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Malassezia pachydermatis and *M nana* predominate amongst the cutaneous mycobiota of Sphynx cats

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Carriage of *Malassezia* species yeasts in healthy Sphynx cats was compared with that in Devon Rex cats (DRC), Cornish Rex cats (CRC) and domestic shorthair (DSH) cats. Swab samples from the external ear, anus and claw folds, and contact plate samples from the axillae and groins, were incubated on modified Dixon's agar at 32°C for 7 days. *Malassezia* species were isolated from all 18 Sphynx cats; *M pachydermatis* accounted for 118/140 isolates. Of 20 isolates of *M nana*, 16 were recovered from the ear canal. *M slooffiae* was isolated from the claw fold of one cat and the left groin of another. The high counts of *M pachydermatis* obtained from the axillae, groins and claw folds of the Sphynx cats exceeded those of healthy DSH, CRC and DRC; axillary populations were comparable to those of seborrhoeic DRC. These data support recent reports of high *Malassezia* species colonisation in Sphynx cats.

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The skin of the domestic cat is colonised by a diverse array of *Malassezia* species yeasts. Whilst the non-lipid-dependent species *Malassezia pachydermatis* is most frequent, the lipid-dependent species isolated from cats include *Malassezia sympodialis*,[1,2] *Malassezia globosa*,[2] *Malassezia furfur*,[3] *Malassezia nana*,[4,5] and more recently, *Malassezia slooffiae*.[6–8] Dermatitis associated with *Malassezia* species in cats is often reported in association with allergic skin diseases or systemic disorders such as endocrine and metabolic diseases, neoplasia, infection with feline leukaemia virus (FeLV) or feline immunodeficiency virus (FIV).[8–12] Devon Rex cats (DRC) that are otherwise healthy may also present with an *M pachydermatis*-associated seborrhoeic dermatitis,[6,13] whereas Cornish Rex cats (CRC) have *Malassezia* species populations comparable to those of domestic shorthair (DSH) cats.[7]

The Sphynx is a breed of cat that is almost hairless and anecdotally reported to be 'oily'[14] and 'greasy'.[15] Phylogenetic analyses of composite short tandem repeat (STR) and single nucleotide polymorphism (SNP) genotypes demonstrated a very close breed relationship between Sphynx and DRC.[16] In a recent study, the claw fold of the single Sphynx cat sampled was shown to be colonised by *M pachydermatis*, *M furfur* and *M sympodialis*.[17] A very recent study of 32 Sphynx cats in Sweden showed a high rate of *M pachydermatis* colonisation.[15] A small number of lipid-dependent *Malassezia* species isolates were obtained, identified as *M sympodialis* ($n = 2$) and *M slooffiae* ($n = 1$) but two isolates could not be identified by the phenotypical methods used,[15] in accordance with several previous reports of difficulties in identifying lipid-dependent *Malassezia* species isolates using physiological tests, especially from animal hosts.[8,18–22]

We hypothesised that the carriage rates of *Malassezia* species yeasts in Sphynx cats in the UK would exceed those of DSH cats, and that lipophilic species would co-exist with *M pachydermatis* in these cats. The purpose of this study was to compare the frequency of isolation and population sizes of *Malassezia* species in Sphynx cats with healthy CRC, healthy DSH and healthy and diseased DRC, and to identify any lipid-dependent *Malassezia* species that might be isolated. The statistical relationships between *Malassezia* species population sizes in Rex and DSH cats have been previously described,[7] and, therefore, this report is focused on differences between these breeds and the Sphynx cats.

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Materials and methods

Cats

The use of the cats was approved by the Royal Veterinary College's Ethics and Welfare Committee and written informed consent from the owner was obtained before sampling.

Sphynx cats were recruited by contacting members of the Sphynx Cat Association. Cats were excluded if they were unwell, had been shampooed in the previous 2 weeks, or had received antifungal or immunosuppressive therapy in the previous 5 months. Data from these cats were compared with those obtained from 33 CRC (aged 2 months − 16 years, 28 females, five males) 21 healthy DRC (aged 1–13 years, 15 females, six males), nine DRC with clinical signs of localised or generalised seborrhoeic skin disease (aged 1–13 years, five females, four males) and 10 healthy DSH cats, comprising one neutered male and nine neutered females, aged 4–12 years.[7] Our data were also compared with data from 32 Sphynx cats in Sweden (aged 7 months–5 years, 14 males, 18 females), five of which had prominent greasy seborrhoea.[15]

Skin sample collection

The skin of the left and right axilla and groin was sampled using contact plates comprising bijou bottle lids filled to the meniscus with modified Dixon's agar and processed as previously described[6]; yeast counts were expressed as colony-forming units (CFU) per cm^2 of skin. The anus, left and right external ear canal, and a claw fold on digit III on the left and right forefoot were swabbed for 5 s using mini-tipped swabs and the samples processed as previously described[6]; yeast counts were expressed as $\log_{10}(\text{CFU swab}^{-1} + 1)$.

