

Malassezia cuniculi sp. nov., a novel yeast species isolated from rabbit skin

F. J. CABAÑES, S. VEGA & G. CASTELLÀ

Veterinary Mycology Group, Department of Animal Health and Anatomy, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain

Members of the genus *Malassezia* have rarely been associated with lagomorphs. During the course of an investigation of the lipophilic mycobiota of rabbit skin, two lipid-dependent isolates which could not be identified were recovered on Leeming and Notman agar medium from different animals. No growth of *Malassezia* yeasts was obtained either on Sabouraud's glucose agar or modified Dixon agar media. In this study, we describe a new taxon, *Malassezia cuniculi* sp. nov., including its morphological and physiological characteristics. The validation of this new species was supported by analysis of the D1/D2 regions of the 26S rRNA gene and the ITS-5.8S rRNA gene sequences. The results of these studies confirm the separation of this new species from the other species of the genus *Malassezia*, as well as the presence of *Malassezia* yeasts on lagomorphs.

Keywords *Malassezia cuniculi*, yeast, lagomorphs, rabbits, skin

Introduction

Members of the genus *Malassezia* may be isolated from the skin and mucosa of a wide range of warm-blooded vertebrates. However, the distribution of different *Malassezia* species, the prevalence of colonization and the density of *Malassezia* populations vary according to the animal host and to the anatomic sites on the hosts. This is probably related to the specific composition of cutaneous lipids and to the competition with different types of microorganisms within the cutaneous microbiota [1]. Therefore, the non lipid-dependent species *M. pachydermatis* is a common inhabitant of the skin and mucosa in dogs and cats but it is not a member of the normal human microbiota [2]. On the other hand, distinct lipid-dependent species form the major component of the cutaneous mycobiota in humans, ruminants and horses [3,4]. However, *Malassezia* has been very rarely recovered from certain animals such as rodent or lagomorph species and some studies have failed to demonstrate the presence of lipophilic yeasts on these animals [1]. At present, the genus *Malassezia* includes 13 species

(Table 1) and of these *M. caprae*, *M. equina* and *M. nana* have only been isolated from domestic animals [5].

In this paper, we describe a new lipid-dependent species in the genus *Malassezia* isolated in the course of an investigation of the lipophilic mycobiota of the healthy skin of rabbits. For these isolates we proposed the name *Malassezia cuniculi* sp. nov.

Material and methods

Animals, sampling and culture media used

The study was conducted during 2009 on 11 healthy New Zealand white male rabbits (*Oryctolagus cuniculus*) at the Rabbit Production Unit of the IRTA-Torre Marimón (Caldes de Monbui, Barcelona, Spain) which were used for breeding and meat production.

Samples were collected from the external ear canals and the perianal, the inguinal and the submandibular areas of the skin. Perianal skin areas and right and left external ear canals of each animal were sampled by using a swab soaked in the wash fluid, i.e., 0.075 mol/l phosphate-buffered physiological saline, pH 7.9 containing 0.1% Tween 80. Areas of the chin and groin were sampled using a modification of the detergent scrub technique [6,7]. Skin areas were gently rubbed for 20 seconds with sterile tubes containing 2 ml of wash fluid. Aliquots of 25 µl of centrifuged wash fluid (4000 rpm, 10 min) were inoculated onto paired quadrants of duplicate agar plates.

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Correspondence: F. J. Cabañes, Grup de Micologia Veterinària, Departament de Sanitat i d'Anatomia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona, E-08193 Bellaterra, Barcelona, Spain. Tel.: +34 935811749; fax: +34 935812006; E-mail: javier.cabanés@uab.es

Table 1 Current described *Malassezia* species and their main hosts.*

<i>Malassezia</i> spp.	Main host/others
<i>M. caprae</i>	Goat/horse
<i>M. cuniculi</i> sp. nov.	Rabbit
<i>M. dermatitis</i>	Man
<i>M. equina</i>	Horse/cow
<i>M. furfur</i>	Man/cow, elephant, pig, monkey, ostrich, pelican
<i>M. globosa</i>	Man/cheetah, cow
<i>M. japonica</i>	Man
<i>M. nana</i>	Cat, cow/dog
<i>M. obtusa</i>	Man
<i>M. pachydermatis</i>	Dog, cat/carnivores, birds
<i>M. restricta</i>	Man
<i>M. slooffiae</i>	Man, pig/goat, sheep
<i>M. sympodialis</i>	Man/horse, pig, sheep
<i>M. yamatoensis</i>	Man

*Adapted from Cabañes [5].

