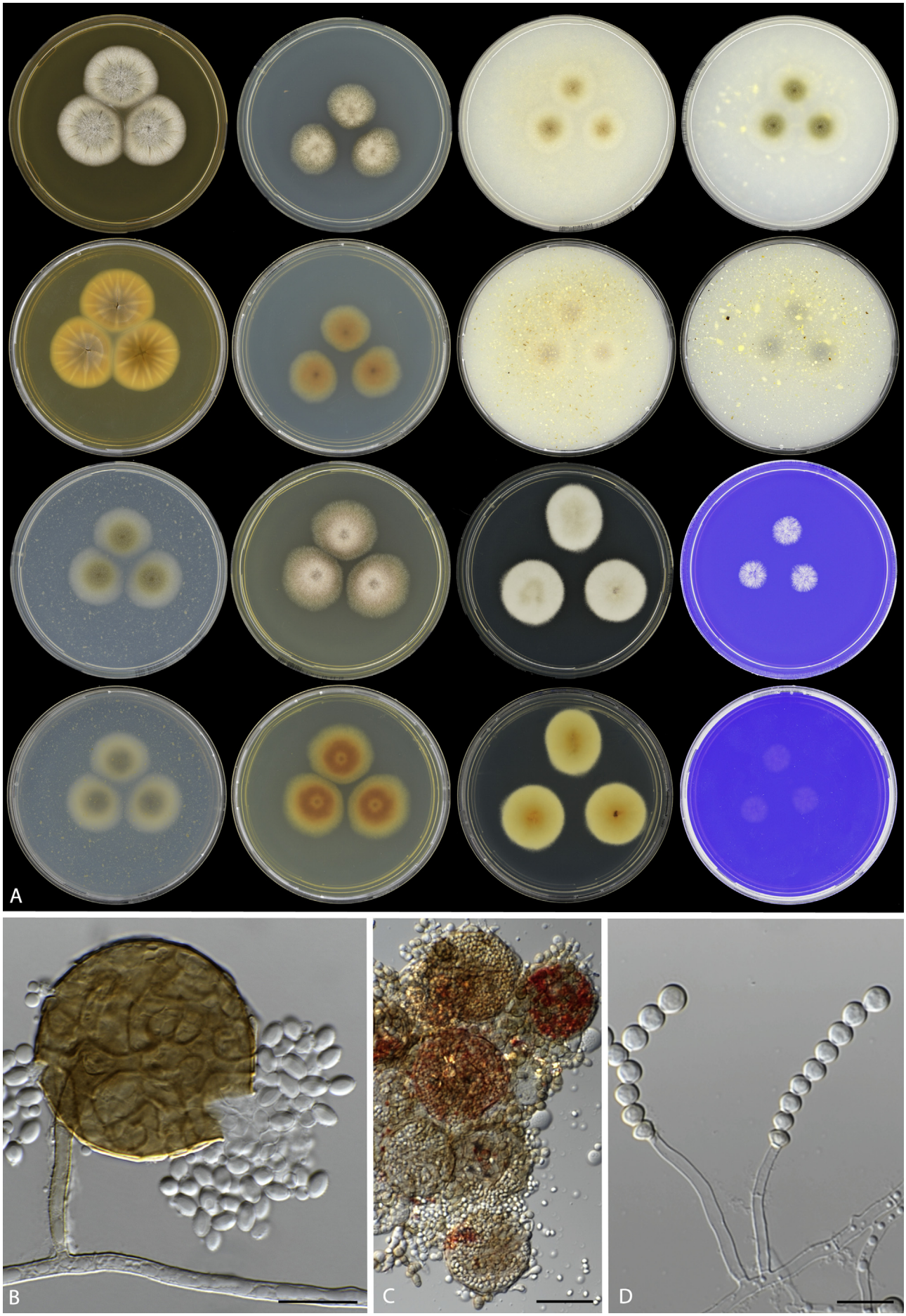

Fig. 5. *Monascus ruber* URM 7525 isolated during the course of this study. **A.** Colonies from left to right (first row) MEA, CYA, OA, CMA; (second row) MEA reverse, CYA reverse, OA reverse, CMA reverse; (third row) PDA, YES, DG18, CREA; (forth row) PDA reverse, YES reverse, DG18 reverse, CREA reverse. **B–C.** Typical ascoma and ascospores. **D.** Conidiophores with conidia chain. Scale bars = 10 µm.

The ITS region is the official DNA barcode for fungi, and is good practice to include ITS sequences whenever new species are described (Schoch *et al.* 2012). However, not all species can be identified using this marker because certain species share identical ITS sequences (e.g. Houbraken *et al.* 2014, Chen *et al.* 2016). All *Monascus* species can be recognized on their ITS sequence only, even though the interspecific differences are low, especially between *M. ruber* and *M. purpureus*. Whether these barcode gaps remain present when a larger set of isolates is investigated remains unknown. A larger sequence variation was observed in the *BenA* gene. This gene is used as secondary barcode for the related genera *Penicillium* and *Talaromyces* and we propose the same for *Monascus* (Visagie *et al.* 2014, Yilmaz *et al.* 2014). The *BenA* gene is easy to amplify in *Monascus* and can distinguish all species. LSU has limited resolving power and *RPB2* is more difficult to amplify and is therefore only recommended in phylogenetic studies.