Yeast identification

M pachydermatis was identified by gross colonial and microscopical morphology, and on the ability to grow when subcultured on Sabouraud's dextrose agar (65 g/l, Oxoid CM0041, Basingstoke, UK) at 32°C. Lipid-dependent *Malassezia* species were identified by gross colonial and microscopical morphology, and in this instance failure to grow when subcultured on Sabouraud's dextrose agar at 32°C. Lipid-dependent isolates were identified by comparing the sequences of the D1/D2 regions of the *26S rRNA* gene amplified using the conserved fungal primers NL1 and NL4[7,23] with those available in GenBank/DNA Databank of Japan using the BLAST programme (www.ncbi.nlm.nih.gov/blast; http://blast.ddbj.nig.ac.jp).

Statistical analyses

Population sizes of *M pachydermatis*, *M slooffiae* and *M nana* were compared between breeds, body regions, and between healthy and seborrhoeic cats using the Mann–Whitney *U*-test of association and the statistical software SPSS 15 for Windows (SPSS, Chicago, IL, USA). Contingency tables and the survey commands of STATA Intercooled 9.2 for Windows (Stata Corporation, College Station, TX, USA) were used to identify associations between the frequency of isolation of *M pachydermatis*, *M slooffiae* and *M nana* isolates and breeds, body regions sampled, and healthy or seborrhoeic cats.

Results

Cats

Eighteen Sphynx cats (aged 5 months–6 years [median 2 years], nine females, nine males) that were in good general health were sampled in three different homes. The cats were noted to have the typical hairlessness of this breed, and a tendency to greasy skin which the owners managed by intermittent bathing with various products, including colloidal-oatmeal or evening primrose oil shampoos, or dish-washing detergent. None had a tendency to significant inflammatory skin disease or greasy seborrhoea. None had been shampooed in the previous 2 weeks and none had received antifungal or immunosuppressive therapy in the previous 5 months.

Yeast isolates

Of the 140 *Malassezia* species isolates obtained, 118 were examples of *M pachydermatis* and 22 were lipid-dependent isolates that formed small, yellow, domed colonies, without precipitates on modified Dixon's agar but failed to grow on Sabouraud's dextrose agar. Twenty of the lipid-dependent isolates were examples of *M nana* (Table 1), with D1/D2 sequences that were identical to that of the *M nana* type culture CBS 9557 (GenBank accession number GQ129203), to 12 UK *M nana* isolates from cats (GenBank accession numbers GQ129204–GQ129206, EU687496–EU687504) and to six Spanish *M nana* isolates (AY743608–AY743611, AY743613–AY743614).[7,24] Two of the lipid-dependent isolates were examples of *M slooffiae* with D1/D2 sequences that were identical to each other and to those of 9/10 *M slooffiae* isolates from cats in our collection (Genbank accession numbers EU687505–EU687507, EU687509–EU687514). The D1/D2 sequences of these isolates differed by 5/559 or 575 bases from those of several type cultures of *M slooffiae* obtained from humans (CBS 7972, AY387247; CBS 7975, AY387245; CBS 7956, AY743606), and by six bases from another cat-derived *M slooffiae* in our collection (DR2C, EU687508).

Large cream-coloured, pasty colonies composed of spherical yeasts resembling *Candida* species were noted on the primary isolation plates from the left claw of cat 17 and the left and right claw of cat 18. These isolates had identical D1/D2 sequences with

Table 1. GenBank accession numbers for the sequences of the D1/D2 regions of the *26S rRNA* gene of the lipid-dependent *Malassezia* species and *Candida* species isolated from Sphynx cats.

Strain	Source	Identification	D1/D2 GenBank accession number
SC2RE	Right ear	*M nana*	HM134160
SC3LE	Left ear	*M nana*	HM134161
SC5LE	Left claw	*M nana*	HM134162
SC5RE	Right ear	*M nana*	HM134163
SC6LE	Left ear	*M nana*	HM134164
SC6RE	Right ear	*M nana*	HM134165
SC7LE	Left ear	*M nana*	HM134166
SC7RE	Right ear	*M nana*	HM134167
SC7LAX	Left axilla	*M nana*	HM134168
SC8LE	Right ear	*M nana*	HM134169
SC8RE	Right ear	*M nana*	HM134170
SC8LG	Left groin	*M nana*	HM134171
SC9LE	Left ear	*M nana*	HM134172
SC10LE	Left ear	*M nana*	HM134173
SC10RE	Right ear	*M nana*	HM134174
SC11LC	Left claw fold	*M nana*	HM134175
SC11RAX	Right axilla	*M nana*	HM134176
SC12LE	Left ear	*M nana*	HM134177
SC13RE	Right ear	*M nana*	HM134178
SC14RE	Right ear	*M nana*	HM134179
SC3LC	Left claw fold	*M slooffiae*	HM134158
SC7LG	Left groin	*M slooffiae*	HM134159
SC17LC	Left claw fold	*C albicans*	HM134155
SC18LC	Left claw fold	*C albicans*	HM134156
SC18RC	Right claw fold	*C albicans*	HM134157

100% matches with sequences of *C albicans* ATCC 14503 (GU319992) and *C albicans* CBS 562 (U45776).