All samples were inoculated onto the following media: Sabouraud's glucose agar (SGA; Biolife s.r.l., Milano, Italy); modified Dixon agar (mDA; 36 g malt extract, 6 g peptone, 20 g desiccated ox-bile, 10 ml Tween 40, 2 ml glycerol, 2 ml oleic acid and 12 g agar per litre, pH 6.0) [8]; and Leeming and Notman agar (LNA; 10 g peptone, 5 g glucose, 0.1 g yeast extract, 4 g desiccated ox-bile, 1 ml glycerol, 0.5 g glycerol monostearate, 0.5 ml Tween 60, 10 ml whole-fat cow's milk and 12 g agar per liter, pH 6.2) [9]. All media contained 0.05% of chloramphenicol and 0.05% of cycloheximide. Plates were incubated at 32°C and examined daily for 20 days. The presence of typical *Malassezia* cells was microscopically determined in swabs with positive growth. The smears were stained with Diff-Quick stain.

When growth of *Malassezia* yeasts was detected, colonies were selected and subcultured to SGA to determine their lipid-dependence and LNA for preservation and storage. The isolates were also stored at -80°C [10].

Morphological and physiological characterization

The characterization of lipid-dependent yeasts was based on their inability to grow on SGA and on their ability to use certain polyoxyethylene sorbitanesters (Tweens 20, 40, 60 and 80), following the identification schemes of Guého *et al.* [8] and Guillot *et al.* [11]. The Cremophor EL assimilation test [12] and the splitting of esculin (β -glucosidase activity) [12,13] were used as additional key identification characteristics. Other tests, such as the catalase reaction, growth at different temperatures (32°C, 37°C, 40°C and 45°C) on LNA and mDA and the morphological characteristics of the isolates after incubation at 32°C for 7 days in the same culture media.

D1/D2 26S rRNA gene and ITS-5.8S rRNA gene sequencing and analysis

DNA was extracted and purified directly from 7-day-old cultures on LNA and according to the FastDNA Spin kit protocol with the FastPrep FP-24 instrument (MP Biomedicals, Biolink, Barcelona, Spain). The DNA was kept at -20°C until used as a template for PCR amplification.

Methods for sequencing the D1/D2 domain of the 26S rRNA gene and the ITS regions and the 5.8S rRNA gene were similar to those described previously [14]. The variable D1 and D2 regions of the 26S rRNA gene were amplified by PCR using the conserved fungal oligonucleotide primers NL1 and NL4 [15]. The amplification process consisted of a pre-denaturation step at 94°C, for 5 min, followed by 30 cycles of denaturation at 94°C/45 s, annealing at 51°C/1 min and extension at 72°C/3 min, plus a final extension of 10 min at 72°C.

ITS-5.8S rRNA gene were amplified by PCR with the primer pair ITS5 and ITS4 [16]. PCR consisted of a pre-denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 1 min and extension at 72°C for 1 min, plus a final extension of 7 min at 72°C. The molecular masses of the amplified DNA were estimated by comparison with a 100-bp DNA ladder (Bio-Rad Laboratories S.A., Barcelona, Spain).

The PCR products were purified with MultiScreen filter plates (Millipore, Barcelona, Spain) following the manufacturer's recommendation and the purified PCR products were used as templates for sequencing which was accomplished with the 'BigDye Terminator v3.1 Cycle Sequencing kit' (Applied Biosystems Hispania S.A., Madrid, Spain). Primers ITS5 and ITS4 were employed to sequence the internal transcribed spacers and 5.8S rRNA gene, and primers NL1 and NL4 were used to sequence the D1/D2 26S rRNA gene. An Applied Biosystems model 3730 sequencer was used to obtain the DNA sequences.

The sequences were aligned by using the software program Clustal X (1.81). The Mega package, version 2.1, was employed to perform a neighbour-joining analysis of a distance matrix (Kimura 2-parameter model complete deletion) with 1,000 bootstrap replicates.

Nucleotide sequence accession numbers

The nucleotide sequences of the D1/D2 26S rRNA gene and ITS-5.8S rRNA gene determined in this study have been deposited in the GenBank and their accession numbers are GU733708 and GU733709, respectively.