Stingless beekeeping, or meliponiculture, is an ancient activity and many species of stingless bees are managed in the Americas, Africa, Asia and Australia; however, it remains a largely under-exploited business and technical knowledge is scarce. Much practical and academic work is being done about the best ways of keeping these bees, multiplying their colonies, and exploring the honey they produce (Cortopassi-Laurino *et al.* 2006, Villanueva-Gutiérrez *et al.* 2013, Jaffé *et al.* 2015). *Melipona scutellaris* is most known in the Northeast of Brazil. Furthermore, these bees are important pollinators in agricultural and natural ecosystems. Recently, a fungus cultivation mutualism in a social bee (*Scaptotrigona postica*) was reported for the first time (Menezes *et al.* 2015). The larvae of *S. postica* have a higher survival rate when they were fed with food grown with *Monascus* mycelium. The symbiotic relationships between microorganisms and stingless bees have been poorly explored, and during our investigation of fungi associated with *Melipona scutellaris* bees, we frequently isolated *M. ruber* from the inside of nests. This indicates that also other bee species, like *Melipona scutellaris*, might also have an (obligatory) relationship with *M. ruber*. Besides *M. ruber*, also *M. mellicola* was frequently isolated from honey, pollen and the inside of nests, followed *M. recifensis* and *M. flavipigmentosus*. This association with bees might be a novel unexplored ecological niche of *Monascus* species and can be the subject of future studies. The antibiotic and antifungal activity of some *Monascus* strains might play a role in the protection of the larvae food from microbial contaminations (Jůzlova *et al.* 1996, Menezes *et al.* 2015) and our discovery of many *Monascus*-unique extrolites in these species (metabolite families M, N, O, and Y) invites structure elucidation and bioactivity testing of those compounds. Stchigel & Guarro (2007) studied several cleistothecial ascomycetes and they concluded that the criterion of the production of closed ascomata without a predefined opening and with an irregular arrangement of asci at the centre is of little systematic value. A recent study about fungi living with association with solitary bees collected in Denmark suggest the convergent evolution of reduced fruiting bodies in Pezizomycotina is adaptive for spore dispersal to the bee habitat (Wynns 2015). Interesting to note in this context is that *Monascus* forms smaller cleistothecia than those produced in the related genera *Aspergillus* and *Penicillium*.

In the past, taxonomic studies on *Monascus* were solely based on phenotypic characters, or when sequence data was used, these were mostly applied for identification purposes. With the transfer of *M. eremophilus* to *Penicillium*, monophyly in *Monascus* is restored. The presented 5-gene phylogeny is a good robust starting point for future taxonomic studies in *Monascus*. Furthermore, a list of accepted species is provided, including information on (ex-)type strains and molecular markers (see Taxonomy section).

TAXONOMY

Phylogenetically, two well-supported clades (*M. floridanus*-clade and *M. ruber*-clade) are present in *Monascus* and these groups can also be differentiated on phenotypic characters. Two sectional names are introduced for these clades and information on this taxonomic decision can be found in the Discussion. Our polyphasic approach revealed the presence of three new species and these are described below. Furthermore, a new combination for *Monascus eremophilus* is proposed.

Section ***Floridani*** R.N. Barbosa & Houbraken **sect. nov.** MycoBank MB820076.

Typus: *Monascus floridanus* P.F. Cannon & E.L. Barnard, Mycologia 79: 480. 1987. MycoBank MB132123.

Diagnosis: Colony diameter on MEA, PDA, CYA, CMA, OA, YES generally below 20 mm, no or restricted growth (<10 mm) on CREA and CYAS, and colony diameter less than 30 mm on MEA incubated at 30 and 37 °C. Colonies in shades of brown; conidia brown pigmented; mycelium white or in shades of brown.

Section ***Rubri*** R.N. Barbosa & Houbraken **sect. nov.** MycoBank MB820077.

Typus: *Monascus ruber* Tiegh., Bulletin de la Société Botanique de France 31: 227. 1884. MycoBank MB234876.

Diagnosis: Colony diameter on MEA, PDA, CYA, CMA, OA, YES generally above 15 mm, no or restricted growth on CREA and CYAS, good growth (>30 mm) on MEA incubated at 30 and 37 °C. Colonies in shades of brown to red; conidia brown pigmented; mycelium white or in shades of red or orange.

Monascus flavipigmentosus R.N. Barbosa, Souza-Motta, N.T. Oliveira & Houbraken **sp. nov.** MycoBank MB820072. Fig. 6.

Etymology: *flavipigmentosus* is referring to yellow pigment produced on CMA and PDA.

Diagnosis: *Monascus flavipigmentosus* is phylogenetically distinct by *BenA*, *CaM* and ITS sequencing and characterized by the absence of growth on CREA 25 °C, and MEA and CYA incubated at 37 °C. Yellow soluble pigments present on CMA and PDA (and old cultures on DG18).

In: *Monascus* section *Floridani*

Typus: **Brazil**, Recife, isolate inside nests of *Melipona scutellaris* Jun 2014, isolated by R.N. Barbosa (**holotype** URM 90064; culture ex-type URM 7536 = CBS 142366 = DTO 353-A2).

Barcodes: ITS barcode: KY511751 (alternative markers: *BenA* = KY709168; *CaM* = KY611929; *RPB2* = KY611968).

Colony diam, 7 d (mm): MEA 10–12; CYA 7–10; CMA 10–12; PDA 6–8; YES 10–11; OA 4–5; DG18 8–10; CYAS No growth; CREA No growth; CYA 30 °C 7–9; CYA 37 °C 0–2; MEA 30 °C 10–12; MEA 37 °C 0–3.

