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### Redefining *Microascus*, *Scopulariopsis* and allied genera

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#### Key words

Ascomvcota Microascaceae Microascales multigene phylogeny taxonomy

Abstract The genera Microascus and Scopulariopsis comprise species commonly isolated from soil, decaying plant material and indoor environments. A few species are also recognised as opportunistic pathogens of insects and animals, including humans. In the past, the taxonomy of these fungi has been based on morphology only. With the aim to clarify the taxonomy and phylogeny of these fungi, we studied a large set of clinical and environmental isolates, including the available ex-type strains of numerous species, by means of morphological, physiological and molecular analyses. Species delineation was assessed under the Genealogical Phylogenetic Species Recognition (GCPSR) criterion using DNA sequence data of four loci (ITS region, and fragments of rDNA LSU, translation elongation factor 1- $\alpha$  and  $\beta$ -tubulin). The genera *Microascus* and *Scopulariopsis* were found to be separated in two distinct lineages. The genus Pithoascus is reinstated and the new genus Pseudoscopulariopsis is erected, typified by P. schumacheri. Seven new species of Microascus and one of Scopulariopsis are described, namely M. alveolaris, M. brunneosporus, M. campaniformis, M. expansus, M. intricatus, M. restrictus, M. verrucosus and Scopulariopsis cordiae. Microascus trigonosporus var. macrosporus is accepted as a species distinct from M. trigonosporus. Nine new combinations are introduced. Microascus cinereus, M. longirostris, P. schumacheri and S. flava are neotypified. A table summarising the morphological features of the species treated and identification keys for each genus are provided.

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### INTRODUCTION

Scopulariopsis was erected by Bainier (1907) for a group of fungi with asexual propagation, with S. brevicaulis as type species and two additional taxa, S. rubellus and S. rufulus. Scopulariopsis brevicaulis was originally described as Penicillium brevicaule by Saccardo (1882) and included in the Penicillium section Anomala (Biourge 1923). In the current sense, the distinctive features of Scopulariopsis are its annellidic conidiogenesis with mostly thick-walled, basally truncate conidia arranged in long, dry chains and its colony colour varying from white to brown or black, but never in bright green shades like Penicillium (Morton & Smith 1963, Samson et al. 2010). Some asexual genera with morphological features similar to those of Scopulariopsis, such as Acaulium, Masoniella, Phaeoscopulariopsis and Torula were considered to be synonymous (Curzi 1930, Morton & Smith 1963). Scopulariopsis currently comprises species with a worldwide distribution that are commonly isolated from soil, air, plant debris and dung (Domsch et al. 2007). In addition, some species have been described as colonisers or pathogens of mammals, including humans and insects (de Hoog et al. 2011, Iwen et al. 2012, Sandoval-Denis et al. 2013). Several authors (Curzi 1930, 1931, Abbott et al. 1998, Abbott & Sigler 2001, Issakainen et al. 2003) have demonstrated

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by culturing, mating studies and molecular methods, that the

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sexual morphs of Scopulariopsis belong to the ascomycete genus Microascus. Abbott & Sigler (2001) confirmed the existence of both homothallic and heterothallic species. Microascus was included in the family Microascaceae (1951), order Microascales, together with other fungi with annellidic conidiogenesis (Lumbsch & Huhndorf 2007). Microascus is characterised by globose to ampulliform perithecial ascomata with cylindrical or papillate necks, and a dark peridium of textura angularis. The asci are ovate to globose, unitunicate, non-pedicellate, and evanescent, formed in basipetal rows and containing eight 1-celled ascospores. The ascospores are typically asymmetrical, reniform, lunate or triangular, dextrinoid when young, often with an inconspicuous germ-pore, and extruded in a long cirrhus or a gelatinous ball at the top of the ascomata (Barron et al. 1961, Morton & Smith 1963, Guarro et al. 2012).

Von Arx (1973a) erected Pithoascus with three species, i.e. P. intermedius, P. nidicola (type species) and P. schumacheri. These three species were previously included in *Microascus* and had ascomata with rudimentary or inconspicuous ostioles, navicular to fusiform ascospores without germ pores, while they lacked asexual morphs. Von Arx (1978) added Pithoascus langeronii, which produced an arthroconidial asexual morph. Nevertheless, species of Pithoascus (i.e. P. intermedius, P. schumacherii) were shown to produce a reduced, scopulariopsis-like asexual morph (Roberts 1985, Valmaseda et al. 1986). Valmaseda et al. (1986) erected the new monotypic genus Pithoascina for the arthroconidia-forming species P. langeronii. Based on these features, P. langeronii was later transferred to the genus Eremomyces (Eremomycetaceae, Dothideomycetes) by Malloch & Sigler (1988) and more recently to Arthrographis, being renamed as Arthrographis arxii (Giraldo et al. 2014).

Several authors consider Pithoascus s.str. as a synonym of Microascus (Malloch & Hubart 1987, Abbott et al. 2002, Guarro et al. 2012) since some species show intermediate morpho-

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logical characteristics. In addition, other asexual genera of the *Microascaceae* phylogenetically close to *Scopulariopsis*, i.e. *Wardomyces* and *Wardomycopsis*, also produce a *Microascus* sexual form (Malloch 1970, Udagawa & Furuya 1978); these authors maintained wider generic concepts.

Barron et al. (1961) and later Morton & Smith (1963) published comprehensive monographic reviews on *Microascus* and *Scopulariopsis* based on morphological criteria. Morphology seems to be insufficient for establishing species limits in these fungi. Although most species can be identified by detailed morphological study, phenotypic characters appear to overlap in several cases (Sandoval-Denis et al. 2013). DNA sequencing and multilocus phylogenetic analysis have considerably improved our understanding of species concepts in many fungal groups (Lackner & de Hoog 2011, Summerbell et al. 2011, Lackner et al. 2014, Samson et al. 2014), but as yet no such study has been undertaken to revise *Microascus, Scopulariopsis* and allied genera.

Presently, 77 species are accepted in *Scopulariopsis* and 32 in *Microascus*. In addition, many described species are of doubtful identity because their type materials are lost and their protologues are uninterpretable. A further complicating factor is that the new International Code of Nomenclature for Fungi, Algae and Plants no longer allows dual nomenclature for those fungal species that present both sexual and asexual morphs (Hawksworth et al. 2011, Hibbett & Taylor 2013). However, to resolve which name has priority, both at genus and species levels, requires understanding of relationships among species, as well as a stable and well-defined generic circumscription. In the case if *Scopulariopsis* and *Microascus* would be congruent, the former name has been recommended (Hawksworth 2012, Sandoval-Denis et al. 2013).

In a recent study on *Scopulariopsis* and *Microascus* species associated with human disease, we characterised several isolates that could not be identified (Sandoval-Denis et al. 2013). The present work aims to clarify the taxonomic position of these putative new species using the Genealogical Phylogenetic Species Recognition (GCPSR) criterion (Taylor et al. 2000). We provide a multigene phylogeny of *Scopulariopsis*, *Microascus* and related fungi based on a large set of isolates, which includes all available ex-type cultures and well-identified reference strains from international culture collections.

### MATERIALS AND METHODS

#### Isolates

In the present study we evaluate a total of 141 fungal strains, representing 67 fungal species (Table 1). The strains were mainly obtained from different international culture collections, but also from human clinical specimens in the USA.

#### DNA extraction, amplification and phylogenetic analysis

All the strains were cultured on YES agar (20 g yeast extract, 150 g sucrose, 20 g agar, 1 L distilled water) for 5 d at 25 °C. Fresh mycelium was removed by scrapping the agar surface and total genomic DNA extraction was obtained using the PrepmanUltra sample preparation reagent (Applied Biosystems, Foster City, CA, USA), according to manufacturer's conditions.

Four nuclear DNA regions were amplified and sequenced. These comprised a fragment (490 bp) including the internal transcribed spacer ITS-1 and ITS-2 and the 5.8S rDNA gene (ITS), a fragment (450 bp) including the D1/D2 regions of the LSU rDNA gene, a fragment (820 bp) of the translation elongation factor 1-alpha (EF-1 $\alpha$ ) and a fragment (470 bp) of the beta-tubulin gene (TUB). The different loci were amplified

using the primer pairs ITS5/ITS4 for the ITS region (White et al. 1990), NL1/NL4b for the LSU region (O'Donnell 1993), 983F/2218R for EF-1α (Rehner & Buckley 2005) and BT2a/ BT2b for TUB (Glass & Donaldson 1995). PCR amplification reaction had a total volume of 40 µL and consisted in 20 mM Tris-HCI (pH 8.4), 50 mM KCI (10X PCR reaction buffer; Invitrogen, Life Technologies Ltd, Paisley, UK) 1.5 mM MgCl (Invitrogen, Life Technologies Ltd, Paisley, UK), 125 µM of each deoxynucleoside triphosphate (GeneAmp® dNTP mix with dTTP, Applied Biosystems, Life Technologies Corporation, Carlsbad, CA, USA), 5 % dimethyl sulfoxyde (DMSO; Panreac Química S.L.U, Barcelona, Spain), 1.2 µM of each primer and 1.25 U of *Taq* DNA Polymerase (Invitrogen, Life Technologies Ltd, Paisley, UK). The amplification programme consisted of an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at a suitable temperature for 1 min, extension for 1 min and 20 s at 72 °C, and a final extension for 1 min at 72 °C. Annealing temperatures for each gene were 55 °C for ITS, 51 °C for LSU and 57 °C for EF-1a and TUB. The amplified products were purified with Diffinity Rapid Tip® purification system (Sigma-Aldrich, St. Louis, MO, USA) and stored at -20 °C until sequencing.

Sequencing was conducted in both directions with the same primer pair used for amplification at Macrogen Europe (Macrogen Inc. Amsterdam, The Netherlands). Consensus sequences were obtained using SeqMan v. 7.0.0 (DNASTAR Lasergene, Madison, WI, USA). The newly generated sequences obtained in this study and their GenBank accession numbers are summarised in Table 1. Additionally, 167 relevant sequences, obtained from public databases (GenBank, NITE) and selected on the basis of BLAST homology search results, were incorporated in the phylogenetic analyses (Table 1).

Sequences were aligned individually for each locus using ClustalW (Thompson et al. 1994), under MEGA v. 5.05 (Tamura et al. 2011), refined with MUSCLE (Edgar 2004) under the same platform and manually adjusted if needed. Phylogenetic reconstructions by maximum likelihood (ML) and bayesian inference were carried out using MEGA v. 5.05 and MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001), respectively. The best nucleotide substitution model for each locus and the combined dataset (GTR+G+I) were estimated using MrModeltest v. 2.3 (Nylander 2004). ML phylogeny was first made separately for each locus (data not shown) and assessed for their concordance by comparing the phylogenetic placement and monophyly of the terminal clades and internal nodes with significant bootstrap (bs) support. Since there was no discordance, the loci were combined into two different datasets. A first analysis was carried out using sequences of both ITS and LSU loci in order to establish the boundaries of the genera with all the available ex-type strains of Microascus / Scopulariopsis species complemented with several sequences of related general of the Microascaceae and Graphiaceae. To establish the species distribution among the genera, a second combined dataset was created including LSU, ITS, EF-1 $\alpha$  and TUB sequences made up of a subset of those previously analysed strains and numerous environmental and clinical isolates morphologically identified as Microascus or Scopulariopsis species.

For ML analysis, the trees were inferred using Nearest-Neighbour-Interchange as a heuristic method and gaps were treated as partial deletion with a 95 % site coverage cut-off. The robustness of branches was assessed by a bootstrap analysis of 1 000 replicates (Felsenstein 1985). Bootstrap values over 70 % were considered significant.

The Bayesian analyses consisted of two parallel runs of four incrementally heated Markov Chains starting from a random tree topology. The analyses lasted for five million generations

rrent name	Original name	Strain number <sup>1</sup>	Source	Origin	Seq	quence acces	ision number <sup>2</sup>	
					ITS	LSU	EF-1α	TUB
ergillus baarnensis	Scopulariopsis halophilica	CBS 380.74 (ex-type)	Undaria pinnatifida	Japan	LM652376	LM652499	I	I
tomyces purpureofuscus	Doratomyces purpureofuscus	CBS 139.42; NBRC 7677	Manure	The Netherlands: Limburg	00767701*	00767701*	I	I
tomyces stemonitis	Doratomyces stemonitis	CBS 127.22; MUCL 4031	Seed	The Netherlands: Wageningen	LM652377	DQ836907	I	I
sia aggregata	Wardomyces aggregatus	CBS 251.69 (ex-isotype)	Dung of carnivore	USA USA	LM652378	LM652500	I	I
sia simplex	Wardomyces simplex	CBS 546.69 (ex-isotype)	Milled Oryza sativa	Japan	LM652379	LM652501	I	I
hium penicillioides	Graphium penicillioides	CBS 102632 (ex-epitype)	Wood, <i>Populus nigra</i>	Czech Republic	AB038432	AF175961	I	I
ocrea atroviridis	Hypocrea atroviridis	CBS 110086; NBRC 101776 (ex-type)	Decorticated wood	France	11776204*	11776205*	I	I
ia nitida	Magnusia nitida	CBS 282.52; NBRC 8200	Chrysolina sanguinolenta	France	00820001*	00820001*	I	I
nia pachvoleura	Kernia pachvoleura	NBRC 30413: UAMH 8858	Soil of paddy field	Japan	03041301*	03041301*	I	I
notrichus macrosporus	Lophotrichus macrosporus	FMR 5571: NBRC 32894	Sheep dung	Iraq	03289401*	03289401*	I	I
notrichus plumbescens	Lophotrichus plumbescens	NBRC 30864; UAMH 8710 (ex-type)	Soil	Thailand; Bangkok	03086401*	03086401*	I	I
oascus alveolaris sp. nov.	Microascus sp.	UTHSC 04-1534; FMR 12354	Human BAL	USA .	LM652380	HG380482	HG380405	LM652596
-	Microascus sp.	UTHSC 05-3416; FMR 12350	Human BAL	USA	LM652381	HG380483	HG380406	LM652597
	Microascus sp.	UTHSC 05-1041; FMR 12351	Human sputum	USA	LM652382	HG380488	HG380411	LM652598
	Microascus sp.	UTHSC 06-3152: FMR 12346	Human BAL	USA	LM652383	HG380487	HG380410	LM652599
	Microascus sp	UTHSC 07-1823: FMR 12342	Human Spurtum	USA	LM652384	HG380489	HG380412	LM652600
	Microascus sp.	CBS 139501: UTHSC 07-3491:	Human BAL	USA	LM652385	HG380484	HG380407	LM652601
	· <del>-</del>	FMR 12252 (ex-tvpe)						
	Microascus sp.	UTHSC 08-886; FMR 12340	Human BAL	USA	LM652386	HG380485	HG380408	LM652602
	Microascus sp.	UTHSC 10-214; FMR 12336	Human BAL	USA	LM652387	HG380486	HG380409	LM652603
	Microascus sp.	UTHSC R-4634; FMR 12333	Human lung Tissue	USA	LM652388	HG380490	HG380413	LM652604
oascus albonigrescens†	Microascus albonigrescens	IHEM 18560	Litter treated with urea	Japan: Nemuro-shi	LM652389	LM652502	I	I
oascus brunneosporus	Microascus sp.	CBS 138276; UTHSC 06-4312;	Human BAL	USA	LM652390	HG380497	HG380420	LM652605
. nov.		FMR 12343 (ex-type)						
oascus campaniformis	Microascus sp.	CBS 138126; UTHSC 10-565;	Human BAL	USA	LM652391	HG380495	HG380418	LM652606
. nov.		FMR 12334 (ex-type)						
oascus caviariformis†	Microascus caviariformis	CBS 536.87: UAMH 5592 (ex-type)	Decaying meat	Belgium	LM652392	LM652503	I	I
oascus chartarus comb. nov.	Masonia chartarum	CBS 294.52; MUCL 9001 (ex-type)	Mouldy wall-paper in a house	England: London.	LM652393	HG380463	HG380386	LM652607
oascus cinereus	Microascus cinereus	UTHSC 06-3278; FMR 12345	BAL	USA	LM652394	HG380347	HG380424	LM652608
	Microascus cinereus	UTHSC 08-3181: FMR 12339	Human sternum tissue	USA	LM652395	HG380348	HG380425	LM652609
	Microascus cinereus	UTHSC 09-573; FMR 12239	Human BAL	USA	LM652396	HG380349	HG380426	LM652610
	Microascus cinereus	UTHSC 10-2805; FMR 12217 (ex-neotype	e) Human BAL	USA	LM652397	HG380350	HG380427	LM652611
	Microascus cinereus	UTHSC 11-383; FMR 12331	Human BAL	USA	LM652398	HG380351	HG380428	LM652612
	Microascus griseus	CBS 365.65; ATCC 16204 (ex-type)	Soil	India: Maharashtra	LM652399	HG380346	HG380423	LM652613
oascus cirrosus	Microascus cirrosus	CBS 217.31 (ex-type)	Leaf of <i>Prunus</i> sp.	Italy	LM652400	HG380429	HG380352	LM652614
	Microascus cirrosus	CBS 277.34; MUCL 9050	Roots of Vitis vinifera	Italy	LM652401	LM652504	LM652556	LM652615
	Microascus desmosporus	CBS 301.61; MUCL 9054	Unknown	UK	LM652402	LM652505	LM652557	LM652616
	Microascus cirrosus	UTHSC 07-1887: FMR 12256	Induced human sputum	USA	LM652403	HG380431	HG380354	LM652617
	Microascus cirrosus	UTHSC 11-14; FMR 12332	Human BAL	USA	LM652404	HG380432	HG380355	LM652618
oascus croci comb. nov.	Scopulariopsis chartarum	FMR 3997	Aquatic sediment, Ebro river	Spain: Tarragona	LM652405	LM652506	LM652558	LM652619
	Microascus cirrosus	FMR 4004	Aquatic sediment, Besós river	Spain: Barcelona	LM652406	LM652507	LM652559	LM652620
	Scopulariopsis croci	CBS 158.44; MUCL 9002 (ex-type)	From Crocus sp.	The Netherlands: Lisse	LM652407	LM652508	LM652560	LM652621
	Masoniella tertia	CBS 296.61; MUCL 9005 (ex-type)	Air	Brazil: Pernambuco	LM652408	LM652509	LM652561	LM652622
oascus expansus sp. nov.	Microascus sp.	UTHSC 06-2519; FMR 12267	Human pleural fluid	USA	LM652409	HG380491	HG380414	LM652623
	<i>Microascus</i> sp.	CBS 138127; UTHSC 06-4472;	Human sputum	USA	LM652410	HG380492	HG380415	LM652624
		FMR 12266 (ex-type)						
oascus giganteus†	Microascus giganteus	CBS /46.69 (ex-type)	Insect trass in dead log	Canada: Untario	LM652411	LM652310	-	-
oascus gracilis comb. nov.	Paecilomyces tuscatus			Japan	LM652412	HG380467	HG380390	CZ92C901
	Scopulariopsis gracilis	UTHSC 09-1351; FMK 12234	Human Joint fluid	USA	LM652413	HG380476	HG380399	LM652626
	Scopulariopsis gracilis	UTHSC 09-1829; FMR 12231	Human BAL	USA	LM652414	HG380477	HG380400	LM652627
	Scopulariopsis gracilis	UTHSC 10-390; FMR 12335	Human BAL	USA	LM652415	LM652511	LM652562	LM652628

 Table 1
 Strains and sequence accession numbers included in this study.

Table 1 (cont.).

