

Rodentomyces, a new hypocrealean genus from Italy

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Received: 16 December 2009 / Accepted: 13 January 2010 / Published online: 21 February 2010
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Abstract An ascomycete with non stromatic, narrowly ovoidal or subpyriform, pale yellow to luteous perithecia, a *Nectria*-like centrum, yellowish-brown reticulate ascospores, and a *Trichothecium*-like anamorph has been isolated from small rodent dung in Italy. Based on morphological, cultural and molecular (nrLSU and ITS sequences) data, a new genus, *Rodentomyces*, and a new species, *R. reticulatus*, are therefore introduced to accommodate this fungus. *Rodentomyces* represents a hypocrealean genus in the *Nectriaceae*. Within this family, and based on molecular data, *R. reticulatus* is placed in the group including *Calonectria*, *Leuconectria*, *Nectriadiella*, *Neonectria* and *Nectria mariannaeae*. Several coprophilous genera are recorded in *Hypocreales* almost evenly distributed among the main families of this order.

Keywords Coprophilous fungi · *Hypocreales* · *Nectriaceae* · *Rodentomyces reticulatus*

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Introduction

Hypocrealean fungi are characterised by usually light to vividly coloured, soft-textured ascomata, which are perithecia or exceptionally cleistothecia with a *Nectria*-pattern organisation of the centrum (Luttrell 1951), i.e. with a basal hymenium of unitunicate, inamyloid, usually persistent and 8-spored, often ring-less asci, interspaced with downward lengthenings of apical, ephemeral, sterile elements (“apical paraphyses”). The ascospores of hypocrealean fungi may be smooth or ornamented, hyaline to yellowish-brown, lacking both pores and germ slits, are usually two-celled, but also one- to poly-celled ascospores or ascospores with a median septum and additional, perpendicular septa can be observed. The associated anamorphs are generally phialidic.

Rogerson (1970) and Samuels and Seifert (1987) recognised *Hypocreaceae* De Not. as the sole family in *Hypocreales* Lindau, within which three main groups of genera were recognised, the first related to *Nectria* (Fr.) Fr., the second with basic features equal to *Hypocrea* Fr., the third centred around *Hypomyces* (Fr.) Tul. & C. Tul.

Molecular studies by Rehner and Samuels (1994, 1995) concerning the phylogenetic relationships in *Hypocreales* proved the existence of four major clades centred around *Bionectria* Speg., *Nectria*, *Hypocrea*, and *Claviceps* Tul. which, according to the systematics suggested by Rossman et al. (1999), respectively became the types of *Bionectriaceae* Samuels & Rossman, *Nectriaceae* Tul. & C. Tul., *Hypocreaceae*, and *Clavicipitaceae* (Lindau) O.E. Erikss. The *Hypocreaceae*, which are the equivalent of the *Hypocrea*- and *Hypomyces*-groups, differ from *Bionectriaceae* and *Nectriaceae* in having ascomata immersed in a stroma and usually disarticulating ascospores, whereas in the latter two the ascospores are not disarticulating (with rare exceptions) and ascomata can be superficial or

immersed, stromatic or non-stromatic. The *Bionectriaceae* can be distinguished from the *Nectriaceae* by their pallid, “white, luteous, orange, moderate brown”, superficial or immersed ascomata, not changing colour in potassium hydroxide (KOH), whereas the *Nectriaceae* are characterised by usually superficial, brightly coloured, “red, scarlet, deep orange, purple ascomata, becoming darker, usually dark vinaceous or blood colour, in 3% KOH” (Rossman 1983; Rossman et al. 1999). The *Clavicipitaceae* can easily be distinguished from the other families of *Hypocreales* by their thread-like and polyseptate ascospores. Rossman et al. (1999) also included in this order the *Niessliaceae* Kirschst., a family characterised by dark ascomata, unusual in *Hypocreales*. Lumbsch and Huhndorf (2007) have also accepted in *Hypocreales* a newly erected family, *Ophiocordycipitaceae* G.H. Sung et al. (2007), segregated from *Clavicipitaceae*, with the majority of species having “darkly pigmented, tough to pliant stromata that often possess aperi-thecial apices”.

The aim of this study is to describe a new fungus collected from rodent dung and to elucidate its phylogenetic position in the *Hypocreales* by morphological, cultural and molecular data based on DNA sequences for the large subunit and internal transcribed spacers of the nuclear rDNA.

Material and methods

Isolation of the fungus

Rodentomyces was obtained from dried rodent dung collected in northern Italy and placed in a non-sterilised damp chamber, following the methods suggested by Richardson and Watling (1997) and Richardson (2001), slightly modified by Doveri (2004). Cultured material, incubated at room temperature (18–25°C) under natural light but not exposed to direct sunlight, was examined every day with a stereomicroscope. Immature ascomata were noticed after 10–14 days’ incubation and mature ascomata after 17–20 days. Mature ascomata were picked up from dung by a sterile needle. Single ascomata were rapidly dipped in 90% ethanol, immediately washed in sterile distilled water and then placed in a drop of sterilised distilled water on a microscope slide. Ascomata were smashed to allow them to release their content into the water that was stirred to evenly distribute asci and ascospores. Drops placed on agar plates by a sterile micropipette were spread over the surface by sterile bent glass rods. PDA (Potato Dextrose Agar, Difco Lab.) amended by 2 mg L⁻¹ streptomycin was used for primary isolation. Plates were incubated for 24/48 h at 24±2°C under cycles of 12 h daylight/12 h darkness. After

incubation plates were observed at the stereomicroscope (up to 100×) and germinating groups of ascospores were selected, picked out of the agar and transferred to sterile agar plates using a glass needle.

Morphological studies

Light microscopy

Cultures were grown under 12/12 h light cycles at 20–26°C on PDA and water agar. To determine growth rates, PDA plates were incubated upside down to avoid growth of secondary colonies from released ascospores. Microscopic examinations were carried out mounting specimens in water, Melzer’s reagent, and methyl blue or cotton blue in lactic acid. 3% KOH and 100% lactic acid have been used for macro-chemical reactions and lactic acid for studying the peridial walls. Spore length and width were calculated on fifty ascospores discharged from mature asci in several ascomata of the holotypus, excluding ornamentations from measurements.

Cryo scanning electron microscopy

Portions of *Rodentomyces* colonies growing on PDA (teleomorph) or WA (anamorph) were mounted on stubs with low-temperature Tissue Tek (Miles Inc., USA), immediately cryo-fixed in liquid nitrogen (LN₂) and stored until observation. Stubs were transferred under LN₂ to a dedicated SEM Cryo Unit (SCU 020, Bal-Tech, Liechtenstein), where the frozen-hydrated samples were partially freeze-dried for 10 min at -80°C under high vacuum ($P < 2 \times 10^{-4}$ Pa), sputter-coated with 20 nm of gold (measured by a quartz thin-film monitor) in an argon atmosphere ($P < 2.2 \times 10^{-2}$ Pa). Specimens were then transferred to the cold stage of the scanning electron microscope (SEM 515, Philips, The Netherlands) and analysed at a temperature below -130°C with an acceleration voltage of 8 kV. Slow-scan images were digitised at a resolution of 768×576 pixels (256 grey levels) and analysed with AnalySIS 2.01 (Soft-Imaging Software GmbH, Germany).