Description: Colonies characters after 7 d. MEA, 25 °C: colony texture velvety to floccose, pulvinate, mycelium white; sporulation absent; exudates absent; soluble pigments absent; reverse yellow. CYA, 25 °C: colony texture floccose low, mycelium white; sporulation absent; exudates absent; soluble pigments absent; reverse white to cream. CMA, 25 °C: colony texture lanose, low, mycelium inconspicuously white at the margin; sporulation weak at centre, conidia *en masse* dull brown; exudate absent; soluble pigments present, yellow; reverse yellow; ascomata abundantly produced, brown. PDA, 25 °C: colony texture floccose to lanose, low, mycelium white; sporulation absent; exudates absent; soluble pigments present, light yellow; colony reverse yellow. YES, 25 °C: colony texture floccose, low, mycelium white; sporulation absent; exudates absent; soluble absent; colony reverse yellow to brownish. OA, 25 °C: colony texture not determinate, mycelium white; sporulation absent, exudates absent; soluble pigments absent; colony reverse white to cream. DG18, 25 °C: colony texture velvety to floccose, low, mycelium white; sporulation absent; exudates absent; soluble pigments absent; reverse white to light yellow. CYAS, 25 °C: no growth. CREA, 25 °C: no growth. MEA, 30 °C: colony texture velvety, umbonate, mycelium white, sporulation absent, exudates absent; soluble pigments absent; reverse light brownish. CYA, 30 °C: mycelium brownish, sporulation weak, conidia *en masse* brownish; ascomata sparsely produced, brown; exudates absent; soluble pigments absent; reverse brownish. MEA, 37 °C: no growth. CYA, 37 °C: no growth.

Mycelium abundant, hyphae irregularly branched, hyaline to pale brown when old, smooth-walled, 1.8–3 µm wide. *Conidiophores* variable in length, smooth, 3–30 × 1.5–2.5 µm. *Conidia* single or formed in short basipetal chains, usually terminal, rarely intercalary, 5.5–7.5 × 5.5–7.5 µm diam, at first hyaline and pale brown to brown with age. *Ascomata*, stalked when young, non-ostiolate, globose to subglobose, 40–60 µm diam, initially light brown and dark brown in the age; peridium brown, developing irregularly polygonal plates, surrounded by short hyaline areas, in time filled with a compact mass of ascospores. *Asci* evanescent or no observed. *Ascospores* hyaline, 1-celled, reniform or allantoid, 4–5 × 1.7–2.5 µm, smooth-walled.

Notes: This species shares morphological features with *M. lunisporas*, but can be distinguished by the production of yellow soluble pigments on CMA and PDA, shorter conidiophores (3–28.5 × 1.5–2.5 µm *vs* 5–500 × 3–5 µm), smaller conidia (5.5–7.5 × 5.5–7.5 µm *vs* 6–11 µm) and ascospores (4–5 × 1.7–2.5 µm *vs* 6–7 × 2–2.5 µm).

Monascus mellicola R.N. Barbosa, Souza-Motta, N.T. Oliveira & Houbraken **sp. nov.** MycoBank MB820073. Fig. 7.

Etymology: *mellicola* refers to honey, the substrate from which the type species was isolated.

Diagnosis: *Monascus mellicola* is phylogenetically distinct by *BenA*, *CaM* and ITS sequencing, a sexual state is not observed in culture, and the species grows restricted on CREA incubated at 25 °C. No exudates and soluble pigments are produced on the agar media used in this study.

In: *Monascus* section *Floridani*

Typus: **Brazil**, Recife, honey from *Melipona scutellaris* Jun 2014, isolated by R.N. Barbosa (**holotype** URM 90065, culture ex-type URM 7510 = CBS 142364 = DTO 350-E6).

Barcodes: ITS barcode: KY511726 (alternative markers: *BenA* = KY709143; *CaM* = KY611904; *RPB2* = KY611943).

Colony diam, 7 d (mm): MEA 11–12; CYA 8–10; CMA 9–10; PDA 9–10; YES 10–11; OA 9–10; DG18 7–10; CYAS No growth; CREA 5–7; CYA 30 °C 10–11; CYA 37 °C 6–8; MEA 30 °C 14–15; MEA 37 °C 5–6.

Description: Colonies characters after 7 d. MEA, 25 °C: colony texture floccose, raised in centre; mycelium white; sporulation strong, conidia *en masse* brown; exudates absent; soluble pigments absent, reverse brown. CYA, 25 °C: colony texture velvety, low; mycelium white, sometimes inconspicuously brown; sporulation weak, conidia *en masse* brown; exudates absent; soluble pigments absent, reverse dark brown at centre to brownish at margins. CMA, 25 °C: colony texture velvety, low; mycelium white sometimes inconspicuously greyish olive, sporulation moderate, conidia *en masse* brown; exudates absent; soluble pigments absent; reverse dark brown. PDA, 25 °C: colony texture velvety, low; mycelium white sometimes inconspicuously brown; sporulation strong, conidia *en masse* brown; exudates absent; soluble pigments absent; reverse brownish. YES, 25 °C: colony texture velvety to floccose, low; mycelium white; sporulation strong, conidia *en masse* brownish; exudates absent; soluble pigments absent; reverse dark brown. OA, 25 °C: colony texture velvety, low; mycelium white, sporulation weak, conidia *en masse* brown; exudates absent; soluble pigments absent, reverse brown. DG18, 25 °C: colony texture velvety to floccose, low, mycelium white; sporulation absent; exudates absent; soluble pigments absent; reverse white in the margins and dark brown at centre. CYAS, 25 °C: no growth. CREA, 25 °C: mycelium white, sporulation absent; no acid production. CYA, 30 °C: colony texture velvety to floccose, low; mycelium brown, sporulation weak, conidia *en masse* brownish; exudates absent; soluble pigments absent; reverse brown. CYA, 37 °C: mycelium white, sporulation absent; exudates absent; soluble pigments absent, reverse brown. MEA 30 °C: mycelium white, sporulation moderate to strong, conidia *en masse* in shades of brown; exudates absent; soluble pigments absent; reverse brownish. MEA, 37 °C: mycelium white, sporulation in centre, weak, conidia *en masse* in shades of brown; exudates absent; soluble pigments absent; reverse yellow-brownish.