Results

Microbiology

Malassezia yeasts were isolated only from two animals, with no growth detected on mDA and SGA. *Malassezia* colonies grew slowly on LNA showing some special lipid-dependence (Fig. 1a). The skin areas from which these yeasts were recovered included the external ear canal and the groin. LNA cultures from the external ear canal yielded numerous punctiform colonies after 7 days of incubation. Similar colonies were detected on LNA cultures inoculated with portions of the groin skin after the same period of incubation. All colonies were white to cream with a waxy consistency and difficult to emulsify. Gram stain of these colonies (Fig. 1b) and Diff-Quick stain of a smear from an ear swab (Fig. 1c) revealed the presence of spherical *Malassezia* yeast like cells.

Twenty four different colonies from LNA cultures of both animals were selected and subcultured on SGA and mDA to determine their lipid-dependence. All were considered as lipid-dependent species because they failed to grow on SGA. These isolates also failed to grow on mDA at 32°C at 7 days and only one isolate showed poor growth with a few colonies at 14 days on this medium.

Two strains, MA 1339 which was isolated from the external ear canal and MA 1353 recovered from the groin, each from a different animal were selected for further taxonomical studies (MA 1339 and MA 1353 are the strain numbers of the Culture Collection of the Veterinary Mycology Group, UAB, Barcelona, Spain).

Morphology and physiology

Malassezia strains belonging to the new species were characterized by using the current morphological and physiological identification scheme. The phenotypic features of the new species *M. cuniculi*, and the other described *Malassezia* species are summarized in Table 2. The strains MA 1339 and MA 1353 did not grow on SGA without lipid supplementation and on mDA at 32°C after 7 days of incubation. These strains were not able to growth on mDA or showed poor growth in this medium after multiple transfers from LNA. Description of the morphology of these strains was based on their appearance LNA due to their poor growth on mDA.

In general, the strains grew slowly and formed small colonies (<0.5–1 mm in diameter; average diameter = 0.5 mm) on LNA at 32°C after 7 days of incubation (Fig. 2a). Colonies were dull, white to cream, raised-to-moderately convex with entire margins, a waxy consistency and were difficult to emulsify. After 14 days of incubation at the same temperature, colonies reached 1–2 mm in diameter. At this time clearing and precipitation zones around the colonies were evident on LNA. The strains

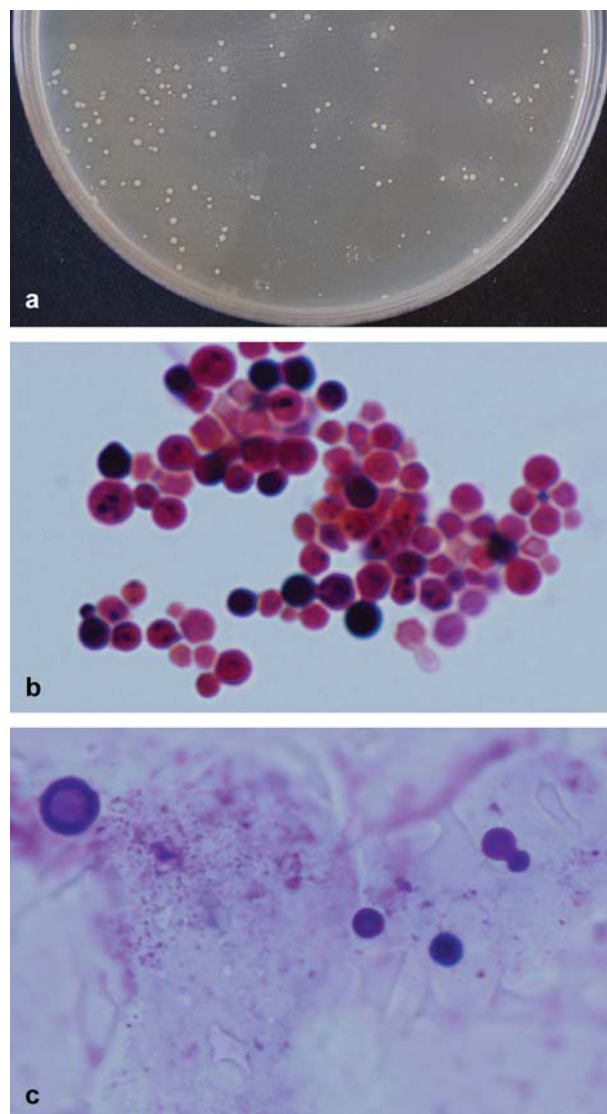


Fig. 1 Direct microscopy and culture of a rabbit ear swab. (a) Culture on Leeming and Notman agar showing abundant *Malassezia* colonies after 10 days of incubation at 32°C, (b) Gram stain of a colony showing *Malassezia* round yeasts cells, and (c) Diff-Quick stain of a smear from an ear swab showing the presence of round yeasts cells.