Current name	Original name	Strain number <sup>1</sup>	Source	Origin	Seq	luence access	tion number <sup>2</sup>	
					ITS	LSU	EF-1α	TUB
	Microascus cinereus	CBS 195.61; MUCL 9048	Soil Seed of Zoa marie	England	LM652416	HG380468	HG380391 1 Meesees	LM652629
<i>Microascus hyalinus</i> comb. nov.	Kernia hyalina	CBS 766.70 (ex-isotype)	Dung of cow	USA	LM652418	LM652513	LM652564	LM652631
<i>dicroascus intricatus</i> sp. nov.	Microascus sp.	CBS 138128; UTHSC 07-156; FMR 12264 (ex-type)	Human BAL	USA	LM652419	HG380496	HG380419	LM652632
	Microascus sp.	FMR 12362	Soil	Argentina: Iguazú	LM652420	LM652514	LM652565	LM652633
Microascus longirostris	Microascus longirostris	CBS 196.61; MUCL 9058 (ex-neotype)	Wasp's nest	USA: Maine	LM652421	LM652515	LM652566	LM652634
Microascus macrosporus	Microascus triaonosporus var.	CBS 662.71	Soil	USA	LM652423	LM652517	LM652568	LM652636
comb & stat. nov.	macrosporus							
<i><b>Nicroascus murinus comb. nov.</b></i>	Scopulariopsis murina	CBS 830.70; IHEM 18567 (ex-type)	Composed municipal waste	Germany: Giessen	LM652424	HG380481	HG380404	LM652637
<i>Microascus paisii</i> comb. nov.	Scopulariopsis brumptii	UTHSC 07-639; FMR 12263	Human BAL	USA	LM652425	HG380451	HG380374	LM652638
	Scopulariopsis brumptii	UTHSC 08-1734; FMR 12248	Human BAL	USA	LM652426	HG380452	HG380375	LM652639
	Scopulariopsis prumptii	UTHSC 09-2391; FIMR 12229 LITHSC 00 457; FMD 42244	Human sputum		1242COML		HG3803/0	0402COM1
	Scopulariopsis brumpii Scopularionsis brumpii	UTHSC 09-437, FINR 12241 LITHSC 09-482, FMR 12240	Human BAI	USA	LIM652420	HG380455	HG380378	LIM652647
	Scopulariopsis brumptii	UTHSC 10-2920; FMR 12215	Human BAL	USA	LM652430	HG380456	HG380379	LM652643
	Scopulariopsis brumptii	UTHSC 11-708; FMR 12210	Human sputum	USA	LM652431	HG380457	HG380380	LM652644
	Scopulariopsis brumptii	CBS 896.68; MUCL 8989	Soil on a Triticum sativum field	Germany: Schleswig	LM652432	HG380449	HG380372	LM652645
	Masonia grisea	CBS 295.52; MUCL 9003 (ex-type)	Culture contaminant	England	LM652433	HG380450	HG380373	LM652646
	Torula paisii	CBS 213.27; MUCL 7915 (ex-type)	Man	Italy	LM652434	LM652518	LM652569	LM652647
	Scopulariopsis brumptii	MUCL 8990	Soil	Germany: Schleswig-Holstein	LM652435	LM652519	LM652570	LM652648
	Scopulariopsis chartarum	CBS 897.68; MUCL 8993	Soil on a wheat field	Germany	LM652436	LM652520	LM652571	LM652649
	Scopulariopsis melanospora	CBS 272.60; MUCL 9040 (ex-isotype)	Milled Oriza sativa	USA	LM652437	LM652521	LM652572	LM652650
	Scopulariopsis sphaerospora	CBS 402.34; MUCL 9045 (ex-type)	Unknown	Austria	LM652438	LM652522	LM652573	LM652651
Microascus pyramidus	Microascus pyramidus	CBS 212.65 (ex-isotype)	Desert soil	USA: California	LM652439	HG380435	HG380358	LM652652
<i>Vlicroascus restrictus</i> sp. nov.	Microascus sp.	CBS 138277; UTHSC 09-2704; FMR 12227 (ex-tvbe)	Human left hallux	USA	LM652440	HG380494	HG380417	LM652653
Microascus senegalensis	Microascus senegalensis	CBS 277.74; IHEM 18561 (ex-type)	Mangrove soil	Senegal	LM652441	LM652523	LM652574	LM652654
Microascus singularis†	Microascus singularis	CBS 414.64	Laboratory contaminant	Japan: Tokyo	LM652442	LM652524	I	I
Microascus trigonosporus	Microascus trigonosporus var.	CBS 218.31 (ex-type)	Unknown	USA	LM652443	HG380436	HG380359	LM652655
	trigonosporus							
	Microascus trigonosporus	CBS 199.61; MUCL 9061	Milled rice	Burma, Japan	LM652444	HG380438	HG380361	LM652656
	Scopulariopsis coprophila	CBS 262.35; MUCL 9841	Mushroom bed		LM652445	LM652525	LM652575	LM652657
wicroascus verrucosus sp. nov.	Microascus sp.	CBS 1382/8; UTHSC 10-2001; FMR 12219 (ex-tvpe)	numan bal	ASU ASU	044700NL	HG380493	HG380410	800700MT
Parascedosporium tectonae	Graphium tectonae	CBS 127.84 (ex-type)	Seed	Jamaica	AY228113	EF151332	EF151409	I
Petriella setifera	Petriella setifera	CBS 437.75	Wood panel in coastal water	Hong Kong	I	DQ470969	DQ836911	I
	Petriella setifera	FMR 7736; NBRC 100025	Soil	Spain; Canary Islands	10002501*	10002501*	I	I
Petriella sordida	Petriella sordida	CBS 124169	Corner of a bathroom	The Netherlands	GQ426957	AY281099	I	I
Petriellopsis africana	Petriellidium africanum	CBS 311.72 (ex-type)	Brown sandy soil	Namibia	AJ888425	EF151331	I	I
Phitoascus ater comb. nov.	Scopulariopsis atra	CBS 400.34; IHEM 18608 (ex-type)	Unknown	Unknown	LM652447	LM652526	LM652576	LM652659
Phitoascus exsertus	Microascus exsertus Microascus exsertus	CBS 583.75 CBS 819 70 (ex-tvne)	From Os <i>mia rufa</i> From Menachile willouchhialla	Denmark: Sjaelland Denmark: Tastrun	LM652448 I M652449	LM652527 1 M652528	LM652577 1 M652578	LM652660 I M652661
Phitoascus intermedius	Microascus intermedius	CBS 217.32 (ex-type)	Root of Fragaria vesca	USA: North Carolina	L M652450	L M652529	LIM652579	I M652662
Phitoascus nidicola	Microascus nidicola	CBS 197.61 (ex-epitype)	From Dipodomys merriami	USA: Utah	LM652451	LM652530	LM652580	LM652663
Pithoascus platysporus†	Pithoascus platysporus	CBS 419.73 (ex-type)	Agricultural soil	The Netherlands	LM652452	LM652531	I	I
Phitoascus stoveri	Microascus stoveri	CBS 176.71 (ex-type)	Root of Beta vulgaris	USA: Ohio	LM652453	LM652532	LM652581	LM652664
Pseudallescheria ellipsoidea	Petriellidium ellipsoideum	CBS 418.73 (ex-type)	Soil	Tajikistan	EF151323	EF151323	I	I
Pseudoscopulariopsis hibernica	Scopulariopsis hibernica	UAMH 2643; ATCC 16690	From soil	Ireland	LM652454	LM652533	LM652582	LM652665
comb. nov.								

Pseudoscopulariopsis schu-	Microascus schumacheri	CBS 435.86 (ex-neotype)	From soil	Spain: Puerto de la Quesera	LM652455	LM652534	LM652583	LM652666
<i>macheri</i> comb. nov.				-				
Scedosporium aurantiacum	Scedosporium aurantiacum	CBS 116910	Ulcer of ankle	Spain	HQ231818	EF151326	I	I
Scedosporium boydii	Pseudallescheria boydii	CBS 330.93	Bronchial secretion	The Netherlands	AY 863 196	AY882372	I	I
Scopulariopsis acremonium†	Scopulariopsis danica	CBS 290.38; MUCL 9028 (ex-type)	Skin of a horse	Denmark	LM652456	HG380439	HG380362	I
	Scopulariopsis acremonium	MUCL 8274	Wheat field soil	Germany: Schleswig-Holstein	LM652457	LM652535	I	I
	Scopulariopsis acremonium	MUCL 8409	Soil	Germany: Schleswig-Holstein	LM652458	LM652536	I	I
Scopulariopsis asperula	Microascus niger	MUCL 40729; UAMH 7879	Indoor air	Canada: Alberta	LM652459	LM652537	LM652584	LM652667
	Microascus niger	MUCL 40746; UAMH 9029	Dung of Mephitis mephitis	Canada: Alberta	LM652460	HG380434	HG380357	LM652668
	Scopulariopsis asperula	CBS 853.68	Compost soil	Germany	LM652461	JQ434669	JQ434621	JQ434558
	Scopulariopsis fusca	UTHSC 10-3405; FMR 12212	Toenail	USA	LM652462	HG380461	HG380384	LM652669
	Scopulariopsis fusca	CBS 401.34; MUCL 9032 (ex-type)	Carcass of rabbit	Austria	LM652463	HG380465	HG380388	LM652670
	Torula bestae	CBS 289.38; MUCL 9012 (ex-type)	From man	Italy	LM652464	LM652538	LM652585	LM652671
Scopulariopsis brevicaulis	Microascus brevicaulis	MUCL 40726 (ex-type)	Indoor air	Canada: Alberta	LM652465	HG380440	HG380363	LM652672
	Scopulariopsis alboflavescens	CBS 399.34 (ex-type)	Diseased skin	Austria	LM652466	LM652539	JQ434600	JQ434537
	Scopulariopsis brevicaulis	UTHSC 06-277; FMR 12273	Human hair	USA	LM652467	HG380441	HG380364	LM652674
	Scopulariopsis brevicaulis	UTHSC 06-619; FMR 12271	Human toenail	USA	LM652468	HG380442	HG380365	LM652675
	Scopulariopsis brevicaulis	UTHSC 06-1072; FMR 12247	Human BAL	USA	LM652469	LM652540	LM652586	LM652676
	Scopulariopsis brevicaulis	UTHSC 07-1812; FMR 12257	Human toenail	USA	LM652470	HG380443	HG380366	LM652677
	Scopulariopsis brevicaulis	UTHSC 07-1888; FMR 12255	Human spine	USA	LM652471	HG380444	HG380367	LM652678
	Scopulariopsis brevicaulis	UTHSC 09-1092; FMR 12236	Human toe	USA	LM652472	HG380445	HG380368	LM652679
	Scopulariopsis brevicaulis	UTHSC 09-1373; FMR 12233	Human sputum	USA	LM652473	LM652541	LM652587	LM652680
	Scopulariopsis brevicaulis	UTHSC 11-1240; FMR 12206	Human lung mass	USA	LM652474	HG380446	HG380369	LM652681
	Scopulariopsis brevicaulis	UTHSC 11-1563; FMR 12204	Human BAL	USA	LM652475	HG380447	HG380370	LM652682
	Scopulariopsis brevicaulis	UTHSC 11-427; FMR 12211	Human sputum	USA	LM652476	HG380448	HG380371	LM652683
	Scopulariopsis insectivora	CBS 335.35; MUCL 9035	Pupa of <i>Pteronus pini</i>	The Netherlands	LM652477	LM652542	LM652588	LM652684
	Scopulariopsis koningii	CBS 208.61	Elephant	Unknown	LM652478	LM652543	LM652589	LM652685
	Scopulariopsis stercoraria	MUCL 14213	Soil	Belgium: Heverlee	LM652479	LM652544	LM652590	LM652686
Scopulariopsis canadensis†	Scopulariopsis canadensis	CBS 204.61 (ex-type)	Seed of Beta vulgaris	Canada	LM652480	LM652545	I	I
Scopulariopsis candida	Scopulariopsis alboflavescens	MUCL 9007	Unknown	Unknown	LM652481	LM652546	LM652591	LM652687
	Scopulariopsis brevicaulis var. alba	CBS 119.43; MUCL 9016	Soil	The Netherlands	LM652482	LM652547	LM652592	LM652688
	Scopulariopsis candelabrum	CBS 205.27; MUCL 9026	Unknown	France	LM652483	LM652548	LM652593	LM652689
	Scopulariopsis candida	MUCL 40743 (ex-epitype)	Indoor air	Canada	LM652484	HG380458	HG380381	LM652690
	Scopulariopsis candida	UTHSC 09-3241; FMR 12226	Scalp	USA	LM652485	HG380460	HG380383	LM652691
	Scopulariopsis candida	UTHSC 09-2576; FMR 12228	Sputum	USA	LM652486	HG380459	HG380382	LM652692
	Microascus manginii	MUCL 41467	Cheese 'Tome de Savoie'	France	LM652487	HG380433	HG380356	LM652693
	Nephrospora manginii	CBS 170.27 (ex-type)	Unknown	France	LM652488	LM652549	LM652594	LM652694
Scopulariopsis carbonaria†	Scopulariopsis carbonaria	MUCL 9027 (ex-type)	Soil	Panama	LM652489	HG380462	HG380385	LM652695
Scopulariopsis coprophila†	Scopulariopsis fimicola	CBS 206.61	Mushroom bed	UK	LM652490	LM652550	I	I
Scopulariopsis cordiae sp. nov.	Scopulariopsis sp.	CBS 138129; UTHSC 09-866;	Human finger	USA	LM652491	HG380499	HG380422	LM652673
		FMR 12338 (ex-type)						
	Scopulariopsis sp.	UTHSC 05-3453; FMR 12349	Human JP Drain	USA	LM652492	HG380498	HG380421	LM652696
Scopulariopsis flava	Scopulariopsis flava	CBS 207.61; MUCL 9031 (ex-neotype)	Cheese	UK	LM652493	HG380464	HG380387	LM652697
Scopulariopsis parva	Scopulariopsis parvula	MUCL 9041 (ex-type)	Soil	Canada	LM652494	LM652551	I	I
Scopulariopsis soppii	Microascus soppii	UAMH 9169 (ex-type)	wood of Populus tremuloides	Canada: Alberta	LM652495	LM652552	LM652595	LM652698
Trichoderma asperellum	Trichoderma asperellum	CBS 433.97; NBRC 101777 (ex-type)	Sclerotia of Sclerotinia minor	USA: Maryland	11776302*	11776301*	I	I
			buried in soil					
Trichurus spiralis	Trichurus spiralis	NBRC 100833	Mushroom	Japan: Kumamoto-shi	11058901*	11058901*	I	I
Trichurus terrophilus	Trichurus terrophilus	NBRC 7660; CBS 448.51; UAMH 8848	Timber of Eucalyptus saligna	South Africa	00766001*	00766001*	I	I
Wardomyces inflatus	Wardomyces hughesii	CBS 216.61 (ex-isotype)	Wood, Acer sp.	Canada: Québec	LM652496	LM652553	I	I

I I I

I I I

> LM652555 LM652554

LM652498 LM652496 LM652497

Canada: Québec Canada: Ontario

Soil Soil

CBS 487.66 (ex-type) FMR 10305

Wardomycopsis inopinata Scopulariopsis humicola Wardomyces hughesii

Wardomycopsis humicola Wardomycopsis inopinata Wardomyces inflatus

Myanmar

ATCC: American type culture collection, Manassas, VA, USA; CBS: CBS Fungal Biodiversity Centre, Utrecht, The Netherlands; FMR; Facultat de Medicina i Ciencies de la Salut, Reus, Spain, IHEM: Biomedical Fungi and Yeasts Collection, Scientific Institute of Public Health, Bel-gium; MUCL: Université Catholique de Louvain, Louvain-Ia-Neuve, Belgium; NBRC: National Biological Resource Centre, Japan; UAMH: University of Alberta Microfungus Collection and Herbarium, Canada; UTHSC: Fungus Testing Laboratory, Department of Pathology, University of Alberta

of Texas Health Science Center, San Antonio, USA.

<sup>2</sup> ITS: Internal transcribed spacer regions of the rDNA and 5.8S region; LSU: partial large subunit of the rDNA; EF-1c: Partial translation elongation factor gene; TUB: partial beta-tubulin gene. † Excluded or doubtful species name. \* Sequences obtained from the NBRC database. Sequences newly generated in this study are indicated in **bold**.



└ NBRC 101777<sup>⊤</sup> Trichoderma asperellum

**Fig. 1** Maximum likelihood (ML) tree obtained from the combined LSU and ITS sequences of 61 representative taxa of *Microascaceae* and *Graphiaceae*. Numbers on the branches are ML bootstrap values (bs) above 70 %, followed by Bayesian posterior probabilities (pp) above 0.95. Full supported branches are indicated in **bold**. Branch lengths are proportional to distance. Strains considered current members of the genera *Microascus* or *Scopulariopsis* genera are represented in **bold**. Ex-type strains are indicated with <sup>T</sup>. The original name of each strain, when applied, is given between parenthesis. The tree was rooted to *Hypocrea atroviridis* (NBRC 101776) and *Trichoderma asperellum* (NBRC 101777).

with a sampling frequency of every 100 generations. The 50 % majority rule consensus trees and posterior probabilities (pp) were calculated from 37 500 trees after discarding 12 500 trees for burn-in. Posterior probability values equal or above 0.95 were considered significant. The resulting trees were plotted using FigTree v.1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/). The alignments originated in this study have been deposited in TreeBASE (http://www.treebase.org).

#### Morphology

All isolates were grown on oatmeal agar (OA; 30 g filtered oat flakes, 20 g agar, 1 L distilled water) and potato-carrot agar (PCA; 20 g each of filtered potatoes and carrots, 20 g agar, 1 L distilled water). They were incubated at different temperatures (5, 15, 25, 30, 35, 37, 40 and 45 °C) and examined at 7 and 14 d to determine colony growth rates. In descriptions, colour notations of the colonies were from Kornerup & Wanscher (1978). Measurements and descriptions of microscopic structures were made using an Olympus CH2 light microscope (Olympus Corporation, Tokyo, Japan) from cultures on PCA or OA at 25 °C for 14 and 21 d to ensure ascomata development. All isolates were examined on slides mounted on 85 % lactic acid. Features of the sexual morph structures were obtained from squash preparations or spore mounts. Photographs of the microscopic structures were made using a Zeiss Axio Imager M1 light microscope (Zeiss, Oberkochen, Germany) with a mounted DeltaPix Infinity X digital camera using Nomarski differential interference contrast and phase contrast optics. Nomenclatural data was deposited in MycoBank (Crous et al. 2004).

### RESULTS

### Generic circumscription of the Microascaceae

To delineate generic boundaries, we conducted a phylogenetic analysis using the combined LSU and ITS datasets including 54 currently accepted species belonging to 12 genera of *Microascaceae* and one species of the family *Graphiaceae*. *Trichoderma asperellum* and *Trichoderma atroviride* were selected as outgroup (Fig. 1). The final alignment consisted of 63 taxa and contained 996 characters (LSU 504, ITS 492), of which 618 were conserved and 297 were phylogenetically informative (LSU 98, ITS 199). Fig. 1 shows the ML tree including bs and pp values. The trees obtained from ML and Bayesian analyses of the individual loci and the combined analysis showed congruent topologies.

The phylogenetic inferences showed that *Microascus / Scopulariopsis* were polyphyletic, with species distributed into several distant lineages. However, most species of *Microascus / Scopulariopsis* clustered into a single large, well-supported lineage (bs = 95 % / pp = 1.00). This lineage comprised four sublineages, which we interpret as three distinct genera, *Microascus, Pithoascus* and *Scopulariopsis*, the fourth one representing a putative undescribed genus.

The members of a sublineage referred to as *Microascus* were characterised by dark-coloured colonies and mostly brown to green-brown mycelia, conidiogenous apparatus and conidia. The conidiogenous cells (annellides) were born singly on aerial hyphae or on penicillate conidiophores. They were ampulliform or lageniform and usually had a long and narrow cylindrical annellated zone tapering gradually to the conidiogenous locus, and produced smooth to roughened conidia. Sexual morphs were observed in 13 species. Ascomata were ostiolate, rarely non-ostiolate, mostly globose to ampulliform, glabrous or hairy, papillate or with long cylindrical necks, and had a dark brown to black peridium of *textura angularis* with exception of the unidentified strains FMR 12362 and UTHSC 07-156, which show-

ed perithecia with peridia of *textura intricata*. The ascospores ranged from reniform to ellipsoidal, triangular or quadrangular, were straw-coloured to pale brown and exhibited a single, mostly inconspicuous germ pore (Fig. 2a-h).

Members of the *Pithoascus* sublineage showed flat, white to grey colonies without aerial mycelia. The mycelium and the conidiogenous apparatus were subhyaline and the latter consisted of solitary, short, mostly ampulliform annellides with a short-cylindrical neck. With the exception of strain IHEM 18608, all strains of the *Pithoascus* clade exhibited a sexual morph characterised by black ascomata with an inconspicuous ostiole and navicular to fusiform ascospores without germ pores (Fig. 2i-n).

The *Scopulariopsis* sublineage included fungi with white, pale grey, tan or brown colonies. The mycelium was mostly hyaline. Annellides were hyaline or pale brown, more or less cylindrical, with a wide, flat conidiogenous opening and mostly formed on densely penicillate conidiophores. Conidia were hyaline or pale brown, smooth or distinctly roughened, often showing a protruding base. A sexual morph was observed in four species and was characterised by dark, globose to subglobose perithecia with a peridium of *textura angularis* and with a papillate or a long cylindrical ostiolar neck. Ascospores were reniform to broadly lunate, hyaline or pale yellow, with a single, inconspicuous germ pore (Fig. 2s–y).

The reference strains of *Microascus schumacheri* (CBS 435.86) and *Scopulariopsis hibernica* (UAMH 2643) formed a well-supported clade (bs 91, pp 1.00), basal to the *Scopulariopsis* clade. Because the former two taxa shared several morphological features that deviated from those of *Scopulariopsis*, they were accommodated in a new genus named *Pseudoscopulariopsis*. Members of this clade were characterised by forming grey or olivaceous colonies and hyaline to subhyaline conidiogenous cells, which usually consisted of annellides arising from swollen basal cells. The annellides were short, more or less ampulliform and with a cylindrical annellated zone. The sexual morph was only observed in *P. schumacheri*, which produced black perithecia and fusiform or navicular, straw-coloured ascospores without germ pores (Fig. 2o-r).

However, this phylogenetic approach had insufficient resolution to establish the limits among the different species included in each genus. Similarly, the ex-type strain of *Scopulariopsis carbonaria* (MUCL 9027) was related to the *Scopulariopsis* clade, but its position was not resolved with this analysis.

The reference strain of *Microascus singularis* (CBS 414.64) formed a solitary branch in an incertae sedis position. The main morphological distinction of this isolate was the production of conidia showing longitudinal bands. A strongly-supported clade, composed by the ex-type strains of *Microascus caviariformis* (CBS 536.87) and Scopulariopsis danica (MUCL 9028), two reference strains of Scopulariopsis acremonium (MUCL 8274 and MUCL 8409) and a reference strain of Microascus albonigrescens (IHEM 18560), clustered apart from the genera included in the study. The ex-type strain of Microascus giganteus (CBS 746.69) was placed very far from the Microascus clade. It formed a well-supported clade with the ex-type strain of Wardomyces inflatus (CBS 216.61) and with another fully supported clade, which included several reference strains of the genera Doratomyces and Trichurus. Our phylogenetic analyses were concordant with the observations made by Abbott (2000), who considered Doratomyces and Trichurus as congeneric with Cephalotrichum, all being characterised by the formation of dryspored synnemata and lacking sexual morphs.