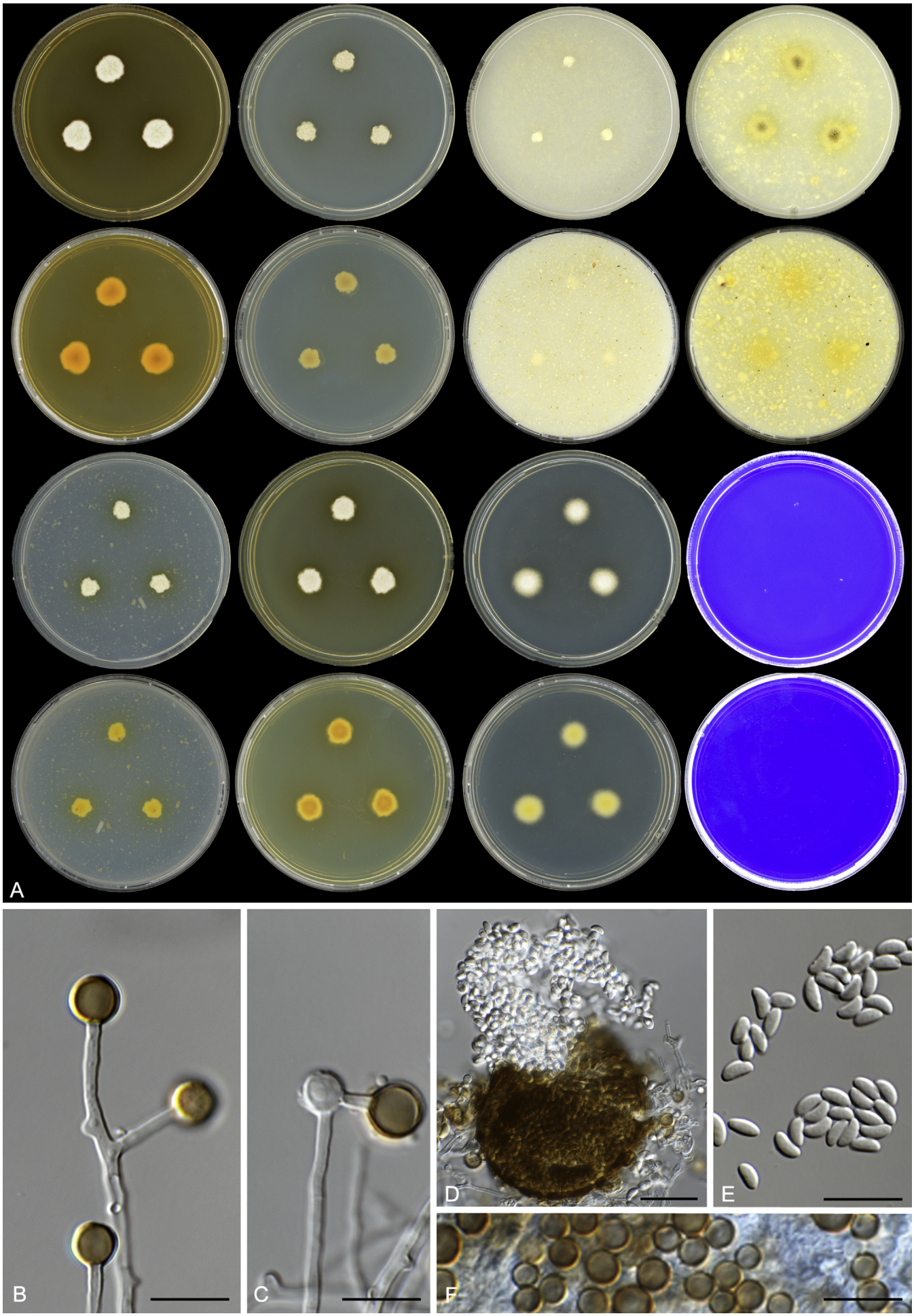

Fig. 6. *Monascus flavipigmentosus*, URM 7536. **A.** Colonies from left to right (first row) MEA, CYA, OA, CMA; (second row) MEA reverse, CYA reverse, OA reverse, CMA reverse; (third row) PDA, YES, DG18, CREA; (forth row) PDA reverse, YES reverse, DG18 reverse, CREA reverse. **B–C.** Conidiophores. **D.** Ascoma. **E.** Ascospores. **F.** Conidia. Scale bars = 10 µm.