grew better at both 37°C (0.5–2 mm in diameter after 7 days; 1–3 mm in diameter after 14 days) and 40°C (<0.5–2 mm in diameter after 7 days; <1–3 mm in diameter after 14 days) than at 32°C but did not grow at 45°C. Cells were spherical, 2–5 µm in diameter, with buds formed in a monopolar pattern on narrow bases (Fig. 2b). Some buds elongated but the base remained always narrower than the bud.

Using the Tween dilution test proposed by Guého *et al.* [8] the strains did not grow on glucose-peptone agar with 10% Tween, 0.5% Tween 40, 0.5% Tween 60 and 0.1% Tween 80 as sole sources of lipids. In the Tween diffusion

Table 2 Main phenotypical characteristics of *Malassezia* species.*

Species	Cell morphology	Growth on mDA	Lipid dependency	T 20 ^a 10%	T 40 ^a 0.5%	T 60 ^a 0.5%	T 80 ^a 0.1%	T 20 ^b	T 40 ^b	T 60 ^b	T 80 ^b	Cremophor EL	Catalase	β-Glucosidase	Growth at 37°C	Growth at 40°C
<i>M. caprae</i>	Globose, ellipsoidal	+	+	-	w (-)	w	w	- ²	+ ¹	+ ¹	+ ¹ , (-)	-	+	+, (-)	-, (w)	-
<i>M. dermatitis</i>	Ellipsoidal, globose	+	+	+	+	+	+	+	+	+	+	W, (+)	+	?	+	+
<i>M. equina</i>	Ellipsoidal	+	+	-	w	w	w	w ²	+	+ ¹	+ ¹	-	+	-	w	-
<i>M. furfur</i>	Globose, ellipsoidal, cylindrical	+	+	+	+	+	+	+, (-)	+, (-)	+, (-)	+, (-)	+, (-)	+, (-)	-, (w)	+	+
<i>M. globosa</i>	Globose	+	+	-	-	-	-	-	- ²	- ²	-	-	+	-	-, (w)	-
<i>M. japonica</i>	Globose, ellipsoidal	+	+	-	w	+	+	-	w	+	-	?	+	?	+	-
<i>M. nana</i>	Ellipsoidal	+	+	+, (-)	+	+	w	v	+	+	w	-	+	-	+	v
<i>M. obtusa</i>	Ellipsoidal, cylindrical	+	+	-	-	-	-	-	-	-	-	-	+	+	-, (w)	-
<i>M. pachydermatis</i>	Ellipsoidal	+	+	-	+	+	+	+ ¹	+	+	+	+ ¹	+, w	+, (-)	+	+
<i>M. restricta</i>	Globose, ellipsoidal	+	+	-	-	-	-	-	- ³	- ³	-	-	-	-	v	-
<i>M. slooffiae</i>	Ellipsoidal, cylindrical	+	+	+, (w)	+	+	-	+, w, +	+	+	-, (w)	-	+	-	+	+
<i>M. sympodialis</i>	Ellipsoidal	+	+	-	+	+	+	+, w ²	+	+	+	-, (w)	+	+	+	+
<i>M. yamatoensis</i>	Ellipsoidal	+	+	+	+	+	+	+	+	+	+	?	+	?	+	-
<i>M. cuniculi</i> sp. nov.	Globose	+, (w)	+	-	-	-	-	-	-	-	-	-	+	+	+	+

*With the exception of *M. cuniculi*, data are from Guého *et al.* [8], Guillot *et al.* [11], Guého *et al.* [13], Sugita *et al.* [17–19], Hirai *et al.* [20], Batra *et al.* [3] and Cabanes *et al.* [4] (+ = positive; - = negative; v = variable; ? = not included in the description of the species; w = weak; v = variable; (-) indicate rare deviations from main pattern; ¹ growth may be inhibited near the well where the substrate is placed; ² growth may occur at some distance from the well where the substrate is placed; ³ opaque zone may occur); ^aTween dilution test proposed by Guého *et al.* [8]; ^bTween diffusion test proposed by Guillot *et al.* [11].