Molecular studies

DNA extraction, amplification and sequencing

Fungal DNA was extracted from lyophilised mycelium by the SDS-CTAB method of Kim et al. (1990) with some modifications (Pecchia et al. 1998). Extracts were treated with a 1% (w/v) polyvinylpyrrolidone solution (soluble PVP, MW 40 000). A fragment of DNA, spanning approximately 1400 bases of the 5’ end of LSU rDNA was symmetrically

amplified by PCR using the following primers: LROR and LR7 (Rehner and Samuels 1994). Complete internal transcribed spacers (ITS) 1 and 2 sequences including 5.8 S of the nuclear ribosomal DNA were amplified with primers ITS5 and ITS4 (White et al. 1990).

Amplification reaction mixtures contained 25–50 ng of template DNA, PCR Master Mix (Promega) 1X, 0.5 μM of each primer in a 25 μl volume. PCR Master Mix 2X includes: 50 U ml^{-1} of Taq DNA Polymerase in a proprietary reaction buffer (pH8.5) supplied by the manufacturer, 400 μM of each dNTP, 3 mM MgCl_2 .

Amplification was performed in a GeneAmp PCR System 2400 (Perkin Elmer) using the following parameters: for LSU initial denaturation step at 94°C for 5 min, 35 cycles consisting of denaturation at 94°C for 1 min, annealing at 50°C for 1 min and extension at 72°C for 2 min, final extension of 72°C for 7 min; for ITS initial denaturation step at 94°C for 1 min, 30 cycles consisting of denaturation at 94°C for 30 s, annealing at 54°C for 1 min and extension at 72°C for 1 min, final extension of 72°C for 4 min. After the final extension of 72°C reactions were cooled to 4°C. Negative controls (no DNA) were included for each set of reactions.

Amplification products were separated by electrophoresis on 1% agarose gels with 0.5X TBE buffer (0.045 M Trizma base, 0.045 M boric acid, 0.001 M EDTA, pH 8.0), and visualised by UV fluorescence after ethidium bromide staining. PCR products were excised, eluted from the gel, and purified using the QIAquick PCR Gel Extraction Kit (QIAGEN).

The purified LSU PCR product was directly cloned into a pGEM[®]-T Easy Vector (Promega) according to the manufacturer's recommendations. Clean ITS PCR product was directly sequenced using ITS5 and ITS4 primers. Sequencing of the PCR products was performed in an Applied Biosystem Prism[®] 3100 DNA sequencer (Perkin Elmer/ABI) by the BigDye[™] terminator v3.1 Cycle Sequencing Ready Reaction Kit. The samples to be sequenced were processed by the DNA Sequence Facility at the Bio Molecular Research (BMR), Servizio Sequenziamento—CRIBI, University of Padova, Italy. Sequences have been deposited in GenBank (Tables 1, 2).

Phylogenetic analysis

Sequence data entry and editing were performed using the VISED program (Visual Software Editor, Version 1.1) (Peters 1996). ITS and LSU sequence alignments were performed with ClustalX 1.81 (Jeannotte et al. 1998) and visually corrected. Additional isolates representing members of the *Hypocreaceae*, *Clavicipitaceae*, *Nectriaceae*, *Bionectriaceae*, were obtained from GenBank (Tables 1 and 2).

Maximum parsimony trees were inferred using heuristic search (random sequence addition, 100 replicates) and branch swapping (Close Neighbour Interchange) options of MEGA 4.0 (Tamura et al. 2007). All characters were

unordered and given equal weight during the analyses. Gaps were treated as missing data. Bootstrap analyses of 1,000 heuristic replications (Felsenstein 1985) with random addition input order were performed in order to assess the robustness of internal branches.

For LSU an alignment of 31 sequences for the major families of the *Hypocreales* was analysed. The data set comprised a region of approximately 870 bp per taxon of the 5' end of the 28 S rDNA, spanning the eukaryote specific divergent regions 1 and 2 (Hassouna et al. 1984). The region analysed corresponds to positions 2–920 of *Saccharomyces cerevisiae* Meyen ex E.C. Hansen (AY048154) (Kurtzman and Robnett 2003). *Verticillium dahliae* Kleb. (*Phyllachorales* M.E. Barr) was used as outgroup.

For ITS an alignment of 31 sequences for the major genera of the *Nectriaceae* was analysed. The data set comprised a region of approximately 531 bp per taxon of the internal transcribed spacers 1 and 2 sequences including 5.8S of the nuclear ribosomal DNA. Members of the *Hypocreaceae*, *Hypocrea lutea* (Tode) Petch and *H. rufa* (Pers.: Fr.) Fr. were used as outgroup species.

Results

Cultural studies

Immature ascomata were visible after 5–7 days on PDA at 20–26°C and under 12/12 h daylight cycles. After ten days, discharged ascospores were present in the drops of water condensate on the bottom of Petri dish lids. These drops were collected and used to make monoascospore isolates. Colonies grown from single ascospores produced fertile ascomata. Sparse ascomata were produced on water agar after 3 weeks to 1 month, both on the surface and immersed in the agar, the latter more frequently near the point of inoculation. Colonies on water agar did not show any aerial mycelium; the anamorph appeared after about 1 week.

Phylogenetic analysis

Alignment for the data set of nrLSU sequences included 31 hypocrealean taxa and one phyllachoralean outgroup taxon. The total length of the sequence alignment was 870 positions and 141 nucleotide positions (16.2%) are parsimony informative. All characters were used in the analysis. MP phylogenetic analysis resulted in 4 parsimonious trees, differing slightly in the arrangement of the terminal taxa within the major lineages (length=625, CI=0.462, RI=0.615 and RC=0.284). Fig. 1 shows the consensus tree (50%) generated from the alignment in which the fungus *Rodentomyces reticulatus* is placed as sister taxon into the clade containing *Nectriaceae* species