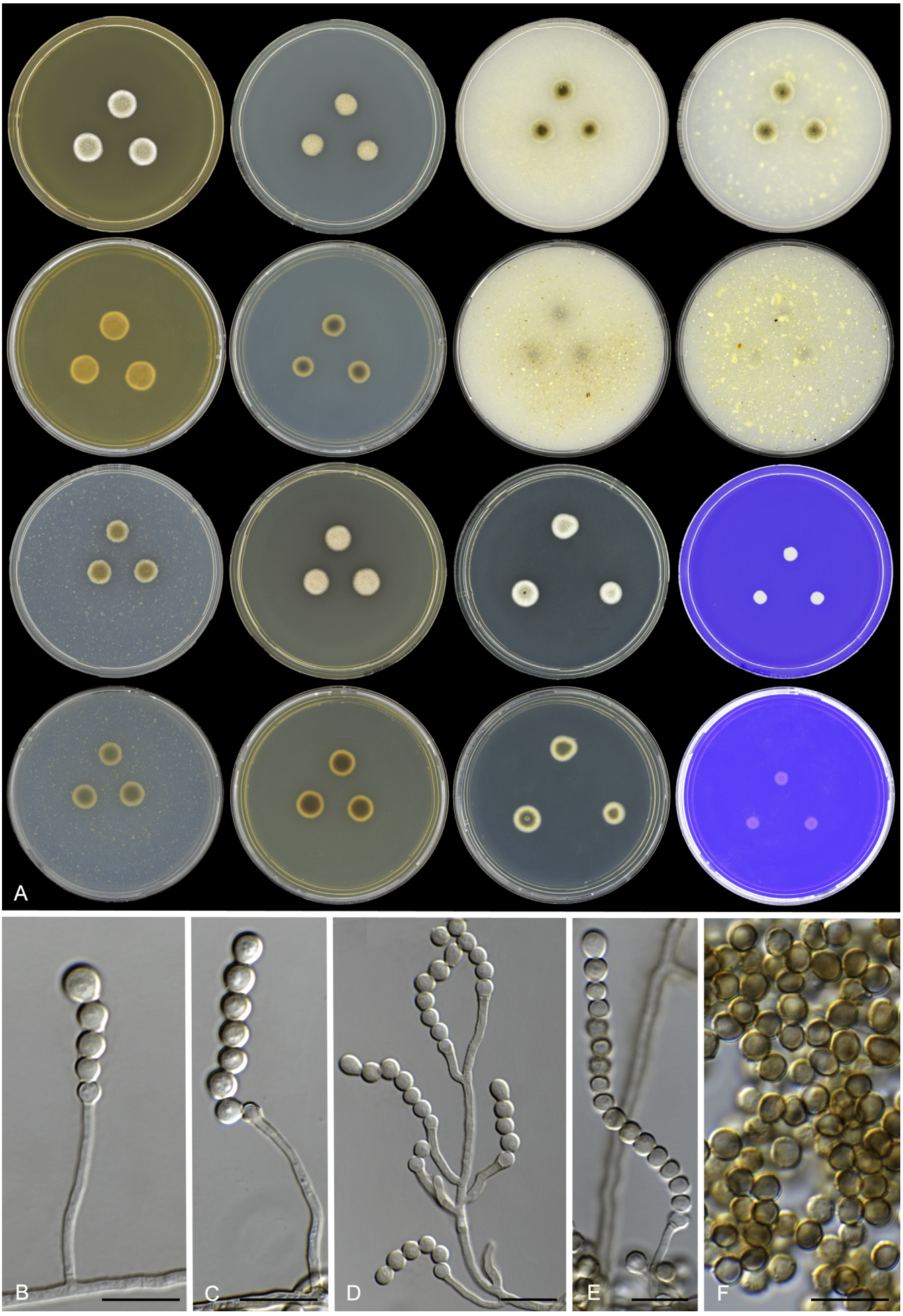

Fig. 7. *Monascus mellicola*, URM 7510. **A.** Colonies from left to right (first row) MEA, CYA, OA, CMA; (second row) MEA reverse, CYA reverse, OA reverse, CMA reverse; (third row) PDA, YES, DG18, CREA; (forth row) PDA reverse, YES reverse, DG18 reverse, CREA reverse. **B–E.** Conidiophores with conidia chain. **D.** Conidia. Scale bars = 10 µm.

Mycelium abundant, hyphae irregularly branched, hyaline to pale brown when old, smooth-walled, 2.5–3 µm wide. *Conidiophores* basipetospora-type, variable in length, smooth, 16–32 × 1.5–2.0 µm. *Conidia* formed basipetally, in long chains, up to 17 conidia, globose to subglobose, smooth-walled, 2.5–5.0 × 3.5–5.0 µm diam, hyaline when young, becoming pale brown to brown with age. Agglomeration of conidia with variable size observed, 45–65 × 55–65 µm diam. Sexual *morph* not observed.

Monascus recifensis R.N. Barbosa, Souza-Motta, N.T. Oliveira & Houbraken **sp. nov.** MycoBank MB820074. Fig. 8.

Etymology: *recifensis* refers to the Brazilian city Recife, the location of the type strain of this species.

Diagnosis: *Monascus recifensis* is phylogenetically distinct by *BenA*, *CaM* and ITS sequencing. The species is characterized by restricted growth on agar media, a sexual state is not observed, and the species doesn't produce exudates and soluble pigments on the agar media used in this study.

In: *Monascus* section *Floridani*

Typus: **Brazil**, Recife, isolated from pollen inside nests of *Melipona scutellaris* Jun 2014, isolated by R.N. Barbosa, (**holotype** URM 90066; culture ex-type URM 7524 = CBS 142365 = DTO 350-G6).