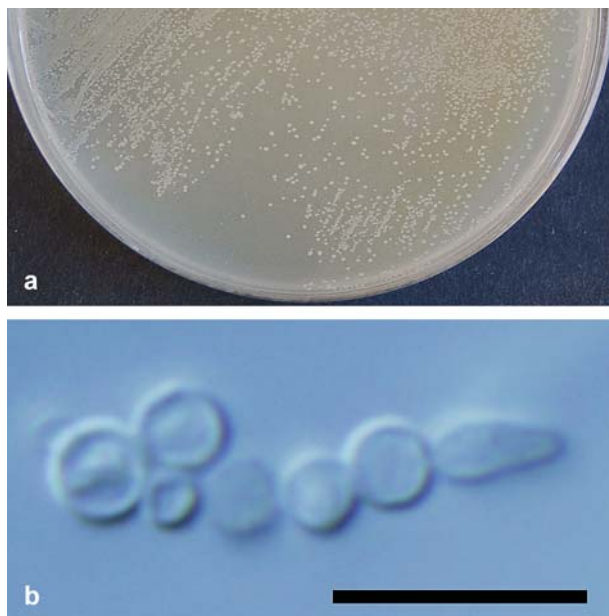


Fig. 2 Colonies and cells of *Malassezia cuniculi* CBS 11721^T (MA 1339) after 7 days of incubation at 32°C on Leeming and Notman agar. (a) culture, and (b) characteristic round cells – note that a cell forms an elongated bud. Bar = 5 µm.

test proposed by Guillot *et al.* [11] none of the strains showed growth around Tweens 20, 40, 60 and 80. Furthermore, neither of the test strains grew around Cremophor EL (Table 2). The two strains of *M. cuniculi* showed a strong β -glucosidase activity, which was revealed by their splitting of esculin.

Molecular analysis

The 26S rRNA gene (D1/D2 regions) from the 24 isolates were sequenced and found to be identical. The 26S rRNA gene (D1/D2 regions) from the type strain of *M. cuniculi* included 583 base pairs.

A search on GenBank database using BLAST [21] revealed that this sequence had a percent identity of 89–90% to all the *Malassezia* species and 94% to the sequence FJ 231747 of a *Malassezia* isolated from a hamster [22]. The latter was the closest match.

Figure 3 shows the molecular phylogenetic tree based on the D1 and D2 regions of the 26S rRNA gene sequences constructed by the neighbor-joining method. The closest *Malassezia* species was *M. slooffiae*. Our sequence differed from that of *M. slooffiae* at 56 positions (dissimilarity 10%), and from the sequence FJ 231747 at 39 positions (dissimilarity 6%).

Figure 4 shows the molecular phylogenetic tree based on the ITS-5.8S rRNA gene sequences. The section of DNA sequenced from the strains analyzed in this study

included 822 base pairs and contained the partial sequence of the 18S rRNA gene, the entire ITS1-5.8S-ITS2 regions and the partial sequence of the 26S rRNA gene. The ITS1 region ranged from nucleotides 9 to 269, the 5.8S rRNA gene from nucleotides 270 to 425 and the ITS2 from nucleotides 426 to 808. These sequences were also identical in all the isolates.

A search on the GenBank database revealed that this sequence had a percent identity of 85% to *M. furfur* but only in 38% of the sequence (a portion of the ITS 1 region, all 5.8S and a portion of ITS 2). When the sequences were aligned, they differed at 319 positions, 133 localized in the ITS 1, 7 in the 5.8S and 179 in the ITS 2. This means that the sequences had a 60% of similarity, reaching 95.5% in the 5.8S rRNA gene. In the phylogenetic tree, the closest species was *M. slooffiae*. However, the ITS-5.8S rRNA gene sequence of the type strain of *M. cuniculi* differed from the sequence of *M. slooffiae* at 377 positions, 144 localized in the ITS1, 7 in the 5.8S and 227 in the ITS2 (dissimilarity 51.7%).

Discussion

The host origin of the strains analyzed in this study (e.g., lagomorphs) is characteristic and novel for this new *Malassezia* species (Table 1). The recovery of *Malassezia* from rabbits has been rarely cited in the literature. During a study of the occurrence of *Malassezia* in the hair of some domestic animals and the feathers of some bird species, Dufait [23] described the isolation of *M. pachydermatis* from rabbits, using Sabouraud broth with chloramphenicol and cycloheximide. This author [23] investigated 19 rabbits and detected *Malassezia* in a high number of stained smears ($n = 9$) and their isolation in culture ($n = 16$). Guillot *et al.* [24] investigated the prevalence of *Malassezia* on different mammal species. The numbers of lipophilic yeasts was found particularly important in some animal species but this study which included four rabbits did not detect *Malassezia* by both direct examination and culture. These authors [24] used SGA and SGA with olive oil, containing chloramphenicol and cycloheximide, for recovering *Malassezia* species. Other investigations have failed to demonstrate the presence of lipophilic yeasts in these animals [1].