Table 1 Fungal species and LSU rDNA data accession numbers used in this study

Classification ^a	Taxon ^b	Source ^c	GenBank number
Nectriaceae	<i>Calonectria kyotensis</i>	ATCC 22677	U17408
	<i>Calonectria morganii</i>	ATCC 11614	U17409
	<i>Leuconectria clusiae</i>	ATCC 22228	U17412
	<i>Nectria australiensis</i>	HMAS 83397	DQ119569
	<i>Nectria cinnabarina</i>	CBS 713.97	AF193237
	<i>Nectria cosmariospora</i> (= <i>Cosmospora coccinea</i>)	AR2741	U17407
	<i>Nectria pseudotrichia</i>	KAS 791	AY283554
	<i>Nectria sinensis</i>	HMAS 83356	DQ119571
	<i>Nectria (Cosmospora) vilior</i>	ATCC 16217	U57348
	<i>Neonectria radicola</i>	CBS 102032	U17415
	<i>Rodentomyces reticulatus</i>	DSM 23301	GU551932
Hypocreaceae	<i>Arachnocrea stipata</i>	TFC 97-43	AF160227
	<i>Hypocrea jecorina</i>	ATCC 13631	AF510497
	<i>Hypocrea schweinitzii</i>	ICMP 5421	AY281095
	<i>Hypomyces australbidus</i>	TFC 97-45	AJ583478
	<i>Hypomyces orthosporus</i>	TFC 97-130	AF160241
	<i>Sphaerostilbella aureonitens</i>	TFC 96-77	AF160246
	<i>Sphaerostilbella broomeana</i>	TFC 97-11	AF160231
Clavicipitaceae	<i>Balansia oblecta</i>	KCBobCEC87-3	U17395
	<i>Claviceps fusiformis</i>	ATCC 26019	U17402
	<i>Claviceps purpurea</i>		U57085
	<i>Cordyceps capitata</i>		U57086
	<i>Epichloe typhina</i>	ATCC 56429	U17396
	<i>Myriogenospora atramentosa</i>		U57084
Bionectriaceae	<i>Bionectria pityrodes</i>	ATCC 208843	AF193240
	<i>Bionectria sesquicillii</i>	ATCC 66880	AF193241
	<i>Heleococcum japonense</i>	ATCC 18157	U17429
	<i>Hydropisphaera erubescens</i>	ATCC44545	AF193231
	<i>Ochronectria calami</i>	CBS 125.87	AY489717
	<i>Selinia pulchra</i>	AR 2750	AF193246
	<i>Stephanonectria keithii</i>	CBS 114057	AY489727
Phyllachorales	<i>Verticillium dahliae</i>	ATCC 16535	U17425

^a Based on classification systems by Rossman et al. 1999; Castlebury et al. 2004; Halleen et al. 2004.

^b Names of genera and species are those from NCBI Genbank database; between brackets the currently accepted names (Index Fungorum=<http://www.indexfungorum.org/Names/Names.asp>) are reported.

^c ATCC American Type Culture Collection, Manassa, VA, USA; HMAS Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences; CBS Centraalbureau voor Schimmelcultures, Utrecht, The Netherland; AR AMY Y. Rossman personal collection; KAS K.A Seifert personal collection; TFC Tartu Fungal Collection; DSM German Resource Centre for Biological Material.

with 73% MP bootstrap support, indicating the close relationship of *R. reticulatus* with other members of the *Nectriaceae*.

Alignment for the data set of ITS sequences included 31 genera of the *Nectriaceae* and two *Hypocrea* species used as outgroups. The total length of the sequence alignment was 531 positions and 195 nucleotide positions (36.7%) are parsimony informative. All characters were used in the analysis. MP phylogenetic analysis resulted in

7 parsimonious trees, differing slightly in the arrangement of the terminal taxa within the major lineages (length=705, CI=0.559, RI=0.723 and RC=0.404). Fig. 2 shows the consensus tree (50%) generated from the alignment in which the fungus *R. reticulatus* is not contained in any of the other known genera/clades represented in the analysis and it was placed on a well supported branching lineage of its own (71% MP bootstrap support).

Table 2 Fungal species and ITS data accession numbers used in this study

Classification ^a	Taxon ^b	Source ^c	GenBank number	
Nectriaceae	<i>Calonectria kyotensis</i>	ATCC 22677	AF261740	
	<i>Calonectria morgani</i>	ATCC 46300	AF493968	
	<i>Cosmospora coccinea</i>	CBS 114050	FJ474072	
	<i>Cosmospora cupularis</i>	HMAS 97514	EF121864	
	<i>Cosmospora ephisphaeria</i>	GJS 98-160	FJ474073	
	<i>Cosmospora gigas</i>	HMAS 99592	EF121863	
	<i>Cyanonectria cyanostoma</i>	CBS 101734	FJ474076	
	<i>Haematonectria haematococca</i>	SUF 1328	AF150477	
	<i>Haematonectria haematococca</i>	SUF 1329	AF150478	
	<i>Lanatonectria flavolanata</i>	HMAS 97516	EF121860	
	<i>Lanatonectria flavolanata</i>	HMAS 76869	EF121862	
	<i>Lanatonectria flocculenta</i>	HMAS 76873	EF121858	
	<i>Lanatonectria flocculenta</i>	HMAS 97517	EF121868	
	<i>Leuconectria clusiae</i>	CBS 451.92	AF220976	
	<i>Leuconectria grandis</i>	HMAS 98302	EF121859	
	<i>Nectria balansae</i>	AR 4446	FJ474074	
	<i>Nectria cinnabarina</i>	NBRC 30679	AB237663	
	<i>Nectria cinnabarina</i>	NRRL 20484	L36626	
	<i>Nectria mariannaeae</i>	NBRC 7060	AB099509	
	<i>Nectria mariannaeae</i>	NBRC 6987	AB111492	
	<i>Nectria pseudotrichia</i>	HMAS 97518	EF121865	
	<i>Nectria (Cosmospora) vilior</i>	RV olrom161	AY618257	
	<i>Nectria (Cosmospora) vilior</i>	ATCC 16217	U57673	
	<i>Nectricladiella camelliae</i>	ATCC 38571	AF220960	
	<i>Nectricladiella camelliae</i>	CPC 10451	AY793452	
	<i>Neocosmospora vasinfecta</i>	NRRL 22499	AY381140	
	<i>Neocosmospora vasinfecta</i>	NRRL 22500	AY381143	
	<i>Neonectria radiciala</i>	AR 2553	AF220968	
	<i>Neonectria radiciala</i>	CTR 7-322	AF220969	
	<i>Rodentomyces reticulatus</i>	DSM 23301	GU551933	
	<i>Rubrinectria olivacea</i>	CBS 120617	FJ 474078	
	Hypocreaceae	<i>Hypocrea lutea</i>	CBS 102037	AY737773
		<i>Hypocrea rufa</i>	CBS 114374	X93980

^aBased on classification systems by Rossman et al. 1999; Castlebury et al. 2004; Halleen et al. 2004.

^bNames of genera and species are those from NCBI GenBank database; between brackets the currently accepted names (Index Fungorum=<http://www.indexfungorum.org/Names.asp>) are reported.

^cATCC American Type Culture Collection, Manassa, VA, USA; CBS Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; HMAS Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences; GJS G.J. Samuels personal collection; NBRC NITE Biological Resource Center, Department of Biotechnology, National Institute of Technology and Evaluation, China, Japan; NRRL National Center for Agricultural Utilization Research, U.S. Dept. of Agriculture, Peoria, IL, USA; RV R. Vasiliauskas personal collection; CTR C.T. Rogerson personal collection; DSM German Resource Centre for Biological Material.