Barcodes: ITS barcode: KY511740 (alternative markers: *BenA* = KY709157; *CaM* = KY611918; *RPB2* = KY611957).

Colony diam, 7 d (mm): MEA 16–18; CYA 12–14; CMA 10–12; PDA 10–11; YES 14–15; OA 3–5; DG18 20–21; CYAS not growth; CREA 1–2; CYA 30 °C 13–15; CYA 37 °C 7–8; MEA 30 °C 17–20; MEA 37 °C 3–4.

Description: Colonies characters after 7 d. MEA, 25 °C: colony texture floccose to lanose, pulvinate, mycelium white, sporulation strong, conidia *en masse* brown; exudates absent; soluble pigments absent; reverse brownish. CYA, 25 °C: colony texture lanose, pulvinate; mycelium white sometimes inconspicuously brown; sporulation weak to moderate, conidia *en masse* brownish; exudates absent; soluble pigments absent; reverse dark brown to light brown close at margins. CMA, 25 °C: colony texture velvety, low; mycelium brown; sporulation moderate to strong at centre, conidia *en masse* dark brown; exudates absent; soluble pigments absent; reverse black. PDA, 25 °C: colony texture velvety to floccose; mycelium white; sporulation strong, *en masse* brownish; exudates absent; soluble pigments absent; reverse white to cream close at margins, dark brown at centre. YES, 25 °C: colony texture velvety, mycelium white sometimes inconspicuously brownish; sporulation strong, conidia *en masse* in shades of brown; exudates absent; soluble pigments present after 10 d. incubation, in shades of brown; reverse dark brown to light brown close at margins. OA, 25 °C: colony texture velvety; mycelium white; sporulation weak, conidia *en masse* dark brown; exudates absent; soluble pigments absent; reverse dark brown. DG18, 25 °C: colony floccose, mycelium white; sporulation weak to moderate, conidia *en masse* brownish; exudates absent; soluble pigments absent; reverse white close to margins and dark brownish at centre. CYAS, 25 °C: no growth. CREA, 25 °C: growth very poor. MEA, 30 °C: colony texture velvety; mycelium white; sporulation moderate to strong, conidia *en masse* brown; exudates absent; soluble pigments absent; reverse brownish. CYA, 30 °C: colony texture velvety, mycelium brownish; sporulation moderate, conidia *en masse* brown; exudates absent; soluble pigments absent; reverse dark brown, white at margins. MEA, 37 °C: colony texture velvety; mycelium white; sporulation absent; exudates absent; soluble pigments absent; reverse cream. CYA, 37 °C: colony texture velvety to floccose; mycelium brownish; sporulation moderate, conidia *en masse* in shades of brown; exudates absent; soluble pigments absent; reverse dark brown.

Mycelium abundant, hyphae irregularly branched, hyaline to pale brown when old, smooth-walled, 1.8–2.5 µm wide. *Conidiophores* variable in length, smooth, 4.5–21.0 × 1.8–2.5 µm, sometimes with additional branch, *Conidia* single, globose, 4.0–7.0 × 4.0–7.0 µm diam, at first hyaline, pale brown to brown with age. *Sexual morph* not observed after 60 d incubation.

Notes: *Monascus lunisporas* and *M. flavipigmentosus* are phylogenetically closely related to *M. recifensis* and the latter species doesn't produce ascomata, exudates and soluble pigments. These species can also be differentiated by their unique extrolite profiles (Table 4).

Penicillium eremophilum (A.D. Hocking & Pitt) Houbraken, Leong & Vinnere-Pettersson **comb. nov.** MycoBank MB820075.

Basionym: *Monascus eremophilus* A.D. Hocking & Pitt, Mycologia 80: 84. 1988. MycoBank MB132383.

Typus: **Australia**, New South Wales, Sydney, isolated from mouldy prunes, isolated by A.D. Hocking, 1986 (Herb.: FRR 3338; Ex-type: IMI 313774 = CBS 123361 = ATCC 62925).

Barcodes: ITS barcode: GU733347 (alternative markers: *BenA* = KY709170; *CaM* = KY611931; *RPB2* = KY611970).

Notes: The colony morphology was identical to that described by Hocking and Pitt in 1998. *Monascus eremophilus* is indeed an obligate xerophile. No growth was observed on either MEA or MA20S at any temperature after incubation of one year. *Monascus eremophilus* grew well on MY50G within the range 10–25 °C. Good growth at 30 °C and absence of growth at 37 °C has been previously reported (Leong *et al.* 2011). Upon microscopy, ascomatal initials were observed after approximately a month of cultivation. However, these cleistothecia never matured and thus no ascospores were observed. No anamorph was observed during the time of cultivation or mentioned in the original description. The fact that we did not observe any fertile cleistothecia may indicate that the type strain (FRR 3338) is deteriorating. Molecular data shows that this species is related to *Penicillium* (Park *et al.* 2004, Vinnere-Pettersson *et al.* 2011, Houbraken *et al.* 2014) and is transferred to *Penicillium* (this study).

List of accepted species in *Monascus*

Monascus argentinensis Stchigel & Guarro, Stud. Mycol. 50: 301. 2004. [MB500076]. — Herb.: FMR 6778. Ex-type: CBS 109402 = FMR 6778. Section *Floridani*. ITS barcode: JF922046 (Alternative markers: *BenA* = KY709174; *CaM* = KY611935; *RPB2* = JN121423).