More recently, a combined *Sarcoptes scabiei* and *Malassezia* spp. infection was diagnosed in a rabbitry affecting about 20 of 500 rabbits [25]. The presence of abundant round yeast cells was detected on direct microscopic exam but there was no attempt in this study to recover the organism in culture. The morphology of the yeast cells shown on various histological stained sections would suggest a *Malassezia* species different from *M. pachydermatis*.

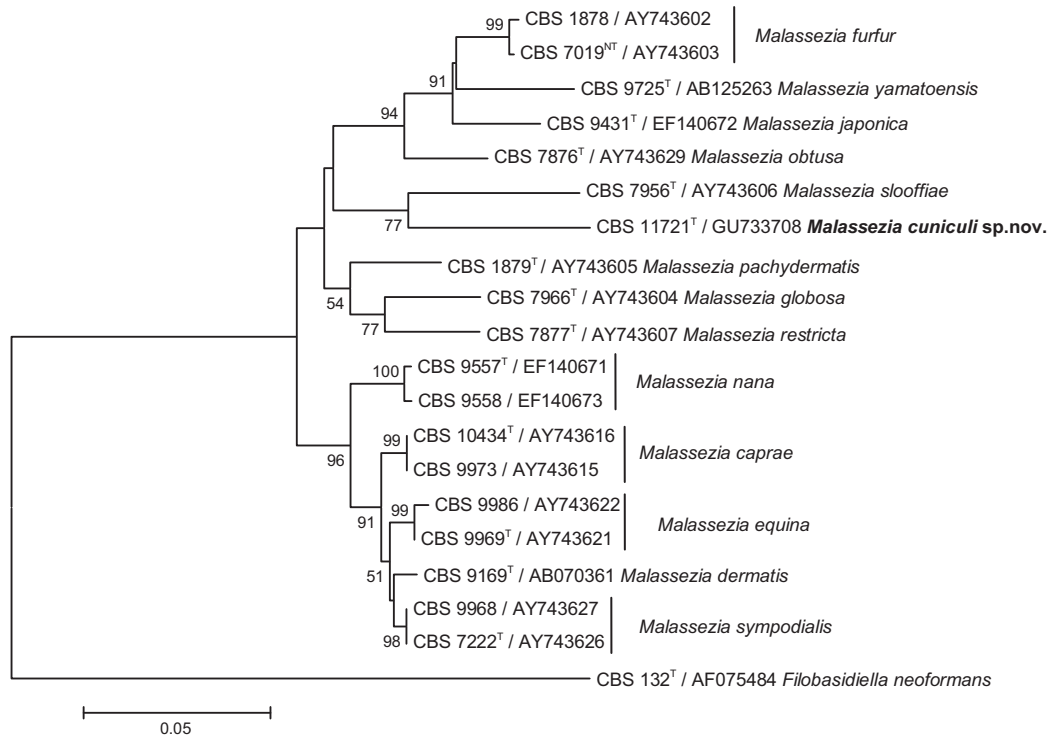


Fig. 3 Neighbour-joining tree based on Kimura 2-p corrected nucleotide distances among D1/D2 sequences of the 26S rRNA gene of *Malassezia* species. Branch lengths are proportional to distance. Bootstrap replication frequencies over 50% (1,000 replications) are indicated at the nodes.

In the present investigation, lipid-dependent isolates from two different animals were obtained on LNA. This medium was described as being better for isolation and enumeration of *M. furfur* in comparison to such media as mDA and Faergemann and Fredriksson's medium [9]. No growth of *Malassezia* species was detected on SGA and mDA culture media, or showed poor growth on mDA. Modified Dixon agar is a common and widely used culture medium for the isolation and characterization of *Malassezia* species [8]. However, we found it to be toxic or inhibitory for the new species. But it should be noted that in the present study *Malassezia* species were isolated only from two of the 11 animals investigated, confirming the difficulty of recovering these lipophilic yeasts from lagomorphs.