Taxonomy

The taxon described below has a *Nectria*-type centrum and a *Trichothecium*-like anamorph, so it can be accommodated in the *Hypocreales*. The unique morphology, supported by

its phylogenetic position, allows us to describe it as a new genus in the *Nectriaceae*:

***Rodentomyces* Doveri**, Pecchia, Sarrocco & Vannacci, **gen. nov.**

Mycobank: MB516520

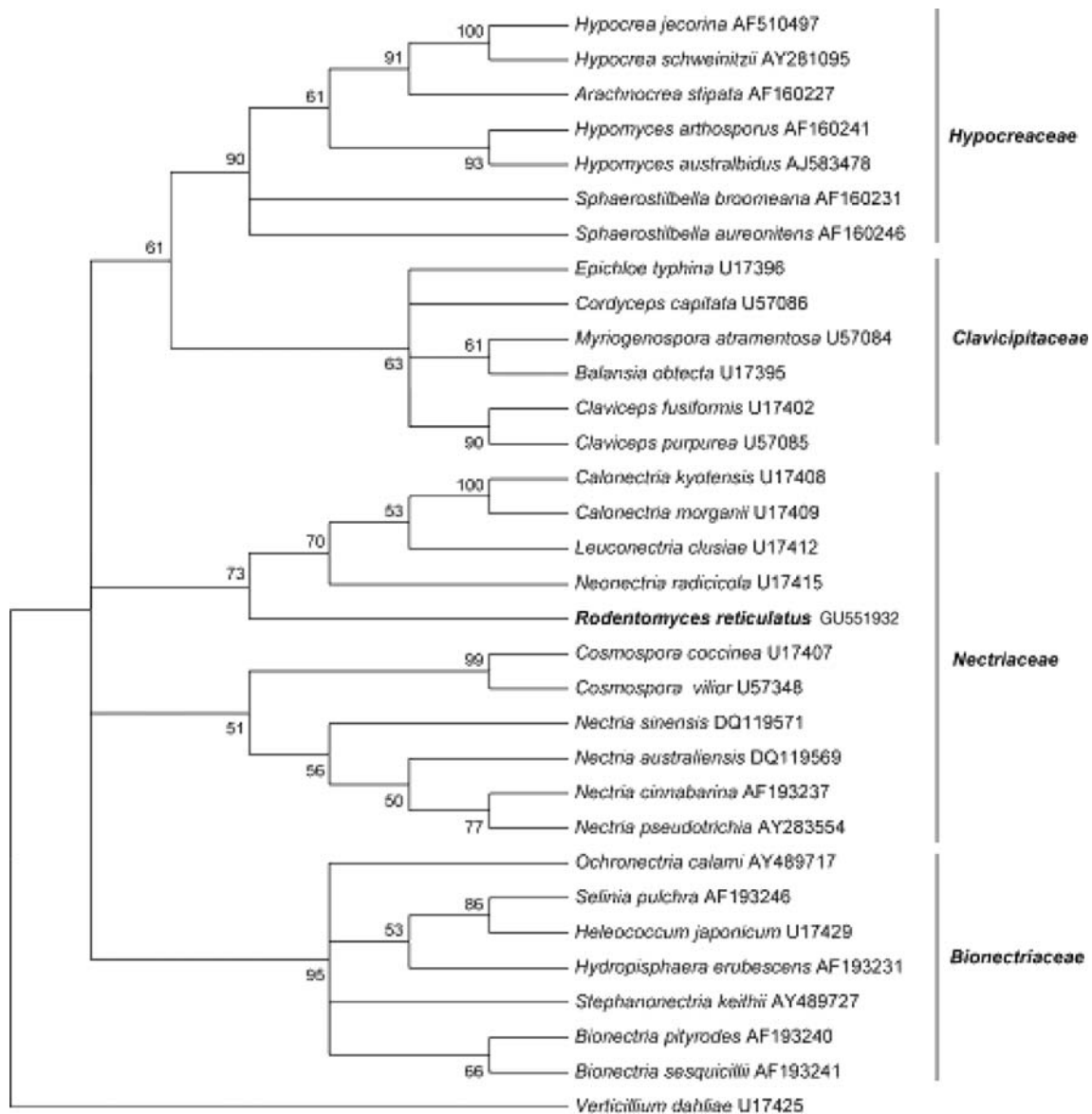


Fig. 1 *Rodentomyces reticulatus* (DSM 23301). Phylogenetic analysis of nrLSU sequences data, including 31 hypocrealean taxa and one phyllocoralean outgroup taxon. Consensus tree of 4 shortest trees

(length=625, CI=0.462, RI=0.615 and RC=0.284) inferred by maximum parsimony analysis. Bootstrap values exceeding 50% are indicated near nodes

Etymology: from the Latin = fungus peculiar to rodents”, as always collected from dung of such animals.

Ascomata in summa substrati parte locata, stromate carentia, ostiolata, pallentia ad lutea, a KOH colore non commutata. *Peridium* valde subtile, minus quam 10µm crassum, ex unica parte compositum, angularibus (textura angulari) atque crasse tunicatis cellulis contextum. *Periphyses* et *apicales paraphyses* praesentes. *Asci* obscuro apicali annulo praediti, unitunicati, in jodo non caerulescentes. *Ascospores* fusco-luteolae, didymae vel muriformes, tuberculis atque reticulo crasse ornatae, et poro et hiatu germinali carentes.

Typus: *Rodentomyces reticulatus* Doveri, Pecchia, Sarrocco & Vannacci.

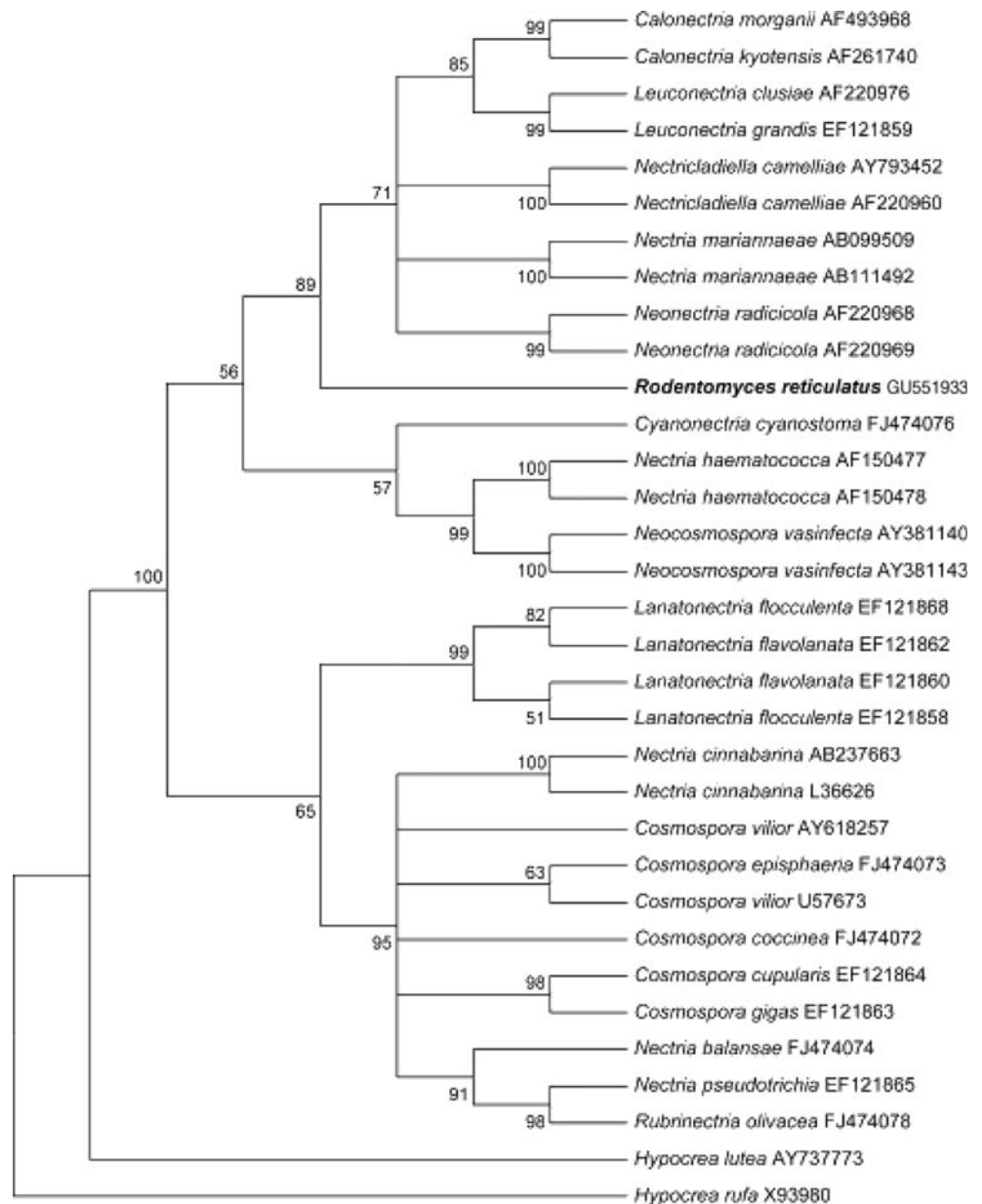
Ascomata superficial, non-stromatic, ostiolate, pale yellow to luteous, not changing colour in KOH. *Peridium* less than 10µm thick, a *textura angularis* of thick-walled cells. *Periphyses* and *apical paraphyses* present. *Asci* unitunicate, inamyloid, with an obscure apical apparatus. *Ascospores* yellowish brown, two-celled to muriform, tuberculate and subreticulate, lacking both a germ pore and slit.