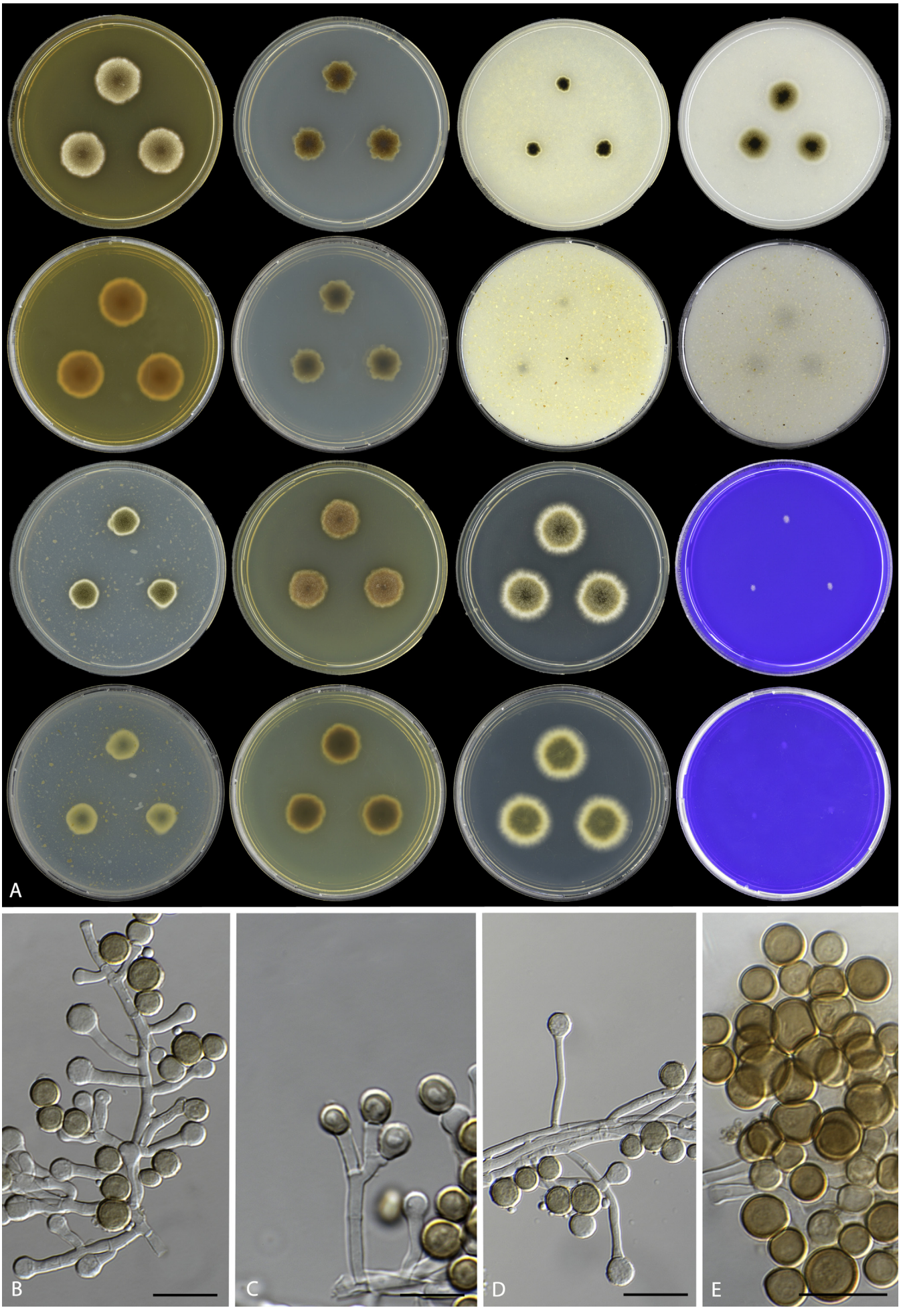

Fig. 8. *Monascus recifensis*, URM 7524. **A.** Colonies from left to right (first row) MEA, CYA, OA, CMA; (second row) MEA reverse, CYA reverse, OA reverse, CMA reverse; (third row) PDA, YES, DG18, CREA; (forth row) PDA reverse, YES reverse, DG18 reverse, CREA reverse. **B–D.** Conidiophores. **E.** Conidia. Scale bars = 10 µm.

Monascus flavipigmentosus R.N. Barbosa, Souza-Motta, N.T. Oliveira & Houbraken (this study). [MB820072]. — Herb.: URM 90064. Ex-type: URM 7536 = CBS 142366 = DTO 353-A2. Section *Floridani*. ITS barcode: KY511751 (Alternative markers: *BenA* = KY709168; *CaM* = KY611929; *RPB2* = KY611968).

Monascus floridanus P.F. Cannon & E.L. Barnard, Mycologia 79: 480. 1987. [MB132123]. — Herb.: IMI 282587. Ex-type: FLAS F54662 = CBS 142228 = CGMCC 3.5843 = BCRC 33310 = UAMH 4180. Section *Floridani*. ITS barcode: KY635848 (Alternative markers: *BenA* = KY709172; *CaM* = KY611933; *RPB2* = KY611972).