Our success in recovering *Malassezia* from rabbits could also be due to the sampling technique, the greater number of skin areas sampled for each animal and the use of different media to allow the isolation of lipid-dependent species. In addition to the external ear canals, we sampled other skin areas, such as the perianal, the inguinal and the submandibular. Rabbits have at least three externally secreting skin glands; (1) the submandibular or chin gland, (2) the inguinal gland in the groin and (3) the anal gland around the terminal part of the rectum. These glands are larger and secrete more actively in males [26].

The strains analyzed in this study did not fit the phenotypic profiles of any described species, and hence could not be identified (Table 2). Among other differences (Tween assimilation profiles included), isolates of the new species can be distinguished from *M. pachydermatis* by their inability to grow in SGA and from the lipid-dependent species by their inability to grow or only poor growth on mDA. Although the new species had similar Tween assimilation profiles to *M. globosa*, *M. obtusa* and *M. restricta*, the strains analysed in the present study did not completely fit the phenotypic profiles of any these described species. The new species can be distinguished from; (1) *M. globosa* by their ability to grow at 37°C and 40°C, and their β -glucosidase activity, (2) *M. obtusa* by their globose cell morphology, their ability to grow at 37°C and 40°C, and (3) *M. restricta* by their globose cell morphology, their ability to grow at 40°C and catalase activity.

Strains of the new species isolated from rabbits were able to grow better at both 37°C and 40°C than at 32°C, with 40°C as the maximum temperature of growth. This physiological feature is also a notable character for this new species. Normal rabbit body temperature ranges from 38.5–40°C [27], the optimal temperature range of the new species.

Special micromorphological characteristics have been cited for some *Malassezia* spp. In the case of *M. furfur*, the micromorphology of the cells is variable in size and shape,

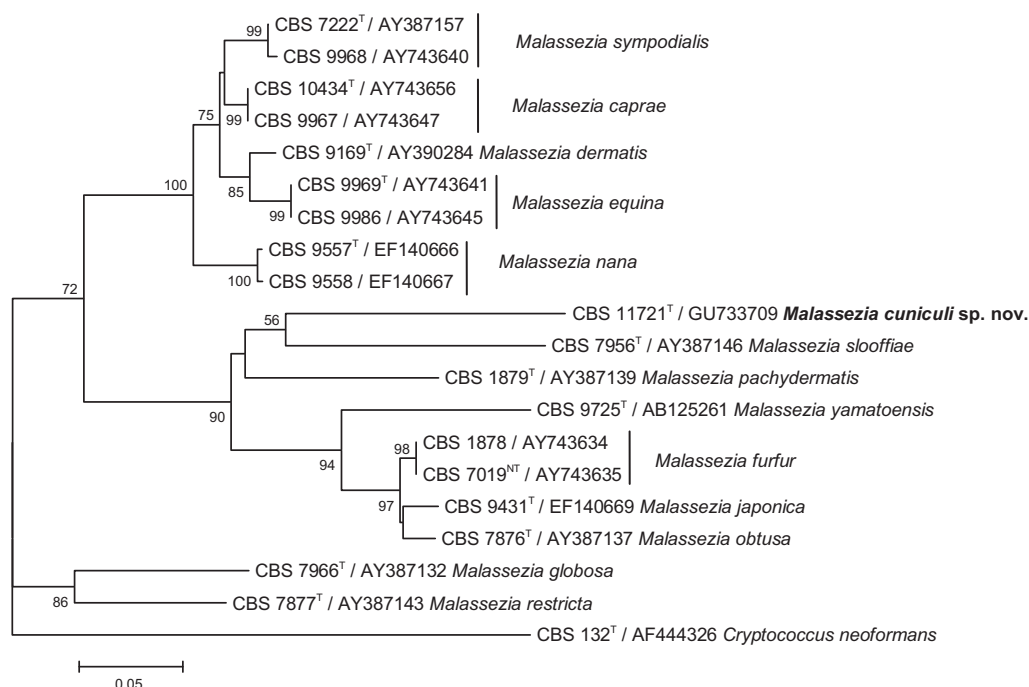


Fig. 4 Neighbour-joining tree based on Kimura 2-p corrected nucleotide distances among ITS-5.8S rRNA gene sequences of *Malassezia* species. Branch lengths are proportional to distance. Bootstrap replication frequencies over 50% (1,000 replications) are indicated at the nodes.

including oval, cylindrical or spherical cells, with buds formed on a broad base [8]. On the other hand, cells of species such as *M. globosa*, have a more stable shape, i.e., spherical with buds formed on a narrow base, similar to the cells of the strains proposed as new species in this study. However, the separation of *Malassezia* species based on morphological characteristics may be considered to be subjective [8] or unreliable [28].