Lophotrichus and Kernia, characterised by hairy ascomata and ellipsoidal ascospores with two germ-pores and graphium- or



**Fig. 2** Key morphological features to distinguish *Microascus* (a–h), *Pithoascus* (*Pi.*) (i–n), *Pseudoscopulariopsis* (*Ps.*) (o–r) and *Scopulariopsis* (s–y). a, i, o, s. Colonies on PCA after 21 d at 25 °C; b, c, j, k, p, t, u. conidiogenous cells; d–f, l, v, w. ascospores (germ pores indicated with arrows); g, h, m, n, q, r, x, y. conidia (a, b, d. *M. cinereus* CBS 365.65; c. *M. restrictus* CBS 138277; e. *M. trigonosporus* CBS 218.31; f. *M. pyramidus* CBS 212.65; g. *M. chartarus* MUCL 9001; h. *M. gracilis* CBS 369.70; i, k, l, n. *Pi. nidicola* CBS 197.61; j, m. *Pi. ater* IHEM 18608; o–r. *Ps. hibernica* UAMH 2643; s, u, x. *S. brevicaulis* MUCL 40726; t, v, y. *S. candida* MUCL 40743; w. *S. soppii* UAMH 9169. — Scale bars: 5 μm.

scopulariopsis-like asexual morphs, respectively, formed wellsupported clades related to *Scedosporium* and allied genera (i.e., *Parascedosporium*, *Petriella* and *Petriellopsis*), characterised by scedosporium-like asexual morphs with slimy conidia.

Some species traditionally included in *Pithoascus* and *Scopulariopsis* clustered in orders different from *Microascales*. The extype strain of *Pithoascus platysporus* (CBS 419.73) and a reference strain of *Scopulariopsis coprophila* (CBS 206.61) were closely related to the *Hypocreales*; the ex-type strain of *Scopulariopsis canadensis* (CBS 204.61) grouped with members of the *Xylariales*; the ex-type strains of *Scopulariopsis parva* (MUCL 9041) and *Scopulariopsis halophilica* (CBS 380.74) clustered outside the *Sordariomycetes*, being related to members of the *Eurotiales* (data not shown).

# Species distribution in Microascus, Pithoascus, Pseudoscopulariopsis and Scopulariopsis

The final alignment of the combined matrix included 106 strains from Microascus, Pithoascus, Scopulariopsis and Pseudoscopulariopsis species and involved 2 219 characters (LSU 437, ITS 493, EF-1a 816, TUB 473), of which 1 493 were conserved, 673 were variable and 486 were phylogenetically informative (LSU 47, ITS 112, EF-1a 176, TUB 151). Petriella setifera and Parascedosporium tectonae were selected as outgroup taxa. The resulting ML tree is shown in Fig. 3 including bs and pp values. The topology of the trees obtained from ML and Bayesian analyses from each individual locus and the combined analysis were concordant. The multilocus analysis confirmed the results obtained from phylogenetic inferences using the combined LSU and ITS dataset. In total 34 well-supported clades were resolved and were distributed among four main lineages corresponding to Microascus, Pithoascus, Scopulariopsis and the new genus Pseudoscopulariopsis proposed here. The Microascus lineage comprised 20 well-supported subclades, 13 of which included an ex-type strain of a known species or a strain considered to be authentic for a particular species, while seven subclades corresponded to new species, which are described here. *Pithoascus* comprised five well-supported monophyletic subclades, each of which included an ex-type strain of a known species. Scopulariopsis encompassed six well-supported subclades, of which five included an ex-type strain or a strain considered as authentic, while one subclade corresponded to a new species described here. The new genus Pseudoscopulariopsis encompassed two subclades, each one including a single reference strain of a species previously identified as Microascus or Scopulariopsis, respectively.

In the combined phylogenetic analysis, the ex-type strain of *Scopulariopsis carbonaria* (MUCL 9027) was basal to the *Microascus* and *Pithoascus* clades. According to the original description (Morton & Smith 1963), this species showed a high similarity in annellidic and conidial morphology with members of the *Microascus* lineage; however, after several attempts to induce sporulation this strain remained sterile, and thus its taxonomic position could not be resolved.

### TAXONOMY

Based on the results of the above multilocus sequence analysis and a morphological analysis, the boundaries of the genera *Microascus*, *Pithoascus* and *Scopulariopsis* have been reassessed accordingly. Their current circumscription is revised and several new taxa and combinations are proposed as follows:

#### Microascus Zukal, Verh. Zool.-Bot. Ges. Wien 35: 339. 1885

= *Peristomium* Lechmere, Compt. Rend. Hebd. Séances Acad. Sci. 154: 178. 1912.

- = Masonia G. Sm., Trans. Brit. Mycol. Soc. 35: 149. 1952.
- ≡ Masoniella G. Sm., Trans. Brit. Mycol. Soc. 35: 237. 1952.

Type species. Microascus longirostris Zukal.

Colonies restricted or spreading, pale grey, brown, olivaceous or black, velvety, floccose or fasciculate, granular and often forming concentric rings due to the production of ascomata. Ascomata perithecial, immersed or superficial, scattered or aggregated, globose to ampulliform, glabrous or covered with scattered hairs, ostiolate, usually with a neck of variable length and shape, sometimes with a tuft of ostiolar hairs; peridium dark brown or black, composed of thick-walled, slightly flattened cells, textura angularis or textura intricata. Asci unitunicate, 8-spored, obovate, barrel-shaped or nearly globose, formed in basipetal rows, evanescent. Ascospores 1-celled, asymmetrical, reniform, heart-shaped, triangular or guadrangular, dextrinoid when young, extruded through the ostiole into a gelatinous drop or a long cirrhus. Conidiogenous cells annellidic, borne singly and laterally on the vegetative hyphae, or in groups of 2-5 on short simple or little branched conidiophores, ampulliform or lageniform, subhyaline or darkening with age, smooth- or rough-walled with a distinct cylindrical annellated zone, Conidia 1-celled, pale yellowish to dark brown, globose to subglobose, obovate or clavate, with a truncate base and rounded or pointed at the apex, smooth- and thin-walled or finely rough- and thick-walled, produced singly or in basipetal dry chains. Solitary conidia present in some species, borne sessile or on short stalks from the vegetative hyphae.

# *Microascus alveolaris* Sandoval-Denis, Gené & Guarro, *sp. nov.* — MycoBank MB809418, Fig. 4

Etymology. In reference to the isolation source of most isolates.

Colonies on OA and PCA at 25 °C attaining 31-36 and 18-29 mm diam, after 14 d, respectively, flat, slightly velvety, somewhat granular at the centre due to the presence of ascomata, white to grey (4B1), abundant submerged mycelium in the outer zone, with a wide white margin; reverse white to grey (4B1). Vegetative hyphae septate, hyaline to light brown, smooth- and thin-walled, 1.5-3 µm wide. Ascomata superficial or immersed, formed predominantly at the centre of the colony, globose to subglobose, 110-290 µm diam, usually with an ostiolar cylindrical neck up to 100 µm long, black, glabrous, the apice sometimes with a tuft of hyaline, septate and acicular hairs, up to 60 µm long; peridium of *textura angularis*. Asci irregularly ellipsoidal, 8–12 × 7.5–11 µm. Ascospores broadly triangular, rarely reniform,  $4-6 \times 3-5 \mu m$ , with a single germ pore, straw coloured, bright yellow in mass. Conidiophores absent or as a basal single cell of  $5-12 \times 2-2.5 \mu m$ , bearing groups of 2-3annellides, rarely slightly branched up to 80 µm long, hyaline to subhyaline, smooth-walled. Annellides mostly sessile, single and lateral on vegetative hyphae, lageniform,  $6-17 \times 1.5-3.5$  $\mu$ m, tapering slightly towards the annellated zone 1–2  $\mu$ m wide, hyaline to subhyaline, smooth- and thin-walled. Conidia ellipsoidal, navicular or bullet-shaped,  $3-5 \times 2-3.5 \mu m$ , with truncate base and rounded apex, subhyaline to pale brown, brown in mass, thin- and smooth-walled, arranged in long chains. Solitary conidia sometimes present, borne laterally from vegetative hyphae, sessile or on short stalks, unicellular, subglobose or obovoidal, 3-5 × 2.5-4 µm, subhyaline or pale brown, smoothand more or less thick-walled.

Cardinal temperature for growth — Optimum 25–30 °C, maximum 40 °C, minimum 15 °C.



**Fig. 3** Maximum likelihood (ML) tree obtained from the combined ITS, LSU, EF-1α and TUB sequences of 105 strains from *Microascus, Pithoascus, Pseudo-scopulariopsis* and *Scopulariopsis* species. Numbers on the branches are ML bootstrap values (bs) above 70 %, followed by Bayesian posterior probabilities (pp) above 0.95. Full supported branches are indicated in **bold**. Branch lengths are proportional to distance. Ex-type strains are indicated with <sup>T</sup>. Ex-neotype strains are indicated with <sup>NT</sup>. The original name of each strain, when applied, is given on parenthesis. The tree was rooted to *Petriella setifera* (CBS 437.75) and *Parascedosporium tectonae* (CBS 127.84).





0.05 Fig. 3 (cont.)

Specimens examined. USA, from bronchoalveolar lavage fluid, 2007, D.A. Sutton (holotype CBS H-22111, culture ex-type CBS 139501 = UTHSC 07-3491 = FMR 12252); from sputum, 2005, D.A. Sutton (UTHSC 05-1041 = FMR 12351); from bronchoalveolar lavage fluid, 2005, D.A. Sutton (UTHSC 05-3416 = FMR 12350); from bronchoalveolar lavage fluid, 2006, D.A. Sutton (UTHSC 06-3152 = FMR 12346); from sputum, 2007, D.A. Sutton (UTHSC 07-1823 = FMR 12342); from bronchoalveolar lavage fluid, 2004, D.A. Sutton (UTHSC 04-1534 = FMR 12354); from bronchoalveolar lavage fluid, 2008, D.A. Sutton (UTHSC 08-886 = FMR 12340); from bronchoalveolar lavage fluid, 2008, D.A. Sutton (UTHSC 08-886 = FMR 12340); from bronchoalveolar lavage fluid, 2010, D.A. Sutton (UTHSC 10-214 = FMR 12336); from lung tissue, D.A. Sutton (UTHSC R-4634 = FMR 12333).

Notes — All the strains included in this species were isolated from the respiratory tract of human patients. Morphologically, *M. alveolaris* is close to *M. campaniformis*, *M. macrosporus*, *M. pyramidus* and *M. trigonosporus*, all showing similar triangularshaped ascospores. *Microascus alveolaris* can be differentiated by its membranous and white colonies, the smaller size of the ascospores and narrower conidia. Microascus brunneosporus Sandoval-Denis, Gené & Guarro, sp. nov. — MycoBank MB809419, Fig. 5

*Etymology.* From the Latin *brunneus-*, brown, referring to the colour of the ascospores.

Colonies on OA at 25 °C attaining 21-25 mm diam in 14 d, flat, velvety, granular at the centre due to the presence of ascomata, dull green (30E3) to olive-brown (4F4), with submerged mycelium towards the outer zone, margin regular; reverse dark green (30F4). On PCA at 25 °C attaining 15-17 mm diam in 14 d, slightly elevated, downy, fasciculate at the centre, dull green (30E3), with a white and regular margin; reverse dull green (30D4). Vegetative hyphae septate, subhyaline to pale brown, smooth- and thin-walled, 1.5–3 µm wide. Ascomata immersed, globose, 110-205 µm diam, with a short cylindrical ostiolar neck up to 40 µm long, black, glabrous; peridium with a textura angularis. Asci irregularly ellipsoidal or ovoidal, 11-14  $\times$  7–8 µm. Ascospores ellipsoidal to allantoid, 5–7  $\times$  2–3 µm, light yellow-brown, brown in mass, with a single and inconspicuous germ pore. Conidiophores absent or as a basal single cell of 5-15 × 1.5-2.5 µm, bearing 1-3 annellides, rarely slightly



**Fig. 4** *Microascus alveolaris* CBS 139501. a, b. Colonies on OA and PCA, respectively, after 21 d at 25 °C; c. ascoma; d. peridium; e-g. asci and ascospores; h-j. conidiophores, annellides and conidia; k, l. solitary conidia. — Scale bars:  $c = 30 \mu m$ ; h, i = 10  $\mu m$ ; all others = 5  $\mu m$ .



**Fig. 5** *Microascus brunneosporus* CBS 138276. a, b. Colonies on OA and PCA, respectively, after 21 d at 25 °C; c. ascoma; d. peridium; e-h. asci and ascospores; i-k. conidiophores, annellides and conidia; l. conidial chain. — Scale bars: c = 50 µm; all others = 5 µm.



**Fig. 6** *Microascus campaniformis* CBS 138126. a, b. Colonies on OA and PCA, respectively, after 21 d at 25 °C; c. ascoma; d. peridium; e, f. asci and ascospores; g-j. conidiophores, annellides and conidia. — Scale bars:  $c = 50 \ \mu m$ ;  $g = 10 \ \mu m$ ; all others = 5  $\mu m$ .

branched up to 30 µm long, subhyaline, smooth-walled. Annellides mostly sessile, single and lateral on vegetative hyphae, more or less lageniform,  $9-14 \times 2-2.5$  µm, tapering to a cylindrical annellated zone 1-1.5 µm wide, subhyaline, smooth- or roughwalled, thin-walled. Conidia subglobose, ellipsoidal or navicular,  $4-5 \times 2.5-5$  µm, with truncate base, light green-brown, thin- and smooth-walled, arranged in long chains. Solitary conidia not observed.

Cardinal temperature for growth — Optimum 25–30  $^\circ\text{C},$  maximum 35  $^\circ\text{C},$  minimum 15  $^\circ\text{C}.$ 

Specimen examined. USA, from bronchoalveolar lavage fluid, 2006, *D.A.* Sutton (holotype CBS H-21783, culture ex-type CBS 138276 = UTHSC 06-4312 = FMR 12343).

Notes — This species is similar to *M. cinereus* and *M. gracilis*. However, the latter two species produce reniform or broadly lunate, straw coloured ascospores with an often conspicuous germ pore.

# *Microascus campaniformis* Sandoval-Denis, Cano & Deanna A. Sutton, *sp. nov.* — MycoBank MB809205, Fig. 6

*Etymology.* From the Latin *campanus*-, bell, referring to the shape of the ascospores.

Colonies on OA at 25 °C attaining 27-34 mm diam in 14 d, flat, velvety to slightly granular at the centre, dull green (30E4), with an irregular margin; reverse dull green (30E4). On PCA at 25 °C, colonies attaining 14-25 mm diam in 14 d, flat, velvety, fluffy at the centre, dull green (28E4) to dark green (30E4), with a white and regular margin; reverse dark green (28F3). Vegetative hyphae septate, subhyaline, smooth- or rough- and thin-walled, 1.5-2.5 µm wide. Ascomata immersed or superficial, usually formed at the periphery of the colony, globose to subglobose, 150-220 µm diam, with a short cylindrical ostiolar neck up to 80 µm long, widening at the ostiolar opening, rarely with a tuft of hyaline, straight and septate hairs up to 50 µm long, black, glabrous; peridium with a textura angularis. Asci irregularly ellipsoidal or subglobose, 18-21 × 10-15 µm. Ascospores broadly triangular,  $6-7 \times 4-4.5 \mu m$ , often with an elongated side towards a single germ pore, straw coloured, bright yellow-orange in mass. Conidiophores absent or as a basal cell of  $5 \times 2 \mu m$ , bearing groups of 5-8 annellides, or slightly branched up to 60 µm long, hyaline to subhyaline, smooth-walled. Annellides somewhat lageniform,  $9-14 \times 2-3 \mu m$ , with a more or less swollen base and tapering abruptly to a cylindrical annellated zone, 1-1.5 µm wide. Conidia subglobose to broadly ellipsoidal,  $4-5 \times 2.5-3.5 \mu m$ , with a truncate base, light green-brown, dark brown in mass, thick-walled, arranged in long chains. Solitary conidia and chlamydospores not observed.

Cardinal temperature for growth — Optimum 25–30 °C, maximum 40 °C, minimum 15 °C.

Specimen examined. USA, from bronchoalveolar lavage fluid, 2010, *D.A. Sutton* (holotype CBS H-21784, culture ex-type CBS 138126 = UTHSC 10-565 = FMR 12334).

Notes — *Microascus campaniformis* is similar to *M. alveolaris*, *M. macrosporus*, *M. pyramidus* and *M. trigonosporus* in having distinctive triangular shaped ascospores. However, *M. campaniformis* can be differentiated by its green colonies and inequilateral ascospores that show an elongation at one side towards the germ pore. In contrast, the ascospores of *M. alveolaris*, *M. macrosporus* and *M. trigonosporus* are almost equilateral with rounded ends, while those of *M. pyramidus* have attenuated ends acquiring a nearly square shape. *Microascus campaniformis* is phylogenetically close to *M. paisii* sharing similar annellides. However, a sexual morph has not been observed in *M. paisii*.

# *Microascus chartarus* (G. Sm.) Sandoval-Denis, Gené & Guarro, *comb. nov.* — MycoBank MB809206

Basionym. Masonia chartarum G. Sm., Trans. Brit. Mycol. Soc. 35: 150. 1952.

≡ Masoniella chartarum (G. Sm.) G. Sm., Trans. Brit. Mycol. Soc. 35: 237. 1952.

≡ Scopulariopsis chartarum (G. Sm.) F.J. Morton & G. Sm., Mycol. Pap. 86: 64. 1963.

Specimen examined. UK, London, isolated from mouldy wall-paper, 1950, K. Maunsell (Masonia chartarum ex-type culture CBS 294.52 = MUCL 9001).

Notes — Microascus chartarus has been reported from soil, dust and indoor-air (Domsch et al. 2007). It was originally described as a member of Masonia G. Sm. (1952a). However, Masonia is an illegitimate homonym of Masonia Hansford (1944), and thus the new genus Masoniella was erected (Smith 1952b). Most members of Masoniella were later transferred to Scopulariopsis (Morton & Smith 1963); both genera share the same conidiogenesis (annellidic, percurrent) and conidiogenous cells, distinctly narrower at the base, then swollen, and ending in a slender annellidic zone. Our phylogenetic analysis shows that M. chartarus is included in the Microascus sublineage and it is closely related to *M. trigonosporus*. *Microascus trigonosporus* can be distinguished by the production of a sexual morph, with triangular ascospores and mostly globose to subglobose and pale brown conidia. No sexual morph is known for *M. chartarus* and its conidia are ovate, often with a pointed end, green-brown (Morton & Smith 1963). Microascus croci and M. paisii resemble *M. chartarus* and also lack a sexual morph. However, these two species can be differentiated from *M. chartarus* by their conidial shape and colour, which are globose and ellipsoidal to short clavate in *M. croci* and *M. paisii*, respectively, and pale brown in both species. In addition, M. croci is able to grow from 5-30 °C and M. paisii grows from 15-37 °C, while M. chartarus has a narrower temperature range growing from 15-25 °C.

### *Microascus cinereus* (Émile-Weil & Gaudin) Curzi, Boll. Staz. Patolog. Veget. Roma 11: 60. 1931.

Basionym. Scopulariopsis cinerea Émile-Weil & Gaudin, Arch. Méd. Exp. Anat. Path. 28: 452. 1919.

- = Scopulariopsis oidiospora Zach, Oesterr. Bot. Z. 83: 182. 1934.
- = Microascus lunasporus P.M. Jones, Mycologia 28: 503. 1936.
- ≡ Scopulariopsis lunaspora P.M. Jones, Mycologia 28: 504. 1936.
- = Microascus pedrosoi C.A. Fuentes & F.A. Wolf, Mycologia 48: 63. 1956.
- = Microascus griseus P.N. Matur & Thirum., Sydowia 16: 49. 1962.
- = Microascus reniformis Orr, Persoonia 8: 194. 1975.

Specimens examined. INDIA, Maharashtra, Poona, from soil, 1965, *M.J. Thirumalachar (M. griseus* ex-type culture CBS 365.65 = ATCC 16204). – USA, from bronchoalveolar lavage fluid, 2010, *D.A. Sutton* (neotype of *M. cinereus* designated here CBS H-21937, MBT198511) culture ex-neotype CBS 138709 = UTHSC 10-2805 = FMR 12217; from bronchoalveolar lavage fluid, 2006, *D.A. Sutton* (UTHSC 06-3278 = FMR 12345); from sternum tissue, 2008, *D.A. Sutton* (UTHSC 08-3181 = FMR 12339); from bronchoalveolar lavage fluid, 2009, *D.A. Sutton* (UTHSC 09-573 = FMR 12239); from bronchoalveolar lavage fluid, 2009, *D.A. Sutton* (UTHSC 11-383 = FMR 12331).