Typus: *Rodentomyces reticulatus* Doveri, Pecchia, Sarrocco & Vannacci.

Rodentomyces reticulatus Doveri, Pecchia, Sarrocco & Vannacci, **sp. nov.** (Figs. 2, 3–10, 11–18, 19–23)

Mycobank: MB516521

Fig. 2 *Rodentomyces reticulatus* (DSM 23301). Phylogenetic analysis of ITS sequences data, including 31 taxa of *Nectriaceae* and two outgroup taxa of *Hypocreaceae*. Consensus tree of 7 shortest trees (length=705, CI=0.559, RI=0.723 and RC=0.404) inferred by maximum parsimony analysis. Bootstrap values exceeding 50% are indicated near nodes



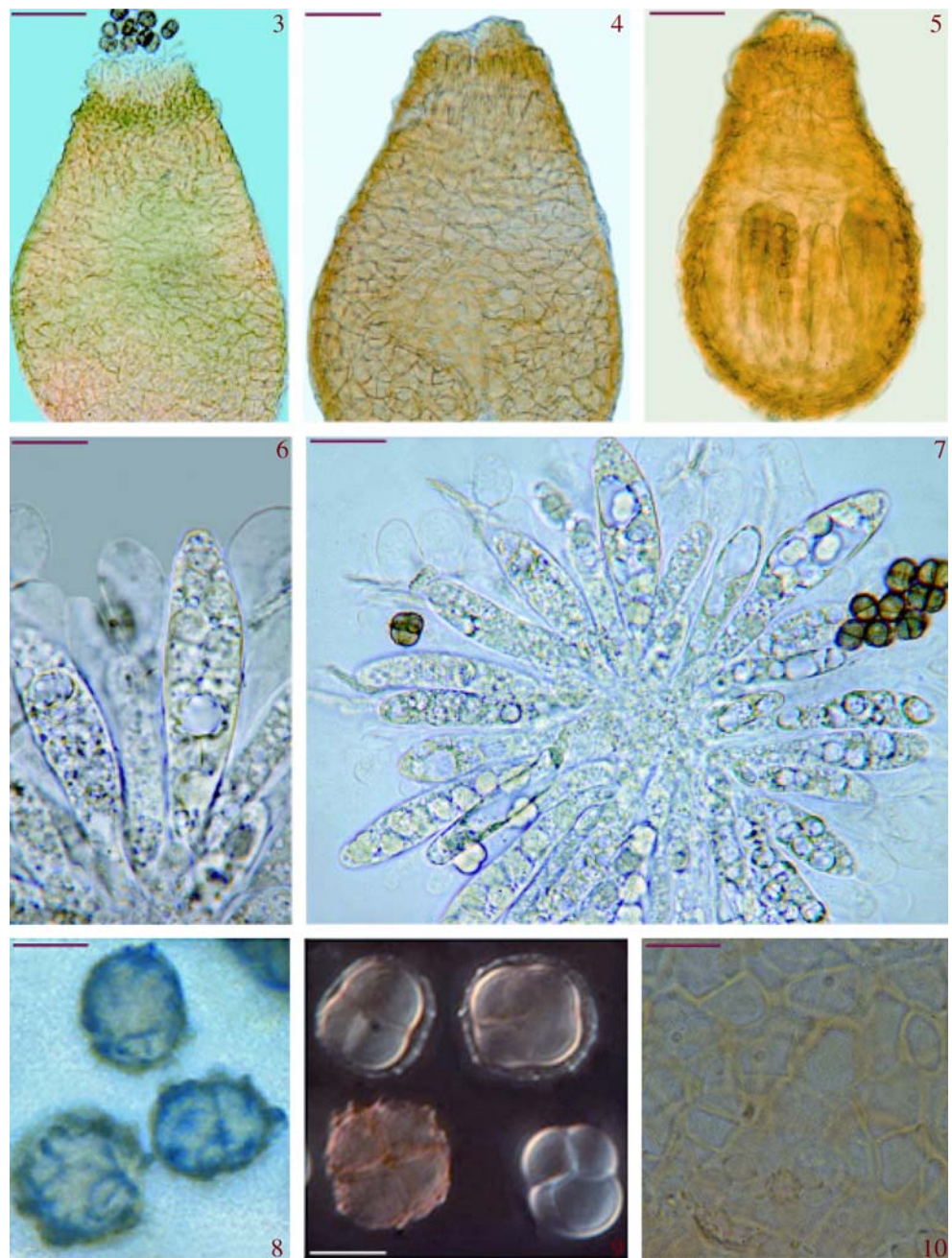
Etymology: from the Latin *reticulatus-a-um* "reticulate", referred to the ascospores ornamented with an almost complete reticulum.

Ascomata 180–320 µm alta, 100–130 µm lata, anguste ovoidea vel subpiriformia, membranacea, glabra, pallentia ad lutea. **Peridium** crasse tunicatis cellulis praeditum. **Apicales paraphyses** septatae, moniliformes, 10–17 µm diam. **Asci** clavati, octospori, 80–90 × 16–20 µm. **Ascospores** 10–13 × 8–10 µm, fusco-luteolae, late ellipsoideae, polis rotundatae, tuberculosae atque fere omnino reticulatae, in media parte septatae, cum utraque hemisphaerica cellula ad septum valde contracta atque saepe adiunctivo septo, ad medianum directo, praedita.