The strains belonging to the new species showed some special lipid-dependence because they were only able to grow on LNA, a rich and complex culture medium. They were also not able to grow on glucose peptone agar supplemented only with Tweens (20, 40, 60 and 80) and Cremophor EL as sole sources of lipids. This may be related to the toxic effects of these compounds at higher concentrations. In fact, the absence of or the poor growth the isolates of the new species on mDA containing Tween 40 at 1%, may be related to its fungistatic properties. LNA is a more complex culture medium than mDA, containing among other components, Tween 60 at a 10-fold lower concentration (0.05%) than that used in the Tween dilution test proposed by Guého *et al.* [8]. The exact nutritional requirements of *Malassezia* species in culture remain to be determined [3,8].

The validation of this new species was supported by analysis of the D1/D2 regions of 26S rRNA gene and the ITS-5.8S rRNA gene, which confirmed the separa-

tion of this new species from the other proposed species of the genus. Phylogenetic analysis of sequences from these novel species showed that they were clearly distinct from the other 13 described *Malassezia* species, exceeding the variation generally observed to occur between species [29].

Malassezia species have, on rare occasions, been recovered from rodents [1]. Recently, a new *Malassezia* isolate from a hamster has been proposed as a new species based on the nucleotide sequence of 26S rRNA gene and ITS1 regions [22]. However, this isolate has not, as of yet, been validly described nor has its physiological characteristics been presented [22]. In the present study, we compared the 26S rRNA sequence deposited in Genbank for this hamster isolate and the percentage of dissimilarity between this sequence and our proposed *M. cuniculi* in their D1/D2 regions of 26S rRNA gene was more than 6%. It was not possible to compare the complete ITS-5.8S rRNA gene sequence because only a partial sequence of this region (FJ 231746) has been submitted. However, the dissimilarity between this hamster sequence (FJ 231746) and *M. cuniculi* in their ITS1 regions was 43.5%.

Our study confirms the presence of *Malassezia* yeasts in lagomorphs. Results of this taxonomic study clearly showed that the yeast isolated from the skin of rabbits represented a hitherto undescribed species

within the genus *Malassezia*. Based on the phenotypic and genotypic data presented above we propose a new species in this genus for which the name *Malassezia cuniculi* sp. nov. is proposed. Here we formally describe this species.

Description of the proposed new species

Malassezia cuniculi Cabañes et Castellá, sp. nov.

Coloniae in agar LNA post 7 dies ad 32°C albida vel cremaea, glabra, hebetata, ceraceae, modice convexae, margine expresso (<0.5–1 mm diam). Cellulae globosae, 2–5 µm, e base angusta gemmantes. In agar glucoso-peptonico Tween 20 (10%), Tween 40 (0.5%), Tween 60 (0.5%) et Tween 80 (0.1%) addito non crescit. In agar mDA non vel paulum crescit. 40°C crescit. H₂O₂ hydrolysatur. Teleomorphus ignota. Typus CBS 11721 (=MA 1339); isolatus ex regione auditoria cuniculi; depositus in collectione zymotica Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

Malassezia cuniculi Cabañes & Castellá, sp. nov.

Mycobank MB 516662

Malassezia cuniculi (cu'ni.cu.li. L.n. cuniculus, rabbit; L. gen. n. cuniculi of the rabbit; this Latin-derived species epithet refers to the host animal from which the yeast was first isolated).

On Leeming and Notman medium, after 7 days at 32°C, colonies are very small (<0.5–1 mm in diameter), whitish to cream-coloured, dull with waxy consistency, raised-to-moderately convex with entire margins. Cells are spherical, 2–5 µm in diameter, with buds formed monopolarly on a narrow base. No growth is obtained on SGA and growth on mDA is weak or absent. No growth occurs on glucose-peptone agar with Tween 20 (10%), Tween 40 (0.5%), Tween 60 (0.5%) and Tween 80 (0.1%). Catalase reaction is positive and β-glucosidase activity is positive. No growth is observed on glucose-peptone agar with Cremophor EL. Good growth occurs at 37°C and at 40°C. The teleomorph is unknown.

The type strain CBS 11721 (originally strain MA 1339) was isolated from healthy skin of the external ear canal of a rabbit in Barcelona, Spain. The strain was deposited in the Collection of the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands as CBS 11721.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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