Notes — *Microascus cinereus* has a widespread distribution and a wide range of substrates. It has been isolated mainly from stored cereals, soil and dung (Barron et al. 1961, Udagawa 1962, Guarro et al. 2012), but it has also been described as an opportunistic pathogen of animals and humans (Baddley et al. 2000, de Hoog et al. 2011, Sandoval-Denis et al. 2013). Descriptions of *M. cinereus* are available in Barron et al. (1961) and Guarro et al. (2012). However, according to our observations, their measurements might have included isolates of *Microascus gracilis* from which *M. cinereus* has to be differentiated (Sandoval-Denis et al. 2013). The isolates of *M. cinereus* studied here showed asci 7–12 × 5–10 µm, ascospores 4–5.5 × 2.5–4 µm and conidia 3–5 × 2–3 µm. In addition, while



**Fig. 7** *Microascus expansus* CBS 138127. a, b. Colonies on OA and PCA, respectively, after 21 d at 25 °C; c-g. conidiophores, annellides and conidia; h. conidial chains. — Scale bars: c, d = 10 µm; all others = 5 µm.

M. cinereus produces pale to dark or black-grey colonies, at first velvety becoming slightly granular due to the presence of ascomata, M. gracilis produces dull green colonies, becoming olive-grey to olive-brown with ascomata mostly covered by aerial mycelium. Since no ex-type material of *M. cinereus* is available, the strain CBS 138709 (UTHSC 10-2805) is proposed here as neotype. Despite the existence of an ex-type culture of M. griseus, a synonym of M. cinereus, we consider it important to neotypify M. cinereus in order to conserve the oldest and most widely used epithet of this taxon. The original description of M. cinereus was based on an isolate obtained from a human nail but none of the isolates available share this original substrate. However, we believe that the isolate CBS 138709 (UTHSC 10-2805), obtained from human bronchoalveolar fluid agrees with the original and modern descriptions of this species (Émile-Weil & Gaudin 1919, Barron et al. 1961, Guarro et al. 2012).

# Microascus cirrosus Curzi, Boll. Staz. Patol. Veg. Roma 10: 308. 1930

Specimens examined. ITALY, from a leaf of *Prunus* sp., 1931, *M. Curzi* (ex-type culture CBS 217.31); from root of *Vitis vinifera*, 1934, *M. Curzi* (CBS 277.34 = MUCL 9050). – UK, from unknown substrate, 1961, *G. Smith* (CBS 301.61 = MUCL 9054). – USA, from sputum, 2007, *D.A. Sutton* (UTHSC 07-1887 = FMR 12256); from bronchoalveolar lavage fluid, 2011, *D.A. Sutton* (UTHSC 11-14 = FMR 12332).

Notes - Microascus cirrosus is a saprobic species with a worldwide distribution, commonly isolated from soil and dung (Barron et al. 1961, von Arx et al. 1988, Guarro et al. 2012). It has also been associated to superficial and respiratory human infections (de Hoog et al. 2011, Sandoval-Denis et al. 2013). Morton & Smith (1963) considered the asexual morph of this species to be conspecific with Scopulariopsis paisii (see Microascus paisii). However, according to our results, the ex-type strain of Torula paisii (MUCL 7915) was shown to be phylogenetically distant to the ex-type strain of M. cirrosus (CBS 217.31), and thus should be considered as a distinct species. Microascus cirrosus can be distinguished by having subglobose to obovate conidia measuring  $4-6.5 \times 4-6 \mu m$ , while those of *M. paisii* are broadly ellipsoidal to short clavate, measuring 4-6 × 2-4.5 µm. Microascus cirrosus is also similar to M. cinereus. However, M. cirrosus produces broadly reniform ascospores measuring  $5-6 \times 3-4 \mu m$  and larger conidia, while M. cinereus produces broadly lunate or almost triangular ascospores measuring  $4-5.5 \times 2.5-4 \mu m$ , and obovate to clavate conidia measuring  $3-5 \times 2-3 \mu m$ .

# *Microascus croci* (J.F.H. Beyma) Sandoval-Denis, Gené & Guarro, *comb. nov.* — MycoBank MB809207

Basionym. Scopulariopsis croci J.F.H. Beyma, Antonie van Leeuwenhoek 10: 52. 1945.

≡ *Masoniella croci* (J.F.H. Beyma) G. Sm., Trans. Brit. Mycol. Soc. 37: 166. 1954.

= Masoniella tertia Bat., J.A. Lima & C.T. Vasconc., Publções Inst. Micol. Recife. 263: 14. 1960.

Specimens examined. BRAZIL, Pernambuco, Recife, isolate from air, 1952, A. Batista (Masoniella tertia ex-type culture MUCL 9005 = CBS 296.61). – SPAIN, Tarragona, Riumar, from aquatic sediment of the Ebro River, May 1991, K. Ulfig & J. Gené (FMR 3997); Barcelona, from aquatic sediment of the Besós river, July 1991, J. Gené (FMR 4004). – THE NETHERLANDS, Lisse, from Crocus sp. Queen of the blues, 1943, H. Diddens (Scopulariopsis croci ex-type culture MUCL 9002 = CBS 158.44).

Notes — The clade representing *M. croci* included isolates from air, aquatic sediments, soil and plants, originating from Europe and South America. *Masoniella tertia* was considered a synonym of *S. melanospora* (Udagawa 1959) and a later synonym of *S. brumptii* (Morton & Smith 1963). However, the current combined analysis showed that the ex-type cultures of *M. tertia* and *S. melanospora* are phylogenetically unrelated, which agrees with their morphological features. All the isolates included in this clade have mostly globose conidia and are able to grow from 5–30 °C. Although no sexual morph has been reported for this species, one of the strains tested here (FMR 4004) was able to produce small and sterile peritecial-like ascomata after 8 mo of incubation on OA.

### Microascus expansus Sandoval-Denis, Gené & Cano, sp. nov. — MycoBank MB809208, Fig. 7

*Etymology.* From the Latin *expansio*-, expansion, referring to the quick growth of the colonies.

Colonies on OA and PCA at 25 °C growing rapidly, 65-81 and 70-75 mm diam, respectively, in 14 d, flat, velvety to powdery, more or less funiculose at the centre, olive (3F3) to grey-brown (4-5F3), with an irregular margin; reverse olive grey (2F2) or olive (2F4). Vegetative hyphae septate, hyaline to pale brown, smooth- and thin-walled, 1.5-3 µm wide. Conidiophores absent or as a basal single cell of  $4-5 \times 2-4 \ \mu m$ , bearing groups of 2-5 annellides, or slightly branched up to 20 µm long, hyaline to subhyaline, smooth-walled. Annellides slightly lageniform or somewhat subulate,  $5-12 \times 1.5-3.5 \mu m$ , tapering to a cylindrical annellated zone 1.5-2 µm wide, smooth-walled. Conidia bullet-shaped or broadly clavate, 4-8 × 2.5-3.5 µm, with a distinctive truncate base and rounded or slightly pointed apex, subhyaline to pale brown in mass, smooth- or finely roughened, thick-walled, arranged in long chains. Sexual morph not observed

Cardinal temperature for growth — Optimum 25–30 °C, maximum 40 °C, minimum 15 °C.

Specimens examined. USA, from sputum, 2006, *D.A. Sutton* (holotype CBS H-21785, culture ex-type CBS 138127 = UTHSC 06-4472 = FMR 12266); from pleural fluid, 2006, *D.A. Sutton* (UTHSC 06-2519 = FMR 12267).

Notes — *Microascus expansus* is known thus far from clinical isolates of human origin. Both isolates are able to grow at 40 °C. Other *Microascus* species able to grow at this temperature are *M. alveolaris*, *M. campaniformis*, *M. cinereus*, *M. cirrosus*, *M. gracilis*, *M. intricatus*, *M. macrosporus*, *M. pyramidus* and *M. restrictus*. However, except *M. restrictus*, all these species produce sexual morphs, while *M. expansus* produces only the asexual morph. *Microascus expansus* can be differentiated from *M. restrictus* by a faster growth rate, reaching > 60 mm diam at 25–30 °C in 14 d.

# Microascus gracilis (Samson) Sandoval-Denis, Gené & Guarro, comb. nov. — MycoBank MB809209

Basionym. Scopulariopsis gracilis Samson, Arch. Mikrobiol. 85: 179. 1972.

≡ Paecilomyces fuscatus N. Inagaki, Trans. Mycol. Soc. Japan 4: 4. 1962.

Specimens examined. JAPAN, from wheat flour, 1970, *N. Inagaki* (*Paecilo-myces fuscatus* ex-type culture CBS 369.70). – UK, isolate from soil, 1959, *J. Mendy* (MUCL 9048 = CBS 195.61). – USA, Iowa, isolate from a seed of *Zea mays*, 1961, *G.L. Barron* (MUCL 9049 = CBS 300.61); from synovial fluid, 2009, *D.A. Sutton* (UTHSC 09-1351 = FMR 12234); from bronchoalveolar lavage fluid, 2009, *D.A. Sutton* (UTHSC 09-1829 = FMR 12231); from bronchoalveolar lavage fluid, 2010, *D.A. Sutton* (UTHSC 10-390 = FMR 12335).

Notes — *Scopulariopsis gracilis* was proposed by Samson & von Klopotek (1972) as a new name for *Paecilomyces fuscatus*, probably to avoid nomenclatural conflict with *Scopulariopsis fusca* (Zach 1934).

*Microascus gracilis* has been isolated mainly from food in Asia, North and South America, and from soil in Europe. Recently, this species was reported from human clinical specimens, but its pathogenicity has not been demonstrated (Sandoval-Denis



**Fig. 8** *Microascus intricatus* CBS 138128. a, b. Colonies on OA and PCA, respectively, after 21 d at 25 °C; c. ascoma; d. peridium; e, f. asci and ascospores; g, h. conidiophores, annellides and conidia; i. conidial chain. — Scale bars: c = 40 µm; all others = 5 µm.

et al. 2013). Microascus gracilis and M. cinereus are very similar making their identification difficult in the absence of the sexual morph; in fact two reference strains (MUCL 9048 and MUCL 9049) and some clinical isolates were previously identified as M. cinereus. However, sequence comparison revealed that these species only showed 98.1 %, 97.8 % and 97 % sequence similarity for ITS, EF-1a and TUB, respectively. Morphologically *M. gracilis* can be differ-entiated from *M. cinereus* by its lunate ascospores, measuring 4.5-6.5 × 2-4 µm (as opposed to reniform to broadly lunate ascospores measuring  $4-5.5 \times 2.5-4$  $\mu$ m in *M. cinereus*), asci measuring 8–18 × 6–10  $\mu$ m (against  $7-12 \times 5-10 \ \mu m$  in *M. cinereus*), the formation of complex conidiophores and the morphology and colour of the colony. The asexual-morph of *M. gracilis* also resembles to that of M. murinus and M. paisii. However, M. gracilis produces annellides  $5-20 \times 1-2.5 \mu m$ , usually formed on well-defined and branched conidiophores, and subglobose to ellipsoidal conidia  $3.5-5.5 \times 2-3.5 \mu m$ ; the annellides of *M. murinus* and *M. paisii* are shorter (6.5–11 × 1.7–2.5 µm and 10–14 × 2–2.5 µm, respectively) borne mostly from the aerial mycelium and producing cylindrical and broadly ellipsoidal conidia, respectively.

### Microascus hyalinus (Malloch & Cain) Sandoval-Denis, Gené & Guarro, comb. nov. — MycoBank MB809210

Basionym. Kernia hyalina Malloch & Cain, Canad. J. Bot. 49: 860. 1971.

Specimen examined. USA, from cow dung, 1964, J.C. Krug (ex-type culture CBS 766.70).

Notes — This species has been isolated from soil and dung in Europe and North America (Malloch & Cain 1971, Guarro et al. 2012). The species was originally described in Kernia by Malloch & Cain (1971), although deviating considerably from the typical features of Kernia such as restricted growth, nonostiolate, hairy ascomata, and ellipsoidal to reniform, orange to copper coloured ascospores with a germ pore at each end (Malloch & Cain 1971, von Arx 1978). Although several species of Kernia have been described with a scopulariopsis-like asexual morph, our phylogenetic analysis based on a combined LSU and ITS sequence dataset (Fig. 1) showed Kernia to be phylogenetically distant to both Scopulariopsis and Microascus. However, K. hyalina is shown to have more affinity with species of Microascus rather than with species of Kernia nested within the Microascus lineage, a relationship previously suggested by Issakainen et al. (2003). The lack of ascomatal appendages, the production of hyaline to yellowish ascospores with a single germ pore, the shape and colour of the annellides and conidia, and the growth rate of the colonies point toward Microascus rather than toward Kernia. Therefore, our phylogenetic and morphological data confirm this taxon as a distinct species in Microascus.

# *Microascus intricatus* Sandoval-Denis, Stchigel & Deanna A. Sutton, *sp. nov.* — MycoBank MB809211, Fig. 8

Etymology. Referring to the textura intricata of the peridium.

Colonies on OA at 25 °C growing rather slowly, attaining 28–30 mm diam in 14 d, flat, finely granular, with scarce aerial mycelium, olive grey (2F2), with a white regular margin; reverse white to grey. On PCA at 25 °C colonies attaining 35–38 mm diam in 14 d, flat, velvety to finely granular, with a densely fasciculate centre, olive brown (4F3/4F4), with a white regular margin; reverse olive brown (4F3/4F4). *Vegetative hyphae* septate, subhyaline to light brown, smooth- and thin-walled, 2–2.5 µm wide. Ascomata immersed or superficial, globose to subglobose, 140–200 µm diam, with a papillate to short cylindrical ostiolar neck up to 40 µm long, black, glabrous; peridium with a *textura intricata*. Asci irregularly ellipsoidal or

subglobose,  $7.5-9.5 \times 5.5-6.5 \mu m$ . Ascospores fusiform,  $5-6 \times 2.5-3.5 \mu m$ , straw coloured, yellow-orange in mass, with one inconspicuous germ pore. Conidiophores absent or as a basal single cell, of  $2.5-3 \times 3-5 \mu m$ , bearing groups of 2-3 annellides, or slightly branched up to 50  $\mu m$  long, septate, subhyaline, smooth-walled. Annellides mostly sessile, single and lateral on vegetative hyphae, more or less ampulliform,  $8-10(-11) \times 2-2.5 \mu m$ , with a swollen base, tapering abruptly to a cylindrical annellated zone,  $1-1.5 \mu m$  wide, subhyaline, smooth-walled. Conidia globose to broadly ellipsoidal,  $4-5 \times 3-3.5 \mu m$ , with truncate base, pale brown, dark brown in mass, and smooth- to rough-walled, thin-walled, arranged in long chains.

Cardinal temperature for growth — Optimum 25–30 °C, maximum 40 °C, minimum 15 °C.

Specimens examined. ARGENTINA, Iguazú, from soil, *Calduch, Guarro* & *Stchigel* (FMR 12362). – USA, from bronchoalveolar lavage fluid, 2007, *D.A. Sutton* (holotype CBS H-21786, culture ex-type CBS 138128 = UTHSC 07-156 = FMR 12264).

Notes — *Microascus intricatus* is described on the basis of two strains, isolated from a clinical (human) sample in the USA and from soil, in Argentina. This species deviates from the other congeneric species in having a perithecial peridium wall with *textura intricata* and by forming short fusiform ascospores. Nonetheless, the abundant conidiation and ascomata with straw-coloured ascospores bearing a single germ pore match with the circumscription of *Microascus*, confirming our phylogenetic results.

# *Microascus longirostris* Zukal, Verh. Zool.-Bot. Ges. Wien 35: 33. 1885

= Microascus variabilis Massee & E.S. Salmon, Ann. Bot., Lond. 15: 349. 1901.

Specimens examined. JAPAN, Tokyo, from soil, 1962, S. Udagawa (CBS 415.64 = NBRC 7554). – USA, Maine, Kittery Point, from a wasp's nest, 1961, *R. Thaxter* (neotype designated here CBS H-14440, MBT198046) culture ex-neotype CBS 196.61 = MUCL 9058.

Notes — Microascus longirostris has been reported from many sources, mostly from dung of several mammals, soil, wood, seeds, air, as well from clinical samples in South and North America, Europe and Australia (Barron et al. 1961). The protologue of this species was made on the basis of ascomata on the natural substrata only (dog dung and rotten wood) (Zukal 1885, Barron et al. 1961). No ex-type strain or holotype material of this species was available. Microascus longirostris is the type of *Microascus* and, in order to stabilize the nomenclature, a neotype is here designated. Although none of the cultures studied here have the same geographical origin or host as the original specimen, the morphological characteristics of the two strains studied agree with the fungus described by Zukal in its original publication (Zukal 1885). The neotype culture selected here also corresponds with the modern descriptions of M. Iongirostris based on cultural characteristics given by Barron et al. (1961), Morton & Smith (1963) and von Arx et al. (1988), being also part of the material revised and considered as authentic by those authors.

# *Microascus macrosporus* (G.F. Orr) Sandoval-Denis, Gené & Guarro, *comb. & stat. nov.* — MycoBank MB809212

Basionym. Microascus trigonosporus C.W. Emmons & B.O. Dodge var. macrosporus G.F. Orr, Canad. J. Bot. 39: 1617. 1961.

Specimen examined. USA, California, from soil, 1971, G.F. Orr (CBS 662.71 = UAMH 9336).

Notes — This species was originally described from desert soil as a variety of *M. trigonosporus*. However, while *M. macro*-



**Fig. 9** *Microascus restrictus* CBS 138277. a, b. Colonies on OA and PCA, respectively, after 21 d at 25 °C; c, f–h. conidiophores, annellides and conidia; d, e. solitary conidia. — Scale bars: c, d = 10 µm; all others = 5 µm.

sporus has ascospores measuring  $5-6.5 \times 5.5-7.5 \mu m$ , those of *M. trigonosporus* are distinctly smaller ( $3-5 \times 3-4 \mu m$ ). *Microascus pyramidus* is another phylogenetically closely related and morphologically similar species. However, its ascospores have distinctly attenuated ends and its conidia are  $4.5-5.5 \times 3-4 \mu m$ . *Microascus macrosporus* produces ascospores with rounded ends and larger conidia ( $5-7 \times 4-5 \mu m$ ).

### *Microascus murinus* (Samson & Klopotek) Sandoval-Denis, Gené & Guarro, *comb. nov.* — MycoBank MB809218

Basionym. Scopulariopsis murina Samson & Klopotek, Arch. Mikrobiol. 85: 175. 1972.

Specimen examined. GERMANY, Giessen, from composed municipal waste, 1970, A. von Klopotek (ex-type culture CBS 830.70 = IHEM 18567).

Notes — This species was originally isolated from domestic waste in Germany. Although *M. murinus* shares morphological features with *M. chartarus*, *M. croci*, *M. paisii*, *M. restrictus* and *M. verrucosus*, it can be differentiated by having smaller cylindrical conidia, measuring  $4-6 \times 1.5-2 \mu m$  and slightly larger annellides, measuring  $6.5-11 \times 1.5-2.5 \mu m$ .

### *Microascus paisii* (Pollacci) Sandoval-Denis, Gené & Guarro, *comb. nov.* — MycoBank MB809213

Basionym. Torula paisii Pollacci (as 'pais'), Atti Ist. Bot. Univ. Pavia, ser. 2, 18: 130. 1921.

≡ Phaeoscopulariopsis paisii (Pollacci) M. Ota, Jap. J. Dermatol. Urol. 28: 5. 1928. nom. inval. (Seifert et al. 2011).

≡ *Scopulariopsis paisii* (Pollacci) Nannf., Repertorio sistematico dei miceti dell'uomo e degli animali 4: 259. 1934.

= Scopulariopsis sphaerospora Zach, Oesterr. Bot. Z. 83: 180. 1934.

= Scopulariopsis brumptii Salv.-Duval, Thèse Fac. Pharm. Paris. 23: 58. 1935.

= *Scopulariopsis versicolor* Salv.-Duval, Thèse Fac. Pharm. Paris. 23: 63. 1935.

= Masoniella grisea (G. Sm.) G. Sm., Trans. Brit. Mycol. Soc. 35: 237. 1952.

≡ Masonia grisea G. Sm., Trans. Brit. Mycol. Soc. 35: 149. 1952, nom. illeg.

= Scopulariopsis melanospora Udagawa, J. Agric. Sci. (Tokyo) 5: 18. 1959.

Specimens examined. AUSTRIA, from unknown substrate, 1934, F. Zach (S. sphaerospora ex-type culture MUCL 9045 = CBS 402.34). - GERMANY, Schleswig-Holstein, Kiel, Kitzeberg, from soil on a Triticum sativum field, 1966, W. Gams (MUCL 8989 = CBS 896.68); Schleswig-Holstein, Kiel, from soil, 1966, W. Gams (MUCL 8990); from soil on a wheat field, 1966, W. Gams (MUCL 8993 = CBS 897.68). - ITALY, from human, 1927, G. Pollacci (T. paisii ex-type culture MUCL 7915 = CBS 213.27). - UK, isolated as a culture contaminant, 1946, G. Smith (M. grisea ex-type culture MUCL 9003 = CBS 295.52). - USA, from milled Oriza sativa, 1955, S. Udagawa (S. melanospora ex-type culture MUCL 9040 = CBS 272.60); from bronchoalveolar lavage fluid, 2007, D.A. Sutton (UTHSC 07-639 = FMR 12263); from bronchoalveolar lavage fluid, 2008, D.A. Sutton (UTHSC 08-1734 = FMR 12248); from sputum, 2009, D.A. Sutton (UTHSC 09-457 = FMR 12241); from bronchoalveolar lavage fluid, 2009, D.A. Sutton (UTHSC 09-482 = FMR 12240); from sputum, 2009, D.A. Sutton (UTHSC 09-2391 = FMR 12229); from bronchoalveolar lavage fluid, 2010, D.A. Sutton (UTHSC 10-2920 = FMR 12215); from sputum, 2011, D.A. Sutton (UTHSC 11-708 = FMR 12210).