Coloniae in culturae elemento (PDA) modice expansae, membranaceae, planae, in summa parte albae vel etiam

lactis pallidi floris colorem accipientes, in aversa parte concolores. **Aerium mycelium** perrarum. **Hyphae** 2–4 µm diam., septatae. **Conidiiferae hyphae** in aquatili culturae elemento mononematae, hyalinae, leves, duobus pluribusve cellulis instructae, usque ad 100 µm longae, raro ramosae, ex septo in basi plerumque definitae. **Aeriae conidiiferae hyphae** parva atque viscosa conidiorum capita raro producentes. Et extremae et interpositae **chlamydo-sporae** presentes. **Conidiogenae cellulae** maxima parte phalacrogenae, usque ad 100 µm longae, ad apicem gradatim angustiores (ad basim 3–4 µm diam.; ad apicem 0.5–1, 5 µm diam.), rectae, leves, hyalinae, crebre vacantes, periclinali amplificatione aliquando praeditae. **Conidia** 10.5–17.5 × 1.5–5.5 µm, hyalina, subtilia, cylindrata, ad apicem rotundata, ad basim truncata, extra medium pos-

Figs. 3–10 *Rodentomyces reticulatus* (DSM 23301). Compound microscope. **3.** Perithecium in methyl blue with free ascospores at its apex. **4, 5.** Pale yellow to luteous perithecia in water. **6.** Detail of centrum with immature asci and remnants of apical paraphyses. **7.** Centrum with a group of eight, pigmented, mature ascospores inside an ascus on the right, and one mature free ascospore on the left. **8.** Mature ascospores with ornamentations stained in methyl blue. **9.** Ascospores in different stages of episporial wall deposition. **10.** Detail of peridial *textura angularis*. Bars: **3, 4, 5**=35 μ m; **6**=20 μ m; **7**=25 μ m; **8, 9**=13 μ m; **10**=12 μ m



itam, patentem abscissionis cicatriculam ostentantia, plerumque transverse 1-septata, raro 0-vel 3-septata, saepe ad septa constricta. *Arthrocatenata thalloconidia* et in aquatili culturae elemento et raro in PDA generata.

Holotypus hic designatus: DSM 23301 (cultura viva), ex fimo parvorum rodentium, in Vicetina Italica terra (Mons Brosimo-Lumignano) invento atque culto, solitaria vel congregata, plus quam quadraginta specimina remota, 12 Iunius 2003.

Perithecia 180–320 \times 100–130 μ m, non-stromatic, membranous, narrowly ovoidal or subpyriform, laterally compressed when dried, pale yellow to luteous, not changing

colour in KOH or lactic acid, almost smooth, with a scarcely differentiated neck, a slightly pointed apex and a roundish base. *Peridium* very thin, less than 10 μ m thick, one-layered, pseudoparenchymatous, of thick-walled, deep yellow, polygonal cells, 7–12 \times 5–8 μ m, forming a *textura angularis*, not joined by pores, interspaced with some cylindric hyphae, 4–5 μ m diam., supporting numerous uncinuate periphyses at ostiolar channel; near ostiole cells are more elongated, cylindric or claviform, hyaline, with roundish apices. *Apical paraphyses* 10–17 μ m diam., moniliform, septate, with cylindric or claviform segments, containing many hyaline vacuoles. *Asci* 80–90 \times 16–20 μ m

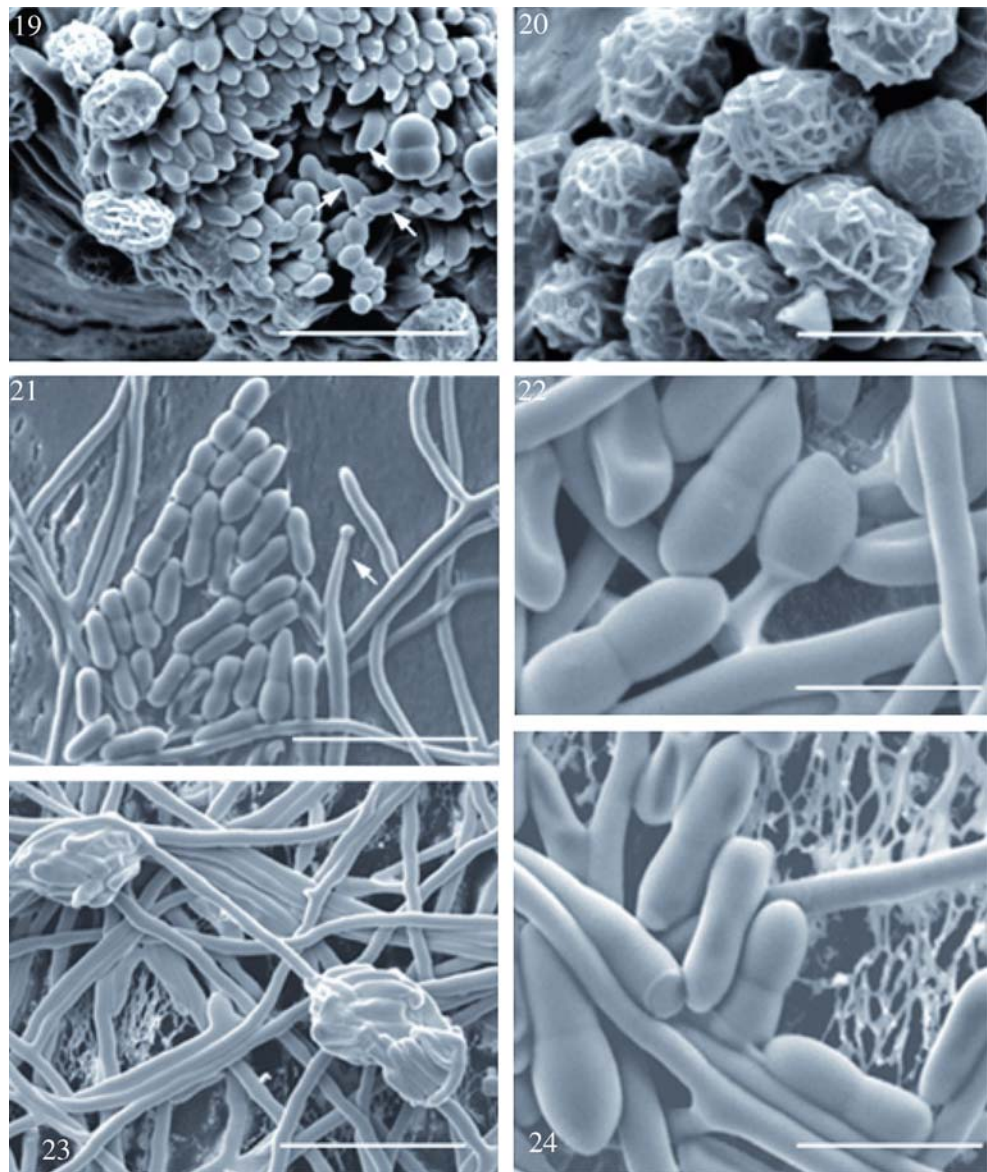
Figs. 11–18 *Rodentomyces reticulatus* (DSM 23301). **11, 12, 13** stereomicroscope (**11, 13**= incident light; **12**=transmitted light). **11**. Nine-day-old colony on water agar. **12**. Nine-day-old colony on PDA with fasciculate hyphae arising from inoculation point. **13**. Fasciculate hyphae of 3-week-old colony. Light to dark brown masses are groups of discharged ascospores. **14, 15, 16, 17, 18** compound microscope. **14**. Conidiophores on water agar. **15**. An empty conidiogenous cell and two mature conidia. **16**. One- and two-celled conidia with truncate, eccentric hilum. **17**. A conidiogenous cell with periclinal thickening (*cotton blue*). **18**. Fasciculate hyphae with anastomoses from nine-day-old colony in PDA. Bars: **11**=1 cm; **12**=0.5 cm; **13**=150 μm ; **14**=25 μm ; **15, 16**=5 μm ; **17**=3 μm ; **18**=2 μm