Monascus lunisporas Udagawa & H. Baba, Cryptogamie Mycol 19: 270. 1998. [MB446999]. — Herb.: SUM 3116. Ex-type: CBS: 142230 = CGMCC 3.7951 = ATCC 204397 = NBRC 33241 = BCRC 33640. Section *Floridani*. ITS barcode: KY635847 (Alternative markers: *BenA* = KY709171; *CaM* = KY611932; *RPB2* = KY611971).

Monascus mellicola R.N. Barbosa, Souza-Motta, N.T. Oliveira & Houbraken (this study). [MB820073]. — Herb.: URM 90065. Ex-type: URM 7510 = CBS 142364 = DTO 350-E6. Section *Floridani*. ITS barcode: KY511726 (Alternative markers: *BenA* = KY709143; *CaM* = KY611904; *RPB2* = KY611943).

Monascus pallens P.F. Cannon, Abdullah & B.A. Abbas, Mycol. Res. 99: 659. 1995. [MB413476]. — Herb.: IMI 356820. Ex-type: BSRA 10266 = CBS 142229 = CGMCC 3.5844 = ATCC 200612 = BCRC 33641. Section *Floridani*. ITS barcode: KY635849 (Alternative markers: *BenA* = KY709173; *CaM* = KY611934; *RPB2* = KY611973).

Monascus purpureus Went, Ann. Sci. Nat., Bot. Ser. 8, 1, 1–18. 1895. [MB235390]. — Herb.: IMI 210765. Ex-type: CBS 109.07 = IF0 45 13 = ATCC 16426 = NRRL 1596 = FRR 1596. Section *Rubri*. ITS barcode: KY635851 (Alternative markers: *BenA* = KY709176; *CaM* = KY611937; *RPB2* = JN121422).

Monascus recifensis R.N. Barbosa, Souza-Motta, N.T. Oliveira & Houbraken (this study). [MB820074]. — Herb.: URM 90066. Ex-type: URM 7524 = CBS 142365 = DTO 350-G6. Section *Floridani*. ITS barcode: KY511740 (Alternative markers: *BenA* = KY709157; *CaM* = KY611918; *RPB2* = KY611957).

Monascus ruber Tiegh, Bull. Soc. Bot. France. 31: 227. 1884. [MB234876]. — Herb.: IMI 81596. Ex-type: CBS 135.60 = IFO 8451 = ATCC 15670. Section *Rubri*. ITS barcode: KY635850 (Alternative markers: *BenA* = KY709175; *CaM* = KY611936; *RPB2* = KY611974).

Overview and status of *Basipetospora* species

Basipetospora chlamydospora Matsush., Icones Microfungorum a Matsushima lectorum 13. 1975. [MB309463]. — Herb.: MFC 2307. Ex-type: CBS 228.84 = MFC 2409. 18S rDNA: AB024045. Note: BLAST analysis of the 18S rDNA sequences shows that this species belongs to *Microascales*.

Basipetospora denticola (C. Moreau) C. Moreau, Bull. Soc. Mycol. France 87: 43. 1971. nom. inval., (Art. 6.10, 41.1 & 41.5) [MB309464]. Basionym: *Chrysosporium keratinophilum* var. *denticola* C. Moreau [as 'denticolum'], Mycopathologia et Mycologia Applicata. 37: 37. 1969. nom. inval., (Art. 39.1 & 40.1) [MB353354]. — Herb.: n/a. Representative culture: CBS 132.78. ITS barcode: LN850801. Note: *Basipetospora denticola* is based on the invalidly described species *C. keratinophilum* var. *denticola*. A representative culture of *B. denticola* (CBS 132.78) belongs to *Microascales* and is a synonym of *Scopulariopsis candida* (Jagielski *et al.* 2016).

Basipetospora halophila (J.F.H. Beyma) Pitt & A.D. Hocking, Mycotaxon 22: 198. 1985. [MB105087]. Basionym: *Oospora halophila* J.F.H. van Beyma Zentralblatt für Bakteriologie und Parasitenkunde Abteilung, Abt. II 88: 134. 1933. [MB266778]. — Herb.: n/a. Representative culture: CBS 232.32 = VKM F-204. Note: This species was formerly described as *Oospora halophila* by van Beyma (1933) and was recently transferred to *Aspergillus* under the new name *A. baarnensis* (Samson *et al.* 2014, Kocsubé *et al.* 2016).

Basipetospora rubra G.T. Cole & W.B. Kendr., Canadian Journal of Botany 46: 991. 1968. [MB326938]. — Herb.: ATCC 18199. Ex-type: FRR 2452. Note: The herbarium and ex-type culture of *B. rubra* and *M. ruber* differ. *Basipetospora rubra* was described as the asexual state of *M. ruber* and is in the single name nomenclature system regarded as a synonym of this species.

Basipetospora variabilis Matsush., Icones Microfungorum a Matsushima lectorum 13. 1975. [MB309465]. — Herb.: MFC 2428. Ex-type: CBS 995.87. 18S rDNA: AF437892. Note: Comparison of the publically available 18S rDNA sequence on GenBank shows that this species belongs to *Microascales*.

Basipetospora vesicarum (Link) Stalpers, Studies in Mycology 24: 91. 1984. [MB106627]. — Herb.: n/a. Ex-type: n/a. Note: This fungus was originally described as *Sporotrichum vesicarum* by Link (Sprengel *et al.* 1818). Stalpers (1984) examined a herbarium specimen from B and this specimen contained the anamorph of *M. ruber*, which he named *B. vesicarum*. This species is tentatively placed in synonymy with *M. ruber*.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.simyco.2017.04.001.

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