Notes — *Microascus paisii* has had a confusing nomenclatural history. As *Scopulariopsis paisii*, it was erroneously considered the asexual morph of *M. desmosporus* by Morton & Smith (1963). We have also observed discrepancies concerning *T. paisii* among fungal databases. In MycoBank, *T. paisii* is considered as the asexual morph of *M. cirrosus* whereas Index Fungorum lists *T. paisii* as a synonym of *Scytalidium thermophilum*. Our phylogenetic analysis showed that the ex-type of *T. paisii* (MUCL 7915) belongs to the *Microascus* lineage. Within this lineage, it belongs to a well-supported subclade together with the ex-type strains of *Masoniella grisea*, *S. melanospora* and *S. sphaerospora* and several reference strains of *S. brumptii*. This subclade might represent the rarely opportunist species *S. brumptii*. However, since there is no type material of *S. brumptii* available, and *T. paisii* being the oldest type strain included in this subclade, the latter name has preference according to the nomenclatural principle that the correct name is the oldest legitimate one (McNeill et al. 2012). Therefore, according to our data the new combination *Microascus paisii* should be adopted.

Regarding data pertaining to *S. brumptii*, *M. paisii* has a worldwide distribution, being isolated from multiple substrates, including air, decaying wood or soil and is an opportunistic pathogen of human and warm-blooded animals (Morton & Smith 1963, de Hoog et al. 2011, Sandoval-Denis et al. 2013). This species morphologically resembles *M. chartarus* and *M. croci*, but it can be differentiated by its dark grey or black colonies and its ability to grow and sporulate well at 37 °C.

# *Microascus pyramidus* G.L. Barron & J.C. Gilman, Canad. J. Bot. 39: 1618. 1961

Specimen examined. USA, from desert soil, 1957, G.L. Barron (ex-type culture CBS 212.65).

Notes — This species was originally isolated from desert soil in North America (Barron et al. 1961). Morphologically, it is similar to other *Microascus* species producing triangular ascospores as *M. alveolaris*, *M. campaniformis*, *M. macrosporus* and *M. trigonosporus*. However, ascospores of *M. pyramidus* are wider  $(5-6.5 \times 5.5-7 \mu m)$ , have attenuated ends and often acquire a nearly square shape (von Arx et al. 1988). The asexual morph of *M. pyramidus* is morphologically similar to those of *M. macrosporus* and *M. campaniformis*. *Microascus macrosporus* produces globose to ovoid conidia measuring  $5-7 \times 4-5 \mu m$ , while those of *M. pyramidus* are markedly narrower measuring  $4.5-5.5 \times 3-4 \mu m$ , and those of *M. campaniformis* are subglobose to broadly ellipsoidal measuring  $4-5 \times 2.5-3.5 \mu m$ .

# *Microascus restrictus* Sandoval-Denis, Gené & Deanna A. Sutton, *sp. nov.* — MycoBank MB809420, Fig. 9

*Etymology*. From the Latin *restringere*-, restrict, referring to the restricted growth of the colony.

Colonies on OA at 25 °C growing rather slowly, attaining 23-25 mm diam in 14 d, flat, downy, olive grey (3F2) to brown-grey (5F2), with an irregular margin; reverse brown-grey (5F2). On PCA at 25 °C growing restrictedly, attaining 3-5 mm diam after 14 d, membranous, lobulate, with an irregular undulate margin, olive brown (4E5) to brown (5E5); reverse brown-grey (5E2). Vegetative hyphae septate, subhyaline becoming dark brown with age, smooth- and thin-walled, 1.5-3 µm wide. Conidiophores absent or as a basal single cell of  $4-6 \times 3-5$ µm, bearing groups of 2-3 annellides, or slightly branched up to 20 µm long, subhyaline, smooth-walled. Annellides mostly sessile borne single and laterally on vegetative hyphae, ampulliform, 7–19  $\times$  2–4.5 µm, with a swollen base, tapering abruptly to a cylindrical and darker annellated zone 1.5-2 µm wide, subhyaline, becoming darker with age, smooth-walled. Conidia globose to obovoidal,  $4.5-6 \times 4-5.5 \mu m$ , with truncate base, dark brown, smooth or finely roughened, thick-walled, arranged in short chains. Solitary conidia sometimes present, borne laterally from vegetative hyphae, sessile or on short stalks, globose or obovate, 5-5.5 × 4.5-5 µm, dark brown, smooth- and thick-walled. Sexual morph not observed.

Cardinal temperature for growth — Optimum 25–30 °C, maximum 40 °C, minimum 15 °C.

Specimen examined. USA, from human left hallux, 2009, *D.A. Sutton* (holotype CBS H-21787, culture ex-type CBS 138277= UTHSC 09-2704 = FMR 12227).



**Fig. 10** *Microascus verrucosus* CBS 138278. a, b. Colonies on OA and PCA, respectively, after 21 d at 25 °C; c-g. conidiophores, annellides and conidia; h, i. solitary conidia. — Scale bars: c, h = 10  $\mu$ m; all others = 5  $\mu$ m.

Notes — *Microascus restrictus* is morphologically very similar to *M. verrucosus*. However, while *M. restrictus* shows larger smooth-walled annellides measuring 7–19 × 2–4.5 µm, smaller conidia ( $4.5-6 \times 4-5.5 \mu$ m) and is able to grow at 40 °C, *M. verrucosus* has annellides measuring 8–10 × 1–3 µm, typically warted when mature, larger conidia ( $5-7 \times 4.5-6 \mu$ m) and is unable to growth at 40 °C.

#### Microascus senegalensis Arx, Persoonia 8: 194. 1975

Specimen examined. SENEGAL, Joel, from mangrove soil, J.A. von Arx (ex-type culture IHEM 18561 = CBS 277.74).

Notes — This species has been reported from soil, seeds and plant debris as well as from human skin, in Africa, India and North America (von Arx et al. 1988). The most remarkable features of *M. senegalensis* are the presence of large reniform ascospores  $(7-9 \times 2-3 \mu m)$  with a single and often protuberant germ pore (von Arx et al. 1988, Guarro et al. 2012).

Microascus trigonosporus C.W. Emmons & B.O. Dodge, Mycologia 23: 317. 1931

≡ Scopulariopsis trigonospora C.W. Emmons & B.O. Dodge, Mycologia 23: 317. 1931.

?= Microascus trigonosporus C.W. Emmons & B.O. Dodge var. terreus Kamyschko, Novosti Sist. Nizsh. Rast. 76: 175. 1966.

?= Microascus trigonosporus C.W. Emmons & B.O. Dodge var. macroperithecia Sage, Steiman, Seigle-Mur. & Guiraud, Mycotaxon 55: 194. 1995, nom inval. Art. 40.5 (Melbourne).

Specimens examined. JAPAN, Burma, from milled rice, 1961, S. Udagawa (MUCL 9061 = CBS 199.61). – UK, from mushroom bed, 1935, *W.M. Ware* (MUCL 9841 = CBS 262.35). – USA, from unknown substrate, 1931, *C.W. Emmons* (*M. trigonosporus* var. *trigonosporus* ex-type culture CBS 218.31).

Notes — This is a cosmopolitan species, commonly reported from soil, seeds and dung (Barron et al. 1961). It is also considered as a human pathogen that has been associated with pneumonia in an immunocompromised patient (Mohammedi et al. 2004) and endocarditis (Wang et al. 2011). Among the species producing triangular-shaped ascospores (*M. alveolaris, M. campaniformis, M. macrosporus* and *M. pyramidus*), *M. trigonosporus* produces the smaller ones, measuring  $3-5 \times 3-4 \mu m$ .

*Microascus verrucosus* Sandoval-Denis, Gené & Cano, *sp. nov.* — MycoBank MB809421; Fig. 10

*Etymology.* From the Latin *verruca-*, wart, referring to the warted ornamentation of the annellides.

Colonies on OA at 25 °C growing slowly, attaining 19-22 mm diam in 14 d, flat, finely granular, olive grey (2–3F2), with an immersed and slightly undulated margin; reverse olive grey (1-3F2). On PCA at 25 °C growing restrictedly, attaining 1–5 mm diam in 14 d, adherent, membranous or slightly downy, hemispherical, cerebriform, with lobulated margin, olive (2E3/2E4); reverse olive (2E2) to olive grey (2E3). Vegetative hyphae septate, subhyaline becoming dark brown with age, smooth- to rough-walled, thin-walled, 1.5-2.5 µm wide. Conidiophores absent or as a basal single cell of  $4-5 \times 2.5-4 \mu m$ , bearing groups of 2–3 annellides, rarely slightly branched up to 25 µm long. Annellides mostly sessile and borne directly on vegetative hyphae, lageniform,  $8-10 \times 1-3 \mu m$ , constricted at the basal septum, followed by a slightly swollen portion and tapering to a more or less cylindrical annellated zone 1-1.5 µm wide, usually sparsely warted. Conidia globose to subglobose, 5-7 × 4.5-6 µm, often with an inconspicuous truncate base, dark brown, smooth or finely roughened, thick-walled, arranged in short chains. Solitary conidia sometimes present, borne laterally from vegetative hyphae, sessile or on short stalks, globose or broadly ellipsoidal,  $5-6 \times 4-5 \mu m$ , dark brown, smooth- and thick-walled. *Chlamydospores* not observed. *Sexual morph* not observed.

Cardinal temperature for growth — Optimum 25–30 °C, maximum 35 °C, minimum 15 °C.

Specimen examined. USA, from bronchoalveolar lavage fluid, 2010, *D.A. Sutton* (holotype CBS H-21788, culture ex-type CBS 138278 = UTHSC 10-2601 = FMR 12219).

Notes — *Microascus verrucosus* can be differentiated from *M. restrictus*, its closest morphological relative, by its sparsely warted annellides and its inability to grow at 40 °C. *Microascus verrucosus* is phylogenetically close to *M. murinus*; however, this latter species has cylindrical conidia with pointed apices.

# Pithoascus Arx, Proc. Kon. Ned. Akad. Wetensch. 76: 295. 1973

Type species. Pithoascus nidicola (Massee & E.S. Salmon) Arx.

*Colonies* restricted, white, becoming grey or darkening due to the production of ascomata; usually with scarce aerial mycelium. *Ascomata* perithecial, immersed or somewhat superficial, gregarious, often grouped on dense crusts, globose, glabrous, often with an inconspicuous ostiolar opening or with a short cylindrical ostiolar neck; peridium black, composed of thick-walled, slightly flattened cells, *textura angularis*. *Asci* unitunicate, 8-spored, broadly clavate or barrel-shaped, evanescent. *Ascospores* 1-celled, asymmetrical, navicular, fusiform or falcate, yellow, straw- or honey-coloured, dextrinoid when young, without germ pores. *Asexual morph* present in some species. *Conidiogenous cells* annellidic, borne singly and laterally on the vegetative hyphae, short, ampulliform, hyaline, smooth-walled. *Conidia* 1-celled, globose to pyriform, with a truncate base, smoothand thin-walled, solitary.

*Pithoascus ater* (Zach) Sandoval-Denis, Cano & Guarro, *comb. nov.* — MycoBank MB809214

Basionym. Scopulariopsis atra Zach, Oesterr. Bot. Z. 83: 184. 1934.

Specimen examined. From human nail, 1934, F. Zach (ex-type culture IHEM 18608 = CBS 400.34).

Notes — A single strain of this species is available. It was isolated from a human nail, but its pathogenic role was not clearly established (Zach 1934). *Pithoascus ater* is the only species of the genus for which a sexual morph is unknown and by contrast shows abundant conidial production. However, the ex-type strain of *P. ater* shows similar morphological characteristics to the known asexual morphs of *Pithoascus* species, such as *P. stoveri* and *P. intermedius*. The main distinctive feature of *P. ater* is the abundant production of solitary, globose and smooth-walled pale brown conidia measuring  $4-9 \times 4.5-8.5$  µm. In contrast, conidia of *P. stoveri* and *P. intermedius* are rarely seen in culture and, when present, are hyaline, obovate to pyriform ( $5-8 \times 3-4$  µm) or globose to subglobose ( $4-8 \times 4.5-7.5$  µm), respectively. Other species, i.e. *P. nidicola* and *P. exsertus* produce only sexual morphs in culture.

#### Pithoascus exsertus (Skou) Arx, Persoonia 7: 373. 1973

Basionym. Microascus exsertus Skou, Antonie van Leeuwenhoek 39: 533. 1973.

Specimens examined. DENMARK, Sjaelland, Bjerge Strand, from Osmia rufa, 1975, J.P. Skou (CBS 583.75); Tastrup, Hojbakkegard, Experimental Station, from *Megachile willoughbiella*, 1970, J.P. Skou (ex-type culture CBS 819.70).

Notes — This fungus is considered as an entomogenous species, which has been isolated from a leaf-cutting bee (*Mega*-

chile willughbiella) and from a Red Mason-bee (*Osmia rufa*), both in northern Europe. Morphologically, *P. exsertus* can be differentiated from the other species of the genus by its larger ascomata (210–450  $\mu$ m diam) and its long, falcate to nearly cylindrical and yellow ascospores,  $6.5-12 \times 1-2.5 \mu$ m.

*Pithoascus intermedius* (C.W. Emmons & B.O. Dodge) Arx, Proc. Kon. Ned. Akad. Wetensch. 76: 292. 1973

Basionym. Microascus intermedius C.W. Emmons & B.O. Dodge, Mycologia 23: 324. 1931.

Specimen examined. USA, North Carolina, Chadbourn, from decaying root of *Fragaria vesca*, 1932, *C.W. Emmons & B.O. Dodge* (ex-type culture CBS 217.32).

Notes — This species has been reported mainly from soil in North America, Europe and Asia, and also as a potential pathogenic species isolated from human hair and nails (von Arx et al. 1988, Guarro et al. 2012). *Pithoascus intermedius* is morphologically similar to *P. nidicola* and *P. stoveri*. However, *P. intermedius* can be identified by its small, fusiform ascospores,  $5-6 \times 2-2.5 \mu m$ .

### *Pithoascus nidicola* (Massee & E.S. Salmon) Arx, Proc. Kon. Ned. Akad. Wetensch. 76: 292. 1973

Basionym. Microascus nidicola Massee & E.S. Salmon, Ann. Bot., Lond. 15: 313. 1901.

Specimen examined. USA, Utah, from Dipodomys merriami, C.W. Emmons (ex-epitype culture CBS 197.61).

Notes - Pithoascus nidicola was originally isolated from a wasp's nest in England and later from soil samples in North America (Massee & Salmon 1901, Barron et al. 1961). The exepitype culture, however, was isolated from a kangaroo rat in the USA (von Arx 1973b, Abbott et al. 2002). This species is similar to P. stoveri; however, P. nidicola can be differentiated by having larger ascomata (90–160 µm diam) with thicker walls (6–10 µm) and navicular to nearly lunate straw coloured ascospores. In contrast, P. stoveri produces ascomata measuring 50-110 µm diam, with a wall 4-7 µm thick, and navicular, golden to brown coloured ascospores (von Arx 1973b, Abbot et al. 2002, Guarro et al. 2012). Although conidia had never been reported for P. nidicola, we observed the development of a reduced asexual morph on PCA forming globose to ampulliform hyaline conidia,  $4-5 \times 2.5-3.5 \mu m$ , borne on short conidiogenous cells (Fig. 2). These asexual structures resemble those of P. intermedius, but the conidia of the latter are globose to subglobose and larger  $(4-8 \times 4-7.5 \ \mu m)$ .

### Pithoascus stoveri Arx, Persoonia 7: 373. 1973

≡ Microascus stoveri (Arx) S.P. Abbott, Mycologia 94: 368. 2002.

Specimen examined. USA, Ohio, from root of Beta vulgaris, W.L. White (ex-type culture CBS 176.71).

Notes — This species was originally isolated from a root of sugar beet in the USA. *Pithoascus stoveri* is morphologically similar to *P. nidicola*; however, the former species forms an asexual morph in culture, has a smaller ascomata (50–110 µm diam) and navicular golden yellow to brown ascospores. *Pithoascus nidicola* produces ascomata 90–160 µm diam, and navicular to nearly lunate straw-coloured ascospores.

Pseudoscopulariopsis Sandoval-Denis, Gené & Guarro, gen. nov. — MycoBank MB809215

Type species. Pseudoscopulariopsis schumacheri (Curzi) Sandoval-Denis, Gené & Guarro. *Colonies* restricted, greyish, dark olive grey to olivaceous black; floccose with abundant submerged mycelium, often becoming crustose and dark. *Ascomata* black, globose or ovate, glabrous, with a short cylindrical ostiolar neck and a peridium of *textura epidermoidea*. *Asci* unitunicate, 8-spored, ovoid, evanescent. *Ascospores* 1-celled, asymmetrical, navicular to fusiform, sub-hyaline, pale yellow or brown, without germ pores. *Conidiogenous cells* short, annellidic, often with a swollen base, mostly borne on a short and swollen supporting cell forming short swollen conidiophores, rarely borne singly on aerial hyphae. *Conidia* 1-celled, subglobose, obovate to short clavate, with truncate base and rounded apex, smooth- and thin-walled, pale brown to brown-grey, arranged in short chains.

#### Pseudoscopulariopsis hibernica (A. Mangan) Sandoval-

Denis, Gené & Cano, comb. nov. — MycoBank MB809216

Basionym. Scopulariopsis hibernica A. Mangan, Trans. Brit. Mycol. Soc. 48: 617. 1965.

Specimen examined. IRELAND, from soil, *A. Mangan* (UAMH 2643 = ATCC 16690).

Notes — This is a species described from soil and only a few isolates are available, all of them derived from the same isolation source (Mangan 1965). The isolate studied here, although not the ex-type culture, was isolated and considered authentic by the same authors (Mangan 1965) and matched in all aspects with the protologue (Mangan 1965). Phylogenetically, *P. hibernica* clustered close to *P. schumacheri*, both species being characterised by short cylindrical annellides mostly formed in small groups on short and swollen supporting cells, commonly darkening with age. *Pseudoscopulariopsis hibernica* can be differentiated mainly by its lack of a sexual morph, the presence of shorter  $(9-15 \times 3-5 \ \mu m)$  and darker annellides, and its larger  $(5-7 \times 5-6 \ \mu m)$  subglobose conidia.

Pseudoscopulariopsis schumacheri (E.C. Hansen) Sandoval-Denis, Gené & Guarro, comb. nov. — MycoBank MB809549

Basionym. Sphaerella schumacheri E.C. Hansen, Vidensk. Meddel. Dansk Naturhist. Foren. Kjøbenhavn: 16. 1876.

≡ Rosellinia schumacheri (E.C. Hansen) Sacc., Syll. Fung. 1: 276. 1882.
≡ Microascus schumacheri (E.C. Hansen) Curzi, Boll. Staz. Patol. Veg.
Roma. N.S. 23: 8. 1931.

≡ *Pithoascus schumacheri* (E.C. Hansen) Arx, Proc. Kon. Ned. Akad. Wetensch. 76: 292. 1973.

= Melanospora stysanophora Mattir., Nuovo Giorn. Bot. Ital. 18: 121. 1886.

≡ Microascus stysanophorus (Mattir.) Curzi, Boll. Staz. Patol. Veg. Roma, N.S. 10: 391. 1930.

≡ *Microascus stysanophorus* (Mattir.) G.L. Barron, Cain & J.C. Gilman, Canad. J. Bot. 39: 1621. 1961.

≡ *Pithoascus stysanophorus* (Mattir.) Valmaseda, A.T. Martínez & Barrasa, Canad. J. Bot. 65: 1805. 1987.

Specimen examined. SPAIN, Puerto de la Quesera, from soil, 1986, A.T. Martínez (neotype designated here MA-Fungi 16319, MBT178643), culture ex-neotype CBS 435.86.

Notes — This species was originally described from dung of rodents, in Denmark, but no ex-type strain was preserved nor herbarium material listed in the protologue. The modern descriptions of the species by Barron et al. (1961), Valmaseda et al. (1986), von Arx et al. (1988) and Guarro et al. (2012) are based on the same isolate studied here; however, a type specimen has never been designated. We agree with the observations of all these authors in that morphological features of CBS 435.86 match with those of the protologue of the species (Hansen 1876). Therefore, due to the scant live material available and the inexistence of ex-type cultures, we have selected this strain as neotype in order to fix the application of the name. Although neither the substrate nor the geographic origin correspond to

that indicated in the protologue of the species, this strain clearly represents *P. schumacheri* according to concepts maintained by subsequent authors (Valmaseda et al. 1986, von Arx 1988, Guarro et al. 2012).

Morphologically, *P. schumacheri* resembles *Pithoascus* species by its navicular to fusiform ascospores lacking germ pores, thus being included in that genus by von Arx (1973a). Nevertheless, *P. schumacheri* can be differentiated by its restricted growth and the *textura epidermoidea* of the ascoma wall. Phylogenetically, *P. schumacheri* is related to *P. hibernica*; however, the former can be differentiated by the presence of ascomata and the production of obovate to short-clavate conidia, measuring  $4.5-6 \times 2.5-4 \mu m$ , on mostly hyaline annellides which measure  $3.5-22.5 \times 1.5-2.5 \mu m$ .

#### Scopulariopsis Bainier, Bull. Soc. Mycol. France 23: 98. 1907

?= Acaulium Sopp, Skr. Vidensk.-Selsk. Christiana, Math.-Naturvidensk. Kl. I, 11: 42. 1912.