(mean=83×17 μm), unitunicate, persistent, inamyloid, with an obscure apical ring, 8-spored, cylindrical at first, becoming claviform, with a roundish apex. *Ascospores* 10–13×8–10 μm (mean=11×8.5 μm), uniseriate in early stages, becoming biseriate in upper two thirds of ascus, uniseriate in the lower third, hyaline at first, yellowish brown at maturity, broadly ellipsoidal with rounded ends, lacking both a germ pore and slit, two-celled, with each cell hemispherical and deeply constricted at equatorial septum, often three- or four-celled due to one (or two) additional septum, which runs perpendicular to equatorial one. Immature ascospores smooth, becoming rugulose, at matu-

rity ornamented with cyanophilic, 0.5–2 μm high tubercles, joined by ridges, 0.5–1 μm diam., which form an almost complete reticulum. *Colonies* moderately spreading (3.7 mm d¹ at 24°C) in PDA, membranous, whitish to pale beige on surface, the same colour on reverse, flat. *Aerial mycelium* scarce, but abundant fasciculate hyphae, with frequent anastomoses, produced from PDA disks on agar plates. *Hyphae* 2–4 μm diam., septate. *Conidiophores* absent or rare on PDA, on water agar mononematous, hyaline, smooth, two- to several-celled, up to 100 μm long, rarely branching, usually delimited by a basal septum, which is 2–20 μm from subtending hypha. *Conidiogenous*

Figs. 19–24 *Rodentomyces reticulatus* (DSM 23301). Cryo scanning electron microscopy images of frozen-hydrated fungus samples. **19, 20** from PDA cultures. **19** Ostiolar channel of a mature perithecium with end cells and emerging periphyses (some indicated by arrows). **20** Mature ascospores with an almost complete reticulum. **21, 22, 23, 24** from water agar cultures. **21**. Arthroconidia produced from mycelium grown on water agar. The arrow indicates a conidiogenous cell producing a conidium. **22**. Arthroconidia released by collapsing of intercalary cells. **23**. Heads of conidia produced in a slimy matrix. **24**. Arthroconidia produced by disarticulation of a pre-existing hypha. Bars: **19**=20 μm ; **20**, **24**=12 μm ; **21**, **23**=30 μm ; **22**=10 μm



cells predominantly phalacrogenous (after Gams 1971), up to 100 μm long, tapering towards their apices (3–4 μm diam. at base, 0.5–1.5 μm diam. at apex), straight, smooth, hyaline, often empty, without patent proliferations, producing conidia in small slimy heads, with a periclinal thickening of apical part, sometimes evident in cells stained by cotton blue. *Conidia* 10.5–17.5 \times 1.5–5.5 μm (mean = 13 \times 2.5 μm), hyaline, cylindrical to subclavate, thin-walled, usually 1-septate, rarely 0- or 3-septate, frequently constricted at the septa, with a rounded head and an evident, truncate, eccentric basal hilum. *Arthric thalloconidia* produced both on water agar and, rarely, on PDA. *Terminal and intercalary chlamydospores* present.

Habitat: small rodent dung.

Known distribution: Italy.

Material examined: ITALY: Vicenza, Mount Brosimo-Lumignano, on dormouse (*Glis glis*) dung in a damp

chamber, 12 June 2003, F. Doveri (HERB 031.04); Vicenza, Mount Brosimo-Lumignano, about forty, superficial, scattered or gregarious (in small groups) specimens on dung of small rodents (probably dormice) in a damp chamber, 15 June 2004, F. Doveri (DSM 23301, cultura viva; **holotype**).

Discussion

Within the *Hypocreales* the major families have been defined based on morphological characteristics and DNA sequence data (Rehner and Samuels 1995; Rossman et al. 1999, 2001; Sung et al. 2007). According to Rossman (2000), *Hypocreales* include over one thousand species with their anamorphs, which develop on several kinds of substrata, particularly on living or rotting plant materials,

soil, and other fungi, sometimes on insects, animal and human tissues, behaving as facultative or obligate saprobes or parasites (Wehmeyer 1975; Samuels and Seifert 1987; Rossman 1996).

Parsimony analyses of 28S and ITS rDNA sequences indicate that the fungus *Rodentomyces reticulatus* represents an hypocrealean genus residing in the *Nectriaceae*. The analyses performed in this study strongly support the placement of *R. reticulatus* in the group including *Calonectria* De Not., *Nectricladiella* Crous & C.L. Schoch, *Neonectria* Wollenw., *Leuconectria* Rossman et al. and *Nectria mariannaeae* Samuels & Seifert isolates. The resulting trees are consistent with those previously reported by Rehner and Samuels (1995), Zhang and Zhuang (2006), Luo and Zhuang (2008) and Samuels et al. (2009).

Genera included in *Nectriaceae* usually change colour in KOH (KOH+) and many have comparatively large, thick walled, often warted, sometimes hairy or furfuraceous ascomata, not morphologically similar to *Rodentomyces*. However, three genera in the *Nectriaceae*, namely *Cosmospora* Rabenh., *Neocosmospora* E.F. Sm. and *Pseudonectria* Seaver, show an affinity with *Rodentomyces* based on their sparse- to non-stromatic, often smooth, and thin-walled ascomata having a peridium of one- or at most two-layers, usually <20 µm thick (Rossman et al. 1999). In *Neocosmospora* the peridial cells are thick-walled, like those in *Rodentomyces*. *Neocosmospora* has brightly coloured, KOH+ ascomata not laterally pinched when dried, a two-layered peridium, and usually one-celled ascospores, unlike *Rodentomyces*. *Cosmospora* and *Pseudonectria* are similar to *Rodentomyces* in having ascomata of one-layer, that collapse laterally, and are sometimes KOH-. *Pseudonectria* has hyaline, one-celled ascospores while many members of *Cosmospora* have septate, ornamented, yellow-brown ascospores. Distinguished from *Rodentomyces*, *Cosmospora* has a peridial structure usually of *textura epidermoidea* (Samuels et al. 1991). The few exceptions in *Cosmospora*, i.e. *C. biasoletiana* (Briosi & Farneti) Rossman & Samuels and *C. obscura* Lowen, with yellow, KOH- ascomata, like those in *Rodentomyces*, differ from the latter in their peridial structure, spore morphology, and woody substrata. Moreover a phylogenetic analysis by Zhang and Zhuang (2006) shows that *Cosmospora coccinea* Rabenh., *C. vilior* (Starbäck) Rossman & Samuels, *Neocosmospora vasinfecta* E.F. Sm. var. *vasinfecta*, and *Pseudonectria* sp. belong to different clades from that including *Leuconectria clusiae* (Samuels & Rogerson) Rossman et al., *Neonectria radicolica* (Gerlach & L. Nilsson) Mantiri & Samuels, and *Calonectria morgani* Crous et al., which in our trees cluster with *Rodentomyces reticulatus*.