= *Phaeoscopulariopsis* M. Ota, Jap. J. Dermatol. Urol. 28: 405. 1928, *nom. inval.* Art 34 (Seifert et al. 2011).

#### Type species. Scopulariopsis brevicaulis (Sacc.) Bainier.

Colonies spreading fast, velvety, funiculose or granular, varying from white and grey-white to several shades of buff, brown or dark brown, but never in shades of green or black. Ascomata perithecial, immersed or superficial, developing slowly, scattered; globose to subglobose or pyriform, glabrous, ostiolate, papillate or with a cylindrical neck; peridium black, composed of thick-walled, slightly flattened cells of textura angularis. Asci unitunicate, 8-spored, subglobose, irregularly ovoidal or ellipsoidal, evanescent. Ascospores 1-celled, asymmetrical, short, broadly reniform or lunate, dextrinoid when young, with or without an inconspicuous germ pore. Conidiogenous cells annellidic, borne on branched penicillate conidiophores, occasionally singly on vegetative hyphae or in groups of 2-3 on short stalks, cylindrical, often with a slightly swollen base followed by a cylindrical annellated portion that ends in a flat and wide conidiogenous opening, hyaline, smooth- or roughwalled. Conidia 1-celled, hyaline, avellaneous or brown, globose to ovate, with a rounded or pointed apex and a conspicuously protuberant and truncate base, smooth- or rough- and thickwalled, arranged in long basipetal dry chains.

# Scopulariopsis asperula (Sacc.) S. Hughes, Canad. J. Bot. 36: 803. 1958

Basionym. Torula asperula Sacc., Michelia 2: 560. 1882.

= Acaulium nigrum Sopp, Skr. Vidensk.-Selsk. Christiana, Math.-Naturvidensk. Kl. I 11: 47. 1912.

≡ Penicillium nigrum (Sopp) Biourge, Cellule 33: 1043. 1923.

≡ *Microascus niger* (Sopp) Curzi, Boll. Staz. Patol. Veg. Roma, N.S. 11: 8. 1931.

- = Scopulariopsis repens Bainier, Bull. Soc. Mycol. France 23: 125. 1907.
- ≡ *Penicillium repens* (Bainier) Biourge, Cellule 33: 225. 1923.

= Monilia arnoldii L. Mangin & Pat., Bull. Soc. Mycol. France 24: 164. 1908.

≡ *Scopulariopsis arnoldii* (L. Mangin & Pat.) Vuill., Bull. Soc. Mycol. France 27: 148. 1911.

= *Scopulariopsis ivorensis* H. Boucher, Bull. Soc. Pathol. Exot. 11: 312. 1918.

= Torula bestae Pollacci, Rivista Biol. 4: 317. 1922.

≡ Phaeoscopulariopsis bestae (Pollacci) M. Ota, Jap. J. Dermatol. Urol. 28: 405. 1928, nom. inval. (Seifert et al. 2011).

≡ Scopulariopsis bestae (Pollacci) Nannf., Repertorio sistematico dei miceti dell'uomo e degli animale 4: 254. 1934.

= Scopulariopsis fusca Zach, Oesterr. Bot. Z. 83: 174. 1934.

= Acaulium nigrum Sopp var. glabrum Salv.-Duval, Thèse Fac. Pharm. Paris. 23: 55. 1935.

= Scopulariopsis roseola N. Inagaki, Trans. Mycol. Soc. Japan 4: 1. 1962.

Specimens examined. AUSTRIA, from a carcass of rabbit, 1934, *F. Zach* (*Scopulariopsis fusca* ex-type culture MUCL 9032 = CBS 401.34). – CANADA, Alberta, Girouxville, from indoor air ex RCS strip, *Apis mellifera* overwintering facility, Jan. 1994, *S.P. Abbott* (MUCL 40729 = UAMH 7879); Alberta, 10 km south of Leduc, from dung of *Mephitis mephitis*, June 1997, *S.P. Abbott* (MUCL 40746 = UAMH 9029). – GERMANY, from compost soil, 1958, *K.H. Domsch* (CBS 853.68). – ITALY, from human, 1938, *G. Pollacci* (*Torula bestae* ex-type culture MUCL 9012 = CBS 289.38). – USA, from toenail, 2010, *D.A. Sutton* (UTHSC 10-3405 = FMR 12212).

Notes — This species has been mostly recovered from environmental samples such as soil, air, mouldy indoor environments, food such as cheese and butter, as well as from human clinical specimens, mainly skin and nails (Ropars et al. 2012, Sandoval-Denis et al. 2013).

Torula bestae was considered by Morton & Smith (1963) to be conspecific with S. koningii. However, although both species show smooth conidia, T. bestae typically exhibits darker fuscous-black colonies and conidia. Later, Abbott & Sigler (2001), using mating experiments, established that S. arnoldii, S. asperula, S. bestae, S. fusca and S. roseola were all synonyms of the heterothallic species Microascus niger. The same authors also selected neotype and epitype cultures for M. niger (UAMH 9489) and S. asperula (UAMH 9037), respectively, which unfortunately were not available for our study. However, all the reference strains studied here were genetically related with the type cultures of S. fusca and S. bestae, both species regarded as synonyms of S. asperula. Recently, Ropars et al. (2012) confirmed the synonymy of these species using a multilocus analysis based on D1/D2, TUB and EF-1α sequences which results are confirmed by our phylogenetic analysis. Morphologically, S. asperula is close to S. brevicaulis and S. flava; however, S. asperula can be differentiated by having dark brown to fuscous or violaceus colonies, and globose to ovate, coarsely verrucose or smooth walled, fuscous to sepia coloured conidia, mostly with a pointed apex and measuring  $5-8 \times 4-6.5 \mu m$ . In contrast, S. brevicaulis shows tan colonies, globose and vertucose pale brown conidia,  $6-9 \times 5.5-9 \mu m$ , while S. flava exhibits white colonies and obovoidal, verrucose and hyaline conidia,  $6-9.5 \times 5-8.5 \mu m$ .

#### Scopulariopsis brevicaulis (Sacc.) Bainier, Bull. Soc. Mycol. France 23: 99. 1907

Basionym. Penicillium brevicaule Sacc., Fungi Ital. 893: 1881.

= Monilia penicillioides Delacr., Bull. Soc. Mycol. France 13: 114. 1897.
 ≡ Penicillium penicillioides (Delacr.) Vuill., Bull. Soc. Mycol. France 27:

75. 1911. ≡ Scopulariopsis penicillioides (Delacr.) Smith & Ramsb., Trans. Brit.

Mycol. Soc. 5: 164. 1915.

= Monilia koningii Oudem., Arch. Neerl. Sci., sér. 2: 287. 1902.

≡ Scopulariopsis koningii (Oudem.) Vuill., Bull. Soc. Mycol. France 27: 143. 1911.

= Penicillium coccophilum Sacc., Ann. Mycol. Berl. 5: 178. 1907.

= Scopulariopsis rufulus Bainier, Bull. Soc. Mycol. France 23: 105. 1907.

■ Penicillium rufulum (Bainier) Sacc., Syll. Fung. 22: 1275. 1913.

Penicillium brevicaule Sacc. var. hominis Brumpt & Langeron in Brumpt,
 E. Précis de parasitologie, Ed. 1: 838. 1910.

≡ Scopulariopsis brevicaulis (Sacc.) Bainier var. hominis (Brumpt & Langeron) Brumpt & Langeron in Brumpt, E. Précis de parasitologie, Ed 2: 902, 1913.

= Scopulariopsis hominis (Brumpt & Langeron) Sartory, Champ. Parasit. Fasc. 8: 612. 1922.

= Acaulium insectivorum Sopp, Skr. Vidensk.-Selsk. Christiana, Math.-Naturvidensk. Kl. I, 11: 60. 1912.

≡ Penicillium insectivorum (Sopp) Biourge, Cellule 33: 103. 1923.

≡ Scopulariopsis insectivora (Sopp) Thom, The Penicillia: 532. 1930.

= Acaulium anomalum Sopp, Skr. Vidensk.-Selsk. Christiana, Math.-Naturvidensk. Kl. I, 11: 65. 1912.

= *Penicillium brevicaule* Sacc.var. *intermedium* Cagnetto, Sperimentale 67, Suppl. to Fasc. 4: 210. 1913.

Sporotrichum stercorarium Ehrenb., Jahrb. Gewächsk. 1: 178. 1818.
 Scopulariopsis stercoraria (Ehrenb.) S. Hughes, Canad. J. Bot. 36: 803, 1958.



Fig. 11 Scopulariopsis cordiae CBS 138129. a, b. Colonies on OA and PCA, respectively, after 21 d at 25 °C; c. ascoma; d. peridium; e, f. asci and ascospores; g-i. conidiophores, annellides and conidia; j, k. conidial chains. — Scale bars:  $c = 50 \mu m$ ; all others = 5  $\mu m$ .

- = Scopulariopsis alboflavescens Zach, Oesterr. Bot. Z. 83: 177. 1934.
- = Microascus brevicaulis S.P. Abbott. Mycologia 90: 298. 1998.

Specimens examined. AUSTRIA, from human diseased skin, 1934, F. Zach (S. alboflavescens ex-type culture CBS 339.34). - BELGIUM, Heverlee, from soil, J. Meyer (as S. stercoraria MUCL 14213). - CANADA, Alberta, from indoor air, March 1994, S.P. Abbott (M. brevicaulis ex-type culture UAMH 7770 = MUCL 40726). - THE NETHERLANDS, Wageningen, from pupa of Pteronus pini, 1935, J. Rozsypal (as S. insectivora CBS 335.35 = MUCL 9035). - Unknown geographical origin, Elephant, 1951, I.M. Scott (as S. koningii CBS 208.61). - USA, from hair, 2006, D.A. Sutton (UTHSC 06-277 = FMR 12273); from toenail, 2006, D.A. Sutton (UTHSC 06-619 = FMR 12271); from toenail, 2007, D.A. Sutton (UTHSC 07-1812 = FMR 12257); from human spine, 2007, D.A. Sutton (UTHSC 07-1888 = FMR 12255); from maxillary sinus, 2008, D.A. Sutton (UTHSC 08-1920 = FMR 12247); from toe, 2009, D.A. Sutton (UTHSC 09-1092 = FMR 12236); from sputum, 2009, D.A. Sutton (UTHSC 09-1373 = FMR 12233); from sputum, 2011, D.A. Sutton (UTHSC 11-427 = FMR 12211); from lung mass, 2011, D.A. Sutton (UTHSC 11-1240 = FMR 12206); from bronchoalveolar lavage fluid, 2011, D.A. Sutton (UTHSC 11-1563 = FMR 12204).

Notes — This is a species with a worldwide distribution. It has been isolated from a wide range of substrates and locations and also recognised as an important human opportunistic pathogen (de Hoog et al. 2011, Sandoval-Denis et al. 2013). The history of this taxon was reviewed by Morton & Smith (1963). These authors synonymised S. insectivora and S. brevicaulis, but regarded S. stercoraria and S. koningii as different species since the latter two taxa exhibited smooth conidia. Later, Abbott & Sigler (2001) based on mating studies demonstrated that S. brevicaulis and S. koningii were conspecific. The present data confirmed the synonymy of the four mentioned species. In contrast, our results showed that the ex-type strain of S. alboflavescens (CBS 339.34), a species currently considered a synonym of S. candida, is conspecific with S. brevicaulis. Scopulariopsis alboflavescens was described as having smooth conidia and colonies at first white becoming pale yellowish, features that distinguished it from S. brevicaulis (Zach 1934). However, our morphological study of the ex-type strain of S. alboflavescens revealed that despite the fact that it forms whitish to pale yellow colonies, it also produces some finely roughened conidia. Ropars et al. (2012) already suggested a relationship between the two species using a phylogenetic analysis based in LSU, TUB and EF-1α sequences, showing that the ex-type strain of S. alboflavescens nested in a clade closely related to S. brevicaulis and far from the S. candida clade. However, those authors concluded that S. candida was a polyphyletic species. Abbott & Sigler (2001) reported the formation of fertile ascomata when crossing the ex-type strain of S. alboflavescens (CBS 339.34) with several strains of S. candida and the ex-types of S. candida (MUCL 40743) and Nephrospora manginii (basionym of M. manginii CBS 170.27), thus supporting the synonymy of S. alboflavescens and S. candida already proposed by Morton & Smith (1963). However, considering that strains of M. manginii (CBS 170.27 and MUCL 12598), the ex-epitype of S. candida (MUCL 40743) and S. alboflavescens (CBS 399.34) were all self-fertile in our study, the phylogenetic results and the placement of S. alboflavescens as a synonym of S. brevicaulis, including strains showing smooth conidia and whitish to pale yellow colonies, is supported.

### Scopulariopsis candida (Guég.) Vuill., Bull. Soc. Mycol. France 27: 143. 1911

Basionym. Monilia candida Guég., Bull. Soc. Mycol. France 15: 271. 1899.

= Nephrospora manginii Loubière, Compt. Rend. Hebd. Séances Acad. Sci. 177: 209. 1923.

≡ Microascus manginii (Loubière) Curzi, Boll. Staz. Patol. Veg. Roma 2: 60. 1931.

= *Monilia candida* auct. non Pers.: Loubière in Compt. Rend. Hebd. Séances Acad. Sci. 177: 209. 1923.

= Chrysosporium keratinophilum (Frey) Carmich. var. denticola C. Moreau, Mycopathol. Mycol. Appl. 37: 37. 1969.

≡ Basipetospora denticola (C. Moreau) C. Moreau, Bull. Soc. Mycol. France 87: 43, 1971.

Specimens examined. CANADA, British Columbia, Chilliwak, from indoor air, March 1997, S.P. Abbott (S. candida ex-type culture MUCL 40743 = UAMH 9004). – FRANCE, from unknown origin, 1927, *L. Mangin (N. manginii* ex-type culture CBS 170.27); from unknown substrate, 1927, *L. Mangin (as S. candelabrum* MUCL 9026 = CBS 205.27); from cheese 'tome de Savoie', Aug. 1998, *C. Decock* (as *M. manginii* MUCL 41467). – Unknown origin, 1966 (as *S. alboflavescens* MUCL 9007). – USA, from sputum, 2009, *D.A. Sutton* (UTHSC 09-2576 = FMR 12228); from scalp, 2009, *D.A. Sutton* (UTHSC 09-3241 = FMR 12226).

Notes — This species has been reported from environmental samples (air, dust and soil) generally from the Northern Hemisphere, especially in Europe and North America; and also from clinical samples, mainly from superficial tissue of humans and animals (de Hoog et al. 2011). It is morphologically close to S. brevicaulis, S. asperula and S. flava. However, S. candida has subglobose to broadly ovate, hyaline, smooth-walled conidia and white colonies. In contrast, S. brevicaulis and S. asperula produce tan or fuscous brown colonies, respectively, while S. flava produces white colonies and obovoidal rough-walled conidia. When the sexual morph is present, it is characterised by globose perithecia, 100-170 µm diam, and reniform to heartshaped ascospores which are somewhat wider  $(4-6 \times 5-6 \mu m)$ than those of its closest relatives such as S. brevicaulis (5-6 × 3.5–4.5 µm), S. cordiae (4.5–5.5 × 3.5–4 µm) and S. soppii  $(6-7 \times 2.5-3 \ \mu m)$ .

# Scopulariopsis cordiae Sandoval-Denis, Gené & Cano, sp. nov. — MycoBank MB809217, Fig. 11

*Etymology.* From the Latin *cordiae-*, heart, referring to the heart-shaped ascospores.

Colonies on OA and PCA at 25 °C attaining 35-36 and 48-50 mm diam, respectively, after 14 d, flat, with scarce aerial mycelium, white to light grey (4B1) and granular due to the abundant production of ascomata, regular margin with abundant submerged mycelium; reverse whitish. Vegetative hyphae septate, hyaline, smooth- and thin-walled, 1.5-3.5 µm wide. Ascomata abundant, superficial or immersed, globose or subglobose, 100-150 µm diam, with a long cylindrical ostiolar neck up to 390 µm, black, glabrous; peridium with a textura angularis. Asci irregularly ellipsoidal,  $9-15.5 \times 7.5-10 \mu m$ . Ascospores broadly lunate to reniform,  $4.5-5.5 \times 3.5-4 \mu m$ , straw coloured, bright yellow in mass, with a single and inconspicuous germ pore. Conidiophores absent. Annellides sessile, borne single and laterally on vegetative hyphae, hyaline, smooth and thinwalled, cylindrical,  $8-15 \times 1.5-3.5 \mu m$ , tapering gradually to a cylindrical annellated zone 1-1.5 µm wide. Conidia broadly ellipsoidal to obovoidal,  $2.5-6 \times 2-5 \mu m$ , with truncate base, hyaline, white in mass, smooth- and thick-walled, arranged in chains. Chlamydospores and solitary conidia not observed.

Cardinal temperature for growth — Optimum 25–30 °C, maximum 40 °C, minimum 15 °C.

Specimens examined. USA, from a human J.P Drain, 2005, *D.A. Sutton* (UTHSC 05-3453 = FMR 12349); from human finger, 2009, *D.A. Sutton* (holotype CBS H-21789, culture ex-type CBS 138129 = UTHSC 09-866 = FMR 12338).

Notes — *Scopulariopsis cordiae* morphologically resembles the sexual morph of *S. candida* in the shape and size of the ascomata, asci and ascospores. *Scopulariopsis cordiae* can be differentiated by its faster growth rate, the sparkled appearance of the colonies, the presence of long cylindrical necks in numerous submerged ascomata, and the slightly reduced size and shape of its ascospores and conidia.

### Scopulariopsis flava (Sopp) F.J. Morton & G. Sm., Mycol. Pap. 86: 43. 1963

Basionym. Acaulium flavum Sopp, Skr. Vidensk.-Selsk. Christiana, Math.-Naturvidensk. Kl. I 11: 53. 1912.

= *Penicillium brevicaule* Sacc. var. *album* Thom, Bull. U.S. Bur. Anim. Ind. 118: 47. 1910.

≡ Scopulariopsis brevicaulis (Sacc.) Bainier var. alba (Thom) Thom, The Penicillia: 520. 1930.

- = Scopulariopsis aurea Sartory, Champ. Parasit. Fasc. 9: 650. 1922.
- = Scopulariopsis casei Loubière, Thèse Fac. Sci. Paris, Sér. 4: 62. 1924.
- = Scopulariopsis grylli Sartory, Ann. Mycol., Berl., 30: 469. 1932.

Specimen examined. UK, from cheese, 1948, *G. Smith* (neotype designated here CBS H-21939, MBT198047) culture ex-neotype CBS 207.61 = MUCL 9031).

Notes — This species is commonly isolated from cheese and soil in Europe and North America (Ropars et al. 2012). However, Sopp's original description of *A. flavum* is based on an isolate obtained from an insect larva. There are also reports of this species as human opportunistic pathogen (de Hoog et al. 2011). *Scopulariopsis flava* is morphologically close to *S. brevicaulis*. However, while *S. brevicaulis* produces tan, powdery to granular colonies, and globose to ovoid conidia with rounded or pointed apices, becoming verrucose and pale brown when mature, *S. flava* produces white floccose to fasciculate colonies, and hyaline globose to obovoidal conidia, with rounded apices and a coarsely roughened wall.

We only could study a single strain of S. flava (MUCL 9031), which is morphologically identical to the asexual morph of A. flavum and fits with the modern concept of S. flava by Morton & Smith (1963). Abbott et al. (2002) considered this strain a probable poorly pigmented variant of S. brevicaulis. However, our analyses show that MUCL 9031 is phylogenetically and morphologically distant from S. brevicaulis. Considering that Morton & Smith (1963) regarded MUCL 9031 as an authentic strain of S. brevicaulis var. alba, the epithet alba would take priority over the younger one flava, however the former epithet was already used in Scopulariopsis (S. alba, currently Doratomyces albus according to Dominik & Majchrowicz (1970)). Thus to avoid nomenclatural confusion we prefer to maintain the epithet flava for the present species. Phylogenetically S. flava is related to S. soppii, but the latter differs morphologically in producing larger conidia  $(5.5-9 \times 5-8 \mu m vs 6.5-7 \times 5.5-6.5)$ µm in S. flava), and falcate to lunate ascospores measuring 6-7  $\times 2.5-3 \mu m$ . It is noteworthy that a short description of a sexual morph was included in the protologue of A. flavum having 'ovalround' ascospores measuring 6-7 µm (Sopp 1912). However, we were not able to induce the production of ascomata in the above-mentioned strain and according to Abbott et al. (2002) no sexual morph has been reported since the original description of the species (Sopp 1912).

### Scopulariopsis soppii S.P. Abbott, Mycologia 94: 364. 2002

≡ Microascus soppii S.P. Abbott, Mycologia 94: 364. 2002.

Specimen examined. CANADA, Alberta, Elk Island National Park, from dry, rotten wood of *Populus tremuloides*, *T. Lumley* (ex-type culture UAMH 9169).

Notes — This species has been isolated from decayed wood and sandy loam (Abbott et al. 2002). It is phylogenetically and morphologically close to *S. flava* from which it can be differentiated based on the size of the conidia and the size and shape of its ascospores (see *S. flava*).

### **IDENTIFICATION KEYS**

According to the morphological features, identification keys were constructed for the different genera including all the phylogenetic species recognised in this study.

### Key to Microascus, Scopulariopsis and allied genera

- Asexual morph always present, conidiophores often branched up to 80 µm long; ascospores with a germ pore . . . .