Three genera in *Nectriaceae* include species that are occasionally coprophilous, however, none are similar to *Rodentomyces*. *Neocosmospora vasinfecta* var. *africana*

(Arx) P.F. Cannon & D. Hawksw. has been recorded from dung only once (Cribb 1991) and *N. vasinfecta* var. *vasinfecta*, a plant pathogen, has been also isolated from soil, sometimes from nematodes (Rossman et al. 1999) and dung (Cailleux 1971; Doveri 2004). Until recently the genus *Nectria* (Fr.) Fr. has included all species with a *Nectria*-like centrum, thus a number of obscure species on dung have been described in this genus. *Nectria eichelbaumii* Henn. has caespitose, reddish brown perithecia and hyaline, fusiform or narrowly ellipsoidal, striate ascospores (Saccardo 1913), *N. fimeti* Cooke is characterised by stromatic, tomentous perithecia and ellipsoidal, non-septate, hyaline ascospores (Cooke 1883), *N. neohenningioides* Rossman has very thick-walled, pallid ascomata with fasciculate hairs and long, fusiform, finely spinulose, 3-septate ascospores (Rossman 1983), *N. tuberculariformis* (Rehm ex Sacc.) G. Winter possesses stromatic, warted ascomata and narrowly ellipsoidal, two-celled ascospores (Winter 1887; Hansen et al. 1998). *Neonectria coccinea* (Pers. : Fr.) Rossman & Samuels has brightly coloured, stromatic, fairly thick-walled ascomata, and two-celled, smooth, hyaline ascospores; it was described on dung by Walkey and Harvey (1965), but usually grows on wood.

Although some basic features of *Rodentomyces* match those of the *Bionectriaceae*, i.e. a *Nectria*-like centrum and comparatively pallid, KOH- ascomata, we have not found any coprophilous or non-coprophilous genus in this family with an association of characteristics like that described in *Rodentomyces*. Although some ascospores of species in the *Bionectriaceae* are ornamented, no genus belonging to them shows the pattern of tubercles forming a reticulum. Coprophilous genera in *Bionectriaceae* are just those with the greatest differences from *Rodentomyces*, particularly for their ascomatal shape (more than half of them have globose cleistothecia) and peridial structure.

The *Bionectriaceae* include several coprophilous genera that are unlike *Rodentomyces* in having cleistothecial ascomata, namely *Emericellopsis* J.F.H. Beyma, *Mycoarachis* Malloch & Cain, and *Roumegueriella* Speg. *Selinia* P. Karst. and *Hydropisphaera* Dumort. are the two perithecial genera in *Bionectriaceae* having species reported on dung. In *Selinia* the ascomata are immersed in a stroma, peridial cells are large, thin-walled and globose (Rossman et al. 1999) and the ascospores are one-celled, smooth, thick-walled, with a gelatinous sheath, quite unlike those of *Rodentomyces*. Some species of *Hydropisphaera* are rarely reported on dung. The ascomatal wall is distinctive in having large, thin-walled cells that result in a distinctly collapsed cupulate ascomata upon drying.

One genus has been described as coprophilous in the *Hypocreaceae*, namely *Aphysiostroma* Barrasa et al. with its *Verticillium*-type anamorph, originally found in Spain on

cattle dung (Barrasa et al. 1985). The features of this fungus, particularly the globose, non-ostiolate ascomata and disarticulating ascospores, are quite different from *Rodentomyces*. Members of the *Hypocreaceae* generally have ascomata immersed in a stroma and ascospores that disarticulate into part-spores. Those genera in the *Hypocreaceae* in which ascospores do not disarticulate, the ascomata are superficial or immersed in a subiculum (Rossman et al. 1999), and ascospores are one-celled and transversely striate, as in *Rogersonia* Samuels and Lodge (1996), or two-celled, often navicular and spinulose, as in *Sphaerostilbella* (Henn.) Saccardo and Saccardo (1905), or fusiform and apiculate, as in *Hypomyces*. These genera do not occur on dung and are unlike *Rodentomyces*.

The *Trichothecium*-like anamorph produced by *Rodentomyces reticulatus* is similar to the asexual states of few other species in the *Hypocreales* that have two- (three-) celled conidia, exceeding $10 \times 3.5 \mu\text{m}$. A *Trichothecium* Link anamorph has been noticed (Samuels and Seifert 1987; Rossman et al. 1999) in species of *Heleococcum* C. A. Jørg. in *Bionectriaceae* and *Hypomyces* (von Arx 1974; Samuels and Seifert 1987) in *Hypocreaceae*, but it had not described in *Nectriaceae* so far.

The study of a new coprophilous genus in *Hypocreales* inevitably led us to consider their natural behaviour “exhibiting a wide diversity of ecology and nutritional specialization” (Rehner and Samuels 1995). Our collections confirm dung as an important substratum for hypocrealean genera, and an overall survey shows that coprophily is almost evenly distributed among the main families of *Hypocreales* (Rossman et al. 1999).

Dung is a particular and complex substratum, whose chemical and physical features change with age and are affected by micro- and macroclimatic factors, which, in turn, can affect the composition of the colonising mycobiota. After Lundqvist (1972) we can state, however, that both a low and high substrate preference exist in *Sordariales* at least, consequently the so called tolerant species can be found on several kinds of dung, whereas the most specialised species are observable on few or even on one kind only. Lundqvist (1972) found a high percentage of tolerant sordariaceous species on rodent droppings, but nothing can be deduced from his work about the occurrence of hypocrealean fungi on small rodent dung. No exhaustive research exists on the ecological aspects of coprophilous *Hypocreales*. A reliable survey is available only for few common species (Rossman et al. 1999; Doveri 2004), whereas for the majority of species, like *Rodentomyces reticulatus*, have rarely been collected or noticed only once, thus the question concerning facultative or obligatory coprophily, tolerance or high specialisation, is still open.

Acknowledgements The authors wish to thank Nils Lundqvist for the first suggestions about the new genus, Amy Rossman for revising the text, Alberto Bizzi for providing abundant substratum, Giuseppe Firrao for useful suggestions in phylogenetic analysis, Marino Zugna, Maurizio Forti and Tiziana Capasso for technical advice.

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