3. Ascomata peridium of *textura angularis*; asexual morph when present forming short, hyaline and single annel-lides ...... *Pithoascus* 

3. Ascomata peridium of *textura epidermoidea*; asexual morph usually abundant, forming long annellides from short swollen conidiophores, darkening with time .....

..... Pseudoscopulariopsis

#### Key to Microascus species

1. 1.	Ascomata present in culture
2. 2.	Growth at 40 °C
3. 3.	Peridium with <i>textura angularis</i>
4. 4.	Ascospores always triangular or quadrangular 5 Ascospores reniform to broadly lunate, rarely triangular . 8
5. 5.	Ascospores with rounded ends
6. 6.	$ \begin{array}{llllllllllllllllllllllllllllllllllll$
7. 7.	Ascospores $4-6 \times 3-5 \mu m$ , yellow in mass <i>M. alveolaris</i> Ascospores $6-7 \times 4-4.5 \mu m$ elongated to one side, yellow to orange in mass <i>M. campaniformis</i>
8. 8.	Colonies dull to olive-green; ascospores lunate $4.5-6.5 \times 2-4 \mu m$ ; conidiophores irregularly branched <i>M. gracilis</i> Colonies light to brown-grey; ascospores reniform to broadly lunate $4-6 \times 2.5-4 \mu m$ ; conidiophores usually simple
9. 9.	Ascomata up to 300 $\mu$ m diam; ascospores pale red-brown in mass; conidia 2–3 $\mu$ m wide
10. 10.	Ascospores triangular $4-5 \times 3-4 \ \mu m \dots M$ . trigonosporus Ascospores otherwise
11. 11.	Ascospores reniform
12. 12.	Ascospores $7-9 \times 2-3 \mu m$ , straw coloured, yellow in mass, with a protuberant germ pore <i>M. senegalensis</i> Ascospores $3-4 \times 2-3.5 \mu m$ , hyaline to subhyaline with an indistinct germ pore <i>M. longirostris</i>
13.	As cospores ellipsoidal $5-7 \times 2-3 \mu m$ , light brown to brown in mass <i>M. brunneosporus</i>
13.	Ascospores broadly ovoid $3.5-5 \times 2-3.5 \mu m$ , pale yellow in mass <i>M. hyalinus</i>
14. 14.	Growth at 35 °C
15. 15.	Colonies fast growing (> 60 mm in 14 d); conidia bullet- shaped to broadly clavate, finely roughened $4-8 \times 2.5-3.5$ µm <i>M. expansus</i> Colonies growing restrictedly: conidia otherwise 16

- Growth at 40 °C; conidia globose to obovoidal 4.5–6 × 4– 5.5 μm, thick- and rough-walled ..... *M. restrictus*

- 18. Conidia cylindrical,  $4-6 \times 1.5-2 \mu m$ , usually with a pointed apex..... *M. murinus*
- 19. Conidia globose, 3.5–5 × 3–4 μm, brown coloured; slow growth at 5 °C ..... *M. croci*
- 19. Conidia ovate, usually with a pointed apex, 4–5.5 × 3–4 μm, green-brown; no growth at 5 °C . . . . . *M. chartarus*

### Key to Pithoascus species

- Colonies black, velvety or powdery; sexual morph not formed in culture; asexual morph abundant showing conidia globose to pyriform, 4–9 × 4.5–8.5 μm ...... P. ater
- 1. Colonies light grey to black, becoming crustose by the formation of ascomata; asexual morph scarce or absent
- 2. Ascomata 210–450 µm diam; ascospores falcate with attenuated ends, up to 12 µm long ..... *P. exsertus*
- 3. Ascomata 50–110 μm diam; ascospores navicular, 6–7.5 × 2–3 μm, golden yellow to brown coloured . . . *P. stoveri*
- 3. Ascomata 90–160  $\mu m$  diam; ascospores fusiform  $\ldots$  . 4
- Ascospores fusiform, nearly lunate 6–8 μm long, subhyaline to straw coloured; asexual morph when present forming globose to ampulliform hyaline conidia 4–5 × 2.5–3.5 μm on short stalks.

#### Key to Pseudoscopulariopsis species

- 1. Colonies grey-white; conidia obovate or short clavate,  $4.5-6 \times 2.5-4 \mu m$ ; sexual morph present in culture, with pale brown fusiform or navicular ascospores, measuring  $8-13 \times 2.5-4 \mu m$ .....*P. schumacheri*
- 1. Colonies olive-grey; conidia subglobose 5–7 × 5–6 μm; sexual morph absent ..... *P. hibernica*

#### Key to Scopulariopsis species

- 1. Colonies white, tending to light grey when ascomata are present ...... 2 1. Colonies tan, pale brown to fuscous brown . . . . . . . 5 3. Conidiophores abundant; conidia 6-9.5 × 5-8.5 µm; ascospores when present hyaline, heart shaped . S. candida 3. Conidiophores scarce; conidia 2.5-6 × 2-5 µm; ascospores straw coloured, reniform to broadly lunate..... 4. Conidia pale yellow in mass, globose to subglobose, 5.5-9  $\times$  5–8 µm; sexual morph present; ascospores falcate to lunate..... S. soppii 4. Conidia white in mass, globose to obovoidal,  $6.5-7 \times$ 5.5–6.5 µm; sexual morph not observed ..... S. flava 5. Conidia brown in mass, verrucose at maturity, globose to
- Conidia brown in mass, verrucose at maturity, globose to ovoid; 6–9 × 5.5–9 µm . . . . . . . . . . . S. brevicaulis

5. Conidia dark brown to fuscous, smooth or verrucose, globose to ovate usually with a pointed apex,  $5-8 \times 4-6.5$  µm . . . . . . . . . . . S. asperula

### **EXCLUDED OR DOUBTFUL SPECIES**

- *Microascus albonigrescens* (Sopp) Curzi, Boll. Staz. Patol. Veg. Roma 11: 60. 1931
- Specimen examined. JAPAN, Hokkaido, from litter, treated with urea, 1967, S. Udagawa (IHEM 18560 = CBS 109.69).

Notes — Our phylogenetic study demonstrates that this taxon does not belong to *Microascus* s.str. It nested in a clade related to the genera *Cephalotrichum*, *Gamsia* and *Wardomyces*, and might represent the former genus *Acaulium*, which was typified by Sopp (1912) with *Scopulariopsis albonigrescens* (sexual morph *M. albonigrescens*). Given the absence of type material, however, we prefer not to introduce any taxonomic changes until a more accurate study of the available reference material can be carried out.

# *Microascus caviariformis* Malloch & Hubart, Canad. J. Bot. 65: 2384. 1987

Specimen examined. BELGIUM, Prov. de Liège, Flemalle, Cave de Ramioul, from decaying meat, 1985, *J.M. Hubart* (ex-type culture CBS 536.87).

Notes — As in the case of *M. albonigrescens*, our phylogenetic data revealed that this taxon shows affinity with members of *Cephalotrichum*, *Gamsia* and *Wardomyces* rather than *Microascus*. It could represent another species of the former genus *Acaulium*.

# *Microascus decorticatus* C. Ram, Nova Hedwigia 21: 226. 1972

Notes — The original description stated a close morphological relationship of this species with *M. cinereus* and *M. gracilis*. *Microascus decorticatus* was described having slightly larger ascomata, while the asci and ascospores are nearly identical in size and shape to those of *M. cinereus*. Because the extype culture (IMUFPe 2194) was not available for study, the taxonomy of this species remains unclear.

# *Microascus desmosporus* (Lechmere) Curzi, Boll. Staz. Patol. Veg. Roma 11: 60. 1931

≡ Peristomium desmosporum Lechmere, Compt. Rend. Hebd. Séances Acad. Sci. 155: 178. 1912.

?= Peristomium desmosporum Lechmere var. verticillium Lechmere, Bull. Trimestriel Soc. Mycol. France: 178. 1913.

*= Microascus desmosporus* (Lechmere) Curzi var. *macroperithecia* Sage, Steiman, Seigle-Mur. & Guiraud, Mycotaxon 55: 191. 1995, *nom inval.* Art. 40.5 (Melbourne).

Notes — This species has a controversial taxonomy. Barron et al. (1961) recognize *M. desmosporus* as a species distinct from *M. cirrosus* while Morton & Smith (1963) regarded both species as conspecific. However, the ex-type strain of *M. cirrosus* was not examined by the latter authors. Von Arx (1975) and von Arx et al. (1988) regarded *M. desmosporus* as a doubtful species considering that *Peristomium desmosporum* and *M. desmosporus* are based on two different fungi. In the absence of type cultures the taxonomy of these fungi remains unknown.

### *Microascus dimonatus* Sage, Steiman, Seigle-Mur. & Guiraud, Mycotaxon 55: 195. 1995

Notes — This name is currently considered as invalid in fungal databases (Index Fungorum, MycoBank) following the

Art. 40.5 of the International Code of Nomenclature for algae, fungi, and plants (Melbourne Code). As stated in the original description, an ex-type culture of this fungus exists (CMPG 1274); however, it was not available for examination. Therefore, according to Guarro et al. (2012) further studies are necessary to clarify the taxonomy of this species.

#### Microascus giganteus Malloch, Mycologia 62: 731. 1970

Specimen examined. CANADA, Ontario, from insect frass in dead log, 1968, D.W. Malloch (ex-type culture CBS 746.69 = UAMH 9425).

Notes — This is a coprophilous species, originally isolated from insect dung (Malloch 1970), which resembles Microascus species in many morphological characteristics, particularly in those of the sexual state. However, it shows large (up to 750 µm) and hairy ascomata, the asci are formed irregularly at the centre of the perithecia and it has a Wardomyces asexual morph. The genus Wardomyces, typified by W. anomalus, is characterised by having swollen conidiogenous cells forming conidia in lateral or basipetal succession, the conidia are subglobose, ovoid or ellipsoid, brown to blackish and present a single longitudinal germ-slit (Brooks & Hansford 1923, Hennerbert 1962). According to our combined LSU and ITS sequence analysis (Fig. 1), M. giganteus is closely related to W. inflatus, thus the morphological features and phylogenetic evidence seem to demonstrate that M. giganteus is a Wardomyces species. However, sequence comparison with the type species of Wardomyces is required for taxonomical changes.

#### *Microascus inopinatus* Udagawa & Furuya, Mycotaxon 7: 91. 1978

= Wardomycopsis inopinata Udagawa & Furuya, Mycotaxon 7: 92. 1978.

Specimen examined. MYANMAR, from soil, 2008, C. Hartung (FMR 10305).

Notes — This taxon was described as unusual among the genus *Microascus* since its asexual morph exhibits annellated conidiogenous cells producing short catenate and globose conidia with a prominent longitudinal germ-slit. Thus, the genus *Wardomycopsis* was established to accommodate its asexual state. Although the ex-type culture was not available, the reference strain included in our study fits clearly with the protologue of the species. The phylogenetic analysis of the LSU and ITS sequences showed that this taxon was located far from the *Microascus* clade forming a highly supported clade with the ex-type strain of *Wardomycopsis humicola* (basionym *Scopulariopsis humicola* CBS 487.66).

*Microascus microcordiformis* Matsush., Matsush. Mycol. Mem. 9: 16. 1996

Notes — The original description correlates with a *Micro-ascus* species; being morphologically close to *M. longirostris*. However, in absence of live type material, the status of this name remains unknown.

# *Microascus pilosus* Valldos. & Guarro, Nova Hedwigia 57: 123. 1993

Specimen examined. SPAIN, Burgos, from rabbit dung, 1986, *M. Hernandez* (ex-isotype FMR 2604).

Notes — No live ex-type material is available for phylogenetic analyses. An isotype specimen (FMR 2604) is preserved at the Universitat Rovira i Virgili. The morphological examination of this specimen corresponded with the original description showing that this species clearly belongs to *Microascus* (Guarro et al. 2012). *Microascus singularis* (Sacc.) Malloch & Cain, Canad. J. Bot. 49: 859. 1971

Basionym. Fairmania singularis Sacc., Ann. Mycol. 4: 276. 1906. = Microascus doguetii Moreau, Rev. Mycol. 18: 177.1953.

Specimen examined. JAPAN, Tokyo, laboratory contaminant, 1962, S. Udagawa (CBS 414.64).

Notes — The morphological features of the asexual morph, with conidia showing longitudinal bands, and the analysis of the LSU and ITS sequences of a reference isolate (CBS 414.64) showed that this taxon formed a phylogenetic lineage related to *Wardomyces* and *Wardomycopsis*. However, given the absence of an ex-type strain, a deeper phylogenetic analysis is required.

# *Microascus tardifaciens* Y. Horie & Udagawa, Mycotaxon 17: 331. 1983

≡ Scopulariopsis tardifaciens Y. Horie & Udagawa, Mycotaxon 17: 331. 1983.

Notes — According to the protologue, this species is similar morphologically in colony features and its asexual morph to *M. albonigrescens* and *S. acremonium*. In this sense, our phylogenetic analysis (Fig. 1) showed that these species could probably correspond to a species of the former genus *Acaulium*. However, the ex-type culture (NHL 2912) of *M. tardifaciens* was not available for study.

# *Pithoascus platysporus* Arx & Veenb.-Rijks, Persoonia 7: 374. 1973

Specimen examined. THE NETHERLANDS, Wageningen, from agricultural soil, date unknown, *J.W. Veenbaas-Rijks* (ex-type culture CBS 419.73).

Notes — This species was described showing reddish brown, broadly cylindrical ascospores that differs from the main characteristics of the genus *Pithoascus*. According to Abbott et al. (2002), the ascospore morphology suggests a closer affinity to *Kernia* or *Lophotrichus*. Although we were unable to obtain sporulation from the ex-type strain (CBS 419.73), the analysis of the LSU and ITS sequences showed that this taxon was phylogenetically far from the *Microascales* and was closely related with the *Hypocreales*.

Scopulariopsis acremonium (Delacr.) Vuill., Bull. Soc. Mycol. France 27: 148. 1911

Basionym. Monilia acremonium Delacr., Soc. Mycol. Fr. 13: 114. 1897. = Scopulariopsis communis Bainier, Bull. Soc. Mycol. France 23: 125.1907.

= Penicillium scopulariopsis Sacc., Syll. Fung. 22: 1275. 1913.

Oospora glabra Hanzawa, J. Coll. Agric. Tohuko Imper. Univ. 4: 1912.
 Scopulariopsis candelabrum Loubière, Rech. Struct. Mucor. (Thesis),

Paris: 63. 1924. = Penicillium brevicaule Sacc. var. glabrum Thom, Bull. U.S. Bur. Anim.

Ind. 118: 48. 1910.

≡ Scopulariopsis brevicaulis (Sacc.) Bainier var. glabra (Thom) Thom in The Penicillia: 250. 1930.

= Scopulariopsis danica J.F.H. Beyma, Zentralbl. Bakteriol., 2 Abt. 99: 390. 1939.

Specimens examined. DENMARK, from horse skin infected with *Trichophyton* sp., 1938, *C. Werdelin* (Scopulariopsis danica ex-type culture MUCL 9028). – GERMANY, from wheat field soil, 1963, *W. Gams* (MUCL 8274); from soil, collector unknown (MUCL 8409).

Notes — This species was transferred to *Scopulariopsis* from *Monilia acremonium* by Vuillemin (1911). No ex-type strain of *M. acremonium* exists. This taxon was later considered conspecific with *Scopulariopsis danica* by Morton & Smith (1963), from which an ex-type culture (MUCL 9028) was available. Our phylogenetic and morphological studies seem to confirm this

synonymy; however, this species is phylogenetically distant from *Scopulariopsis* and related to the genera *Cephalotrichum*, *Gamsia*, *Trichurus* and *Wardomyces*, clustering with a reference strain of *S. albonigrescens* and probably corresponding to a species of the former genus *Acaulium* (see *M. albonigrescens*).

Scopulariopsis argentea Szilvinyi, Zentralbl. Bakteriol., 2 Abt. 103: 173. 1941

Notes — Type material was studied by Morton & Smith (1963) and considered as 'unidentifiable'. The protologue suggest a species of *Paecilomyces*. No living ex-type material is available.

Scopulariopsis bertaccini Redaelli, Giorn. Ital. Derm. Syph. 75: 825. 1934

Notes — Type material was studied by Morton & Smith (1963), and considered not to be a *Scopulariopsis* species. No living type material is available.

Scopulariopsis canadensis F.J. Morton & G. Sm., Mycol. Pap. 86: 55. 1963

Specimen examined. CaNADA, British Columbia, from seed of *Beta vulgaris*, 1958, *S.J. Hughes* (ex-type culture CBS 204.61).

Notes — Our phylogenetic data showed that the ex-type culture (CBS 204.61) is related to the *Xylariales*.

Scopulariopsis carbonaria F.J. Morton & G. Sm., Mycol. Pap. 86: 59. 1963

Specimen examined. PANAMA, from soil, 1961, *R. Coghill* (ex-type culture MUCL 9027 = CBS 205.61).

Notes — Our phylogenetic data on LSU, ITS, EF-1 $\alpha$  and TUB showed that the ex-type strain (MUCL 9027) formed an isolated lineage basal to the *Microascus* and *Pithoascus* clades. However, the ex-type culture was sterile, impeding further comparisons.

Scopulariopsis castellanii M. Ota & Komaya, Dermatol. Wochenschrift 78: 163. 1924

Notes — Morton & Smith (1963) considered its original description as 'unidentifiable'. Since no living type material exists, the identity of this taxon remains unknown.

### Scopulariopsis coprophila (Cooke & Massee) W. Gams, Cephalosporium-artige Schimmelpilze (Stuttgart): 207. 1971

Basionym. Monosporium coprophilum Cooke & Massee, Grevillea 16:

10. 1887. = *Monilia fimicola* Costantin & Matr., Rev. Gén. Bot. 6: 292. 1894.

■ Monima inficola costantin & Matr.) Cub. & Megliola, C. R. Accad.
 Lincei: 440. 1903.

≡ Scopulariopsis fimicola (Costantin & Matr.) Vuill., Bull. Soc. Mycol. France 27: 143. 1911.

Specimen examined. UK, from mushroom bed, 1946, C.J. La Touche (as Scopulariopsis fimicola MUCL 9030 = CBS 206.61).

Notes — Originally described as *Monosporium coprophilum*, this species was transferred to *Scopulariopsis* by Gams (1971) who also considered it to be conspecific with *Monilia fimicola*. The latter taxon had been previously transferred to *Scopulariopsis* by Vuillemin (1911) as *Scopulariopsis fimicola*, a species that was regarded as valid by Morton & Smith (1963); however, these authors did not consider *M. coprophilum*, whose oldest epithet has priority over *fimicola*. According to their observations, *S. fimicola* shares colonial and micromorphological characteristics with *Scopulariopsis baarnensis*, both species being included in different series of *Scopulariopsis* (Morton & Smith 1963). The latter species was transferred to the genus *Gliomastix*, as *G. murorum* var. *polychroma* by Dickinson (1968), currently named *Gliomastix polychromum* (*Bionectriaceae*, *Hypocreales*) (Summerbell et al. 2011).

Although no ex-type culture of *S. coprophila* is available, two reference strains were included in this study, one of them (MUCL 9641) was reidentified here as *M. trigonosporus*. Interestingly, the second strain (CBS 206.61), according to its LSU and ITS sequences it is phylogenetically related with members of *Bionectriaceae*.

Scopulariopsis finkii Sartory & R. Sartory ex Vuill., Encycl. Mycol. 2: 65. 1931

Notes — Original description of this fungus is too vague and inadequate for recognition of the species (Morton & Smith 1963). Type material does not exist.

Scopulariopsis halophilica Tubaki, Trans. Mycol. Soc. Japan 14: 367. 1973

Specimen examined. JAPAN, Osaka, from Undaria pinnatifida, 1974, K. Tubaki (ex-type culture CBS 380.74).

Notes — This name was considered by Pitt & Hocking (1985) to be a synonym of *Basipetospora halophila*, a fungus formerly described as *Oospora halophila* by van Beyma (1933). Recently, Samson et al. (2014) transferred this species to the genus *Aspergillus* under the new name *A. baarnensis*. Our phylogenetic data on LSU and ITS confirmed those results, showing its relationships with the *Eurotiales*.

Scopulariopsis hanii Moustafa & Abdul-Wahid, Nova Hedwigia 51: 476. 1990

Notes — According to the protologue, this fungus is morphologically compatible with the asexual morph of *Microascus* and should be considered as member of this genus. In many aspects, this species resembles *M. restrictus* and *M. verrucosus* from which it can be differentiated by having annellides, conidia and solitary sessile conidia at least two times larger. The protologue indicates that holotype material was deposited in the Herbarium of the Royal Botanic Gardens (IMI 326933). However, this accession number corresponds to an isolate of *Scopulariopsis croci*. No living culture of *S. hanii* was available for study.

Scopulariopsis lanosa J.F.H. Beyma, Zentralbl. Bakteriol., 2 Abt. 99: 423. 1937

Notes — This species was excluded from the genus by Morton & Smith (1963). No ex-type strain is available.

**Scopulariopsis lilacea** Szilvinyi, Zentralbl. Bakteriol., 2 Abt. 103: 174. 1941

Notes — Morton & Smith (1963) regarded the species as 'unidentifiable'. The illustrations included in the protologue seem to represent a conidial apparatus similar to *Fusarium*, while none of the described structures fits with those of *Scopulariopsis*. No live cultures were available.

### Scopulariopsis lingualis Neto bis & C. Martins, Compt. Rend. Seanc. Soc. Biol. 106: 1179. 1931

Notes — Morton & Smith (1963) regarded the species as 'unidentifiable'. No living type material was available.

Scopulariopsis longipes H.Q. Pan & T.Y. Zhang, Mycosystema 33: 2. 2014

Notes — This species was recently described based only on morphological features but no phylogenetic study was carried out. Unfortunately, the type material listed in the protologue (holotype HMAS 196252, dried culture HSAUP II <sub>07</sub>4334) is not available for comparison. According to the authors, *S. longipes* morphologically resembles *S. fusca* (syn. *S. asperula*), but differs in having smaller conidia (3.5–5 µm wide vs 5–8 µm in *S. fusca*).

Scopulariopsis maduramycosis Q.T. Chen, Chin. Med. J. 99: 378. 1986

Notes — This name has been invalidated since it was published without an adequate description or holotype information.

**Scopulariopsis mencieri** C.W. Dodge, Medical Mycology. Fungous diseases of men and other mammals: 648. 1935

Notes — According to Morton & Smith (1963) the description is inadequate. No living type material is available.

Scopulariopsis minima Sartory, Hufschm. & J. Mey., Bull. Acad. Méd. Paris, sér. 3, 103: 606. 1930

Notes — This species is not a *Scopulariopsis* according to Morton & Smith (1963). No living material is available.

### Scopulariopsis mottai Vuill., Encycl. Mycol. 2: 62. 1931

Notes — Considered as a doubtful species by Dodge (1935), and probably not a *Scopulariopsis* according to Morton & Smith (1963). No living material is available.

# Scopulariopsis musae Matsush., Matsush. Mycol. Mem. 5: 27. 1987

Notes — The protologue of this species has morphological features that could correspond with an asexual state of *Microascus*. However, it shows asymmetrical, curved and extremely large conidia, features that do not match with the typical characteristics of the genus. No living culture is available for study.

Scopulariopsis nicotianae J.F.H. Beyma, Zentralbl. Bakteriol., 2 Abt. 91: 354. 1933

Notes — According to Morton & Smith (1963) the fungus probably belongs to a fungal genus different from *Scopulariopsis*. No living material is available.

Scopulariopsis nivea Demelius, Verh. Zool.-Bot. Ges. Wien 66: 490. 1916

Notes — According to the original description the fungus might represent a *Scopulariopsis* species; however, the description is too vague. No living specimen is available.

Scopulariopsis olivacea Szilvinyi, Zentralbl. Bakteriol., 2 Abt. 103: 174. 1941

Notes — Description and illustrations of the protologue seem to indicate that this is a *Penicillium* species. No living cultures are available.

Scopulariopsis parva (A.H.S. Br. & G. Sm.) Samson, Stud. Mycol. 6: 102. 1974

Basionym. Paecilomyces parvus A.H.S. Br. & G. Sm., Trans. Brit. Mycol. Soc. 40: 58. 1957.

Specimen examined. CANADA, Alberta, from soil, 1961, *J.W. Carmichael* (Scopulariopsis parvula ex-type culture MUCL 9041 = CBS 209.61).

Notes — This species was originally described as *Scopulariopsis parvula* by Morton & Smith (1963). Samson (1974) considered this taxon as synonym of the older species *Paecilomyces parvus*, transferred to *Scopulariopsis* since it produces conidia with a truncate base, thus the new combination *S. parva* was established. However, the analysis of the LSU and ITS sequences of the ex-type culture (basionym *Scopulariopsis parvula* MUCL 9041) showed that this fungus is related with the *Eurotiomycetes*.

Scopulariopsis penicillioides H.Q. Pan, Y.L. Jiang, H.F. Wang & T.Y. Zhang, Mycosystema 33: 3. 2014

Notes — This name is an illegitimate later homonym of Scopulariopsis penicillioides (Delacroix) Smith & Ramsbottom (1915), a species considered a synonym of S. brevicaulis in Morton & Smith (1963), but not listed in the repositories for fungal names such as Index Fungorum or MycoBank. Comparing the original descriptions of these two fungi we conclude that they are different species. While S. penicillioides (Delacroix) Smith & Ramsbottom, a species based on *Monilia penicillioides* Delacroix (1897), shows pale-yellow, oval and echinulate conidia, the fungus described by Pan et al. (2014) has pale-brown, ellipsoidal to broadly obovoid and smooth-walled conidia. The latter fungus resembles Pseudoscopulariopsis hibernica but mainly differs in having narrower conidia (3-4.5 µm vs 5-6 µm in *P. hibernica*). The molecular study of these fungi has not been carried out because the type material listed in the protologue of S. penicillioides (holotype HMAS 196253, dried culture HSAUP II <sub>07</sub>4299) is not available for comparison.

Scopulariopsis polychromica Szilvinyi, Zentralbl. Bakteriol., 2 Abt. 103: 175. 1941

Notes — According to Morton & Smith (1963) the fungus is unrecognisable from the original description. The original illustrations seem to represent a 'degenerated' strain. A type does not exist.

Scopulariopsis rosacea Szilvinyi, Zentralbl. Bakteriol., 2 Abt. 103: 175. 1941

Notes — 'Unidentifiable' according to Morton & Smith (1963). No living material is available.

Scopulariopsis rubellus Bainier, Bull. Soc. Mycol. France 23: 104. 1907

Notes — Inadequately described according to Morton & Smith (1963). An ex-type strain does not exist.

Scopulariopsis sehnsuchta Mello, Bull. Soc. Pathol. Exot. 25: 296. 1932

Notes — This fungus was poorly described according to Morton & Smith (1963). No living material is available.

Scopulariopsis silvatica (Oudem.) Apinis, Nova Hedwigia 5: 73. 1963

Basionym. Spicaria silvatica Oudem., Arch. Néerl. Sci., sér. 2, 7: 291. 1902.

Notes — The description of *S. silvatica* (Apinis 1962) and the illustrations of the basionym *Spicaria silvatica*, seem to represent a fungus not belonging to *Scopulariopsis*, showing a phialidic conidiogenesis with abundant intercalary phialides. No living material is available for examination.

Scopulariopsis spinosa E. Müll. & Pacha-Aue, Nova Hedwigia 18: 161. 1970

Notes — The original illustrations of the fungus suggest the conidial apparatus typical of a *Penicillium* species. No living culture is available.

**Scopulariopsis sputicola** (Galippe) C.W. Dodge, Medical Mycology. Fungous diseases of men and other mammals: 648. 1935

Basionym. Monilia sputicola Galippe, J. Anatomie 21: 538. 1885.

Notes — 'Unidentifiable' according to Morton & Smith (1963). No living material is available.

Scopulariopsis tritici K.B. Deshp. & K.S. Deshp., Curr. Sci. 34: 222. 1965

Notes — The original description and illustration seem to belong to a species of *Stachybotrys* or related taxa. According to the authors of the species, the holotype and an ex-type culture were deposited in the Herbarium Cryptogamae India Orientalia (New Delhi) and in the Herbarium of the Botany Department, Marathwada University (Aurangabad) from India. However, no records of this species were found in the respective catalogues.

### **Scopulariopsis venerei** Greco, Origine des Tumeurs (Etiologie du Cancer, etc.) et Observations de Mycoses (Blastomycoses, etc.) Argentines (Buenos Aires): 716. 1916

Notes — Morton & Smith (1963) considered this fungus as a possible species of *Botrytis*. No living material is available.

Scopulariopsis verticillioides Kamyschko, Notul. Syst. Sect. Cryptog. Inst. Bot. Acad. Sci. U.S.S.R. 14: 225. 1961

Notes — Living material of this species is not available.

**Scopulariopsis verrucaria** ('verrucifera') H.F. Wang & T.Y. Zhang, Mycosystema 33: 4. 2014

Notes — This species was recently described based only on morphological features. This has not been included in our phylogenetic study because the type material listed in the protologue (holotype HMAS 196254, dried culture HSAUP II <sub>06</sub>4334) is not available for comparison. According to the original description and illustration of the species, it is similar to *Microascus verrucosus*. However, *S. verrucaria* differs in having dark brown colonies, and conidia of  $3-5 \,\mu$ m wide covered by a gelatinous membrane, while *M. verrucosus* has olive grey colonies and rough, somewhat wider conidia ( $5-7 \,\mu$ m).

# **Scopulariopsis vignolo-lutatii** (Matr.) C.W. Dodge, Medical Mycology. Fungous diseases of men and other mammals: 650. 1935

Basionym. Acaulium vignolo-lutatii Matr. (as Vignoli-Lutatii), in Vignolo-Lutati, Arch. Derm. Syph., Berlin 118: 690. 1913.

Notes — Dodge (1935), although with no strong conviction due to its inadequate description, transferred *Acaulium vignolilutatii* to *Scopulariopsis*. Morton & Smith (1963) considered this species as a possible member of the *Scopulariopsis brumptii*  series, but with an inadequate description for recognition of the fungus. The original description and illustration seem to refer to a fungus morphologically close to *M. paisii*. No living cultures are available for study.

Scopulariopsis yunnanensis Q.T. Chen & C.L. Jiang, Acta Mycol. Sin. 4: 167. 1985

Notes — The original description seems to refer to a *Scopulariopsis* species. However, it was described forming bifurcate chains of conidia. In addition, the conidia are smaller than those of the currently known *Scopulariopsis* species. According to the authors (Jiang et al. 1985), *S. yunnanensis* is similar to *S. parva*, a species that according to our results is phylogenetically related with the *Eurotiomycetes*. The ex-type strain of this species is not available.

#### DISCUSSION

In this study we have reviewed the taxonomic circumscription of *Microascus* and *Scopulariopsis*, traditionally referred to as sexual and asexual morphs, respectively, and related genera using a polyphasic approach based on the evaluation of molecular, physiological and morphological data. These results show that *Microascus* and *Scopulariopsis* constitute two phylogenetically distant lineages, which are clearly different from *Pithoascus*, a genus revalidated in the present work, and from the lineage proposed here as the novel genus *Pseudoscopulariopsis*. Furthermore, combining the results of a multilocus sequence analysis and phenotypic data, we were able to delineate the accepted species of the four genera, proposing several new ones.

One of the first attempts to clarify phylogenetically the relationships among the different genera of the Microascales by the use of partial LSU sequences was that of Issakainen et al. (2003). That study demonstrated polyphyly of several genera of Microascaceae and raised questions concerning correct positions of several members of the family and their generic circumscriptions, suggesting a possible subdivision of Microascus and Scopulariopsis into several smaller genera. However, the LSU fragments used were too small and poorly informative, thus no final conclusions were made. Nevertheless, our phylogenetic analysis based on combined, longer LSU and ITS sequences proved to be useful to resolve topology of the different genera in the Microascacae. Confirming the findings of Issakainen et al. (2003), we demonstrated that *Microascus* and *Scopulariopsis* are clearly polyphyletic and that their currently accepted species are grouped in at least seven different lineages. In addition, the combined LSU and ITS phylogeny showed that the dry-spored synnematous genera Cephalotrichum, Doratomyces, Stysanus, and Trichurus are conspecific, which agrees with Abbott (2000) who synonymised and integrated these four genera under the name Cephalotrichum. However, given the lack of ex-type strains for most of the species, no formal decision is made at the moment. The genus Cephalotrichum, as well as several other genera of the Microascaceae, such as Kernia, Wardomyces and Wardomycopsis, have been considered in the past as probably congeneric with Scopulariopsis or Microascus (Morton & Smith 1963, Abbott 2000). Our phylogeny demonstrates that, although these genera share similar morphological and ecological traits, they are in fact genetically distant. The phylogenetic data is supported by relevant morphological differences, such as the presence of germ slits, synnemata or conspicuously hairy ascomata (Abbott 2000, Issakainen et al. 2003).

The recognition of *Pithoascus* as a valid genus has always been a matter of discussion. Probably one of the strongest reasons to synonymise the genus with *Microascus* was the

 Table 2
 Relevant phenotypic features of members of Microascus, Pithoascus, Pseudoscopulariopsis and Scopulariopsis.

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species	Coloriy	mornh	Asexual	diam (IIIm)	Ascospore size (rim)	Ascospore	Jerrin pore	conidial size (iim)	Contata shane	2010		5		
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Microascus		4	4	000 011	с : 2	broodly trionautor	4	ט ט ט ע ע	ollinocidal to build of board		-	-	4	
NI PERSONALIS									empourar to participation	I			-	
w. prunneosporus	dull green to olive prown	+	+	GUZ-011	5-7×7-G	ellipsoldal	+	G-G.2 × G-4	subglobose to navicular	I	+	+	I	
M. campaniformis	dull green	+	+	150-220	6-7 × 4-4.5	triangular with an elongated side	+	$4-5 \times 2.5-3.5$	subglobose to broadly ellipsoidal	I	+	+	+	
M. chartarus	grey to smoke grey	I	+					$4-5.5 \times 3-4$	ovate	I	+			
M. cinereus	light to dark grey	+	+	95–300	4-5.5 × 2.5-4	reniform, broadly lunate, rarely triangular	+	$3-5 \times 2-3$	obovate or clavate	I	+	+	+	
M. cirrosus	brown-grey	+	+	140-230	$5-6 \times 3-4$	broadly reniform	+	$4-6.5 \times 4-6$	subglobose to obovate	I	+	+	+	,
M. croci	grey to mouse grey	I	+					$3.5 - 5 \times 3 - 4$	globose	+	+	י ד		
M. expansus	olive to grey-brown	I	+					$4-8 \times 2.5 - 3.5$	bullet shaped to broadly clavate	+	+	+	+	
M. gracilis	dull to olive green	+	+	130-300	$4.5 - 6.5 \times 2 - 4$	lunate	+	$3.5 - 5.5 \times 2 - 3.5$	subglobose or ellipsoidal	I	+	+	+	
M. hyalinus	dark grey	+	+	50-180	$3.5-5 \times 2-3.5$	broadly ovoid	+	$3.5-5 \times 2-3.5$	ovoid	I	+	+		,
M. intricatus	white to olive grey	+	+	140-200	$5-6 \times 2.5 - 3.5$	fusiform	+	$4-5 \times 3-3.5$	globose to broadly ellipsoidal	I	+	+	+	
M. Iongirostris	grey-black to brown-black	+	+	180-300	$3-4 \times 2-3.5$	reniform	+	$4-6 \times 3-4$	obovate	I	+	י ד		
M. macrosporus	grey opaque	+	+	130-250	$5-6.5 \times 5.5-7.5$	triangular	+	$5-7 \times 4-5$	globose to ovoid	I	+	+	+	
M. murinus	pale to dark grey	I	+					$4-6 \times 1.5-2$	cylindrical	I	+	+		,
M. paisii	grey-brown grey-black	I	+					$4-6 \times 2-4.5$	broadly ellipsoidal to short clavate	I	+	+	1	
M. pyramidus	grey to violaceus grey	+	+	125–250	$5-6.5 \times 5.5-7$	triangular or quadrangula with attenuated ends	+	$4.5 - 5.5 \times 3 - 4$	obovate	I	+	+	+	Ŧ
M. restrictus	olive to brown-grey	I	+					$4.5-6 \times 4-5.5$	globose to obovoidal	I	+	+	+	-1
M. senegalensis	pale brown	+	+	180-250	$7-9 \times 2-3$	reniform	+	$4.5 - 5.5 \times 3.5 - 4$	obovate	I	+	+		
M. trigonosporus	grey	+	+	130-250	$3-5 \times 3-4$	triangular	+	$2.5 - 3.5 \times 3.5 - 5.5$	globose to ovoid	I	+	+		
M. verrucosus	olive grey	I	+					$5-7 \times 4.5-6$	globose to subglobose	I	+	+		
Pithoascus P. ater	brown-black	I	+					$4-9 \times 4.5 - 8.5$	globose to pyriform	+	+	+		
P. exsertus	white	+	I	210-450	$6.5 - 12 \times 1 - 2.5$	falcate	I			I	+	+	+	_
P. intermedius <sup>1</sup>	white to light grey	+	+	95-150	$5-6 \times 2-2.5$	fusiform	I	$4-8 \times 4-7.5$	globose to subglobose	I	+	+		
P. nidicola	white to light grey	+	+	90–160	$6-8 \times 2-2.5$	fusiform, nearly lunate	I	$4-5 \times 2.5-3.5$	globose to ampulliform	I	+	+	+	
P. stoveri <sup>2</sup>	light to dark grey	+	+	50-110	$6-7.5 \times 2-3$	navicular	I	$5-8 \times 3-4$	obovate to pyriform	I	+	-	۱	
Pseudoscopulariopsis								0 1 1 1			-			
P. IIIDeIIIICa D. sobumochori3	onve grey	1 4	+ +	140 100	0 13.7F 1	fueiform or navioular		0-0×1-0	subgrouose	I	' + 4			
Scopulariopsis									0004000,0000,000					
S. asperula <sup>4</sup>	dark brown	+	+	130-190	$4.5 - 6.5 \times 3.5 - 4$	broadly reniform		$5-8 \times 4-6.5$	globose to ovate	I	+	+		,
S. brevicaulis	tan	+	+	70-150	$5-6 \times 3.5-4.5$	broadly reniform	+	$6-9 \times 5.5-9$	globose to ovate	I	+	+	+	
S. candida	white	+	+	100-170	$4-6 \times 5-6$	reniform, heart shaped	+	$6-9.5 \times 5-8.5$	subglobose to broadly ovate	I	+	+		
S. cordiae	white to light grey	+	+	100-150	$4.5 - 5.5 \times 3.5 - 4$	reniform	+	$2.5 - 6 \times 2 - 5$	broadly ellipsoidal to obovoidal	I	+	+	+	
S. flava	white	I	+					$6.5 - 7 \times 5.5 - 6.5$	globose to obovoidal	I	+	+		
S. soppii	white	+	+	130-200	$6-7 \times 2.5-3$	lunate	+	$5.5 - 9 \times 5 - 8$	globose to subglobose	I	+	+		
<sup>1</sup> Asexual morph data obtair <sup>2</sup> Asexual morph data obtain <sup>3</sup> Sexual morph data obtain <sup>4</sup> Sexual morph data obtain	red from Roberts (1985). The from von Arx et al. (1988). The von Arx et al. (1988). The Abbott & Sigler (2001).													

publication of the species M. caviariformis by Malloch & Hubart (1987), a fungus exhibiting intermediate characteristics between Microascus and Pithoascus. Microascus caviariformis showed the typical ascospores of *Pithoascus*, although with an inconspicuous germ pore, and produced abundant conidia. Our study demonstrated that both genera were clearly separated, thus confirming the original observations made by Skou (1973) and von Arx (1973a, b). Moreover, our data showed that M. caviariformis was located outside the main clades, which represent the genera Microascus, Pithoascus, Scopulariopsis and Pseudoscopulariopsis, forming a distant and strongly supported clade with S. acremonium and a reference strain of M. albonigrescens. The latter fungus was described as the sexual morph of the type species of Acaulium (Sopp 1912), and this genus was considered as congeneric with Microascus (Curzi 1930, Barron et al. 1961, Morton & Smith 1963). However, our phylogenetic analysis suggests that the old genus Acaulium might be revalidated. Considering that neither original cultures of Sopp, nor an ex-type strain of *M. albonigrescens* were available for analysis, we think it is best to not propose further taxonomic changes at the moment. Furthermore, M. singularis proved to be related to the putative Acaulium clade, which also constituted a new lineage among the Microascaceae. More study is needed on members of these clades and their closest phylogenetic relatives.

Although the morphological differences distinguishing the genera treated here are subtle, they correlate with the phylogenetic data, shape, size and colour of annellides and conidia, shape of ascospores and presence of germ pores being the most informative morphological characters (Table 2). Other features, such as shape and size of ascomata and number and shape of ostiolar necks, are frequently associated with environmental changes related to incubation conditions (Barron et al. 1961). On the basis of our data, we recommend using PCA or OA culture media to achieve the best growth and sporulation ratio. On PCA these fungi produce fast and abundant sporulation but some species, particularly those with little growth of their asexual morphs, might sporulate better on OA. It is most likely that variable morphological differences observed in the past might have led to incorrect identification of strains due to overlapping characteristics between closely related species. For example, two strains (MUCL 9048 and MUCL 9049) that were received as *M. cinereus* were finally reidentified as belonging to the undescribed sexual morph of M. gracilis, a species morphologically close to M. cinereus but differentiated by size and complexity of conidiophores and shape of ascospores (Table 2). Barron et al. (1961) and Udagawa (1962) mentioned a wide variation in size and shape of ascospores of isolates identified as M. trigonosporus, i.e. from triangular to nearly quadrangular, with some isolates showing spores distinctly longer at one side, but judging from our data this ascospore shape variation might correspond to different phylogenetic species. Most of the species of Microascus newly described here showed triangular ascospores of variable size. The new species M. campaniformis produced inequilateral spores with the longer side towards the germ pore, although the measurements do not coincide with those previously described by the authors mentioned above.

One of the main objectives of the present work was to assess the phylogenetic relationships among members of *Microascus* and *Scopulariopsis* in order to comply with the requirements of the new International Code of Nomenclature for fungi, algae and plants (Hawksworth et al. 2011). As has been discussed extensively by Hawksworth (2012), for this particular dual-name combination an option might be to retain the most 'widely-used' name. Accordingly, *Scopulariopsis* should have priority over *Microascus*, primarily because of the abundant medical literature on this genus (Hawksworth 2012, Sandoval-Denis et al. 2013). Our proposal to separate both genera is an alternative approach that maintains the names of the most relevant species of each genus, including those of the species that are significant in medicine as well as some important plant pathogens.

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