Association between Malassezia *species and breed*

The frequency of isolation of the different *Malassezia* species was significantly associated with breed of cat (Fisher's exact test: $P < 0.001$; Table 2). All Sphynx cats were colonised by one or more species of *Malassezia*, contrasting with only 50% of the DSH cats and 61% of the CRC. *M pachydermatis* was the most frequently isolated yeast in all breeds, and was isolated from all the Sphynx cats. Remarkably, 12 Sphynx cats (66%) were also colonised by the lipid-dependent species *M nana*, and two of these 12 were also colonised by *M slooffiae*. The frequency of isolation of lipid-dependent *Malassezia* species from the UK Sphynx cats (12/18) significantly exceeded ($P < 0.001$) that reported amongst Swedish Sphynx cats (5/32).

Association between Malassezia *species and body region*

M pachydermatis was frequently isolated from the axilla (left, 89% of Sphynx cats; right, 94%), groin (left, 78%; right, 83%), and claw (left, 89%; right, 94%), but infrequently from the anus (22%), and ear (left, 11%; right, 17%). *M nana* was quite often isolated from the ear canal (left, 44%; right, 44%), but infrequently from other sites (left axilla, 6%; right axilla, 6%; left groin, 6%; left claw, 6%). *M slooffiae* was isolated from two sites only (left groin, 6%; left claw, 6%).

Population sizes of *M pachydermatis* isolated from the ear and right groin did not differ significantly between Sphynx cats and the other breeds (Mann–Whitney *U*-test: $P > 0.05$) (Table 3). Significantly larger populations of *M pachydermatis* were isolated from the claw of healthy Sphynx cats than from healthy CRC, DRC and DSH cats (Mann–Whitney *U*-test: all $P < 0.001$) but these were significantly lower than those of diseased DRC (Mann–Whitney *U*-test: $P = 0.02$) even though this difference in population size was small.

Population sizes of *M pachydermatis* isolated from the anus differed significantly between Sphynx and CRC, DSH (Mann–Whitney *U*-test: all $P < 0.001$), healthy DRC ($P = 0.001$) and diseased DRC ($P = 0.009$). *M pachydermatis* counts from the left and right axillae of Sphynx cats were significantly larger than those of healthy CRC and DRC (both $P < 0.001$), but were comparable to those of seborrhoeic DRC ($P = 0.145$ and $P = 0.463$, respectively). *M pachydermatis* populations isolated from the left groin in Sphynx cats exceeded those of CRC

Table 2. Frequency of isolation of *Malassezia pachydermatis*, *Malassezia slooffiae* and *Malassezia nana* from healthy CRC ($n = 33$), DRC ($n = 21$), DSH ($n = 10$) and Sphynx ($n = 17$) cats, and seborrhoeic DRC ($n = 9$).

Malassezia species	Healthy				Seborrhoeic
	Sphynx *N* (%)	Cornish Rex *N* (%)	DSH *N* (%)	Devon Rex *N* (%)	Devon Rex *N* (%)
No *Malassezia* species	0	20 (61)	5 (50)	3 (14)	0
M pachydermatis	6 (33)	5 (15)	4 (40)	13 (62)	6 (67)
M slooffiae	0	1 (3)	1 (10)	1 (5)	0
M nana	0	1 (3)	0	0	0
M pachydermatis and *M slooffiae*	0	0	0	4 (19)	3 (33)
M pachydermatis and *M nana*	10 (55)	5 (15)	0	0	0
M slooffiae and *M nana*	0	0	0	0	0
M pachydermatis, *M slooffiae* and *M nana*	2 (11)	1 (3)	0	0	0
Total	18 (100)	33 (100)	10 (100)	21 (100)	9 (100)

($P < 0.001$), healthy DRC ($P = 0.007$) and DSH cats ($P = 0.001$), but were lower than those of seborrhoeic DRC ($P = 0.012$).

Discussion

The results of the present study confirm and extend many of the recent observations on the cutaneous mycobiota of Sphynx cats from Sweden, although there are also some important differences. The frequent isolation of high counts of *M pachydermatis* from the axillae and groin of the Sphynx cats in both UK and Sweden is striking, and contrasts markedly with its infrequent isolation from the same sites in CRC and DSH cats. Sphynx cats appear to share the same tendency to *M pachydermatis* skin colonisation noted in DRC. It has been suggested that the recessive gene *hr* implicated in the hypotrichosis of Sphynx cats is either closely linked to, or an allele of, the Devon Rex gene *re*, but this is distinct from the Cornish Rex trait, denoted *r*.[25] In view of the close phylogenetic relatedness of the Sphynx and DRC,[16] it is possible that similar or identical genetic factor(s) in DRC and Sphynx cats linked to these haircoat genes promote *M pachydermatis* colonisation in these breeds.

It has been clearly established that *M pachydermatis* is the most frequent *Malassezia* species found in the skin and external ear canal in both healthy cats and in cats with skin disease or otitis externa, but lipid-dependent *Malassezia* species have also been commonly isolated, albeit at a lower frequency, in studies where lipid-supplemented mycological media have been used.[1,2,6,8,13,15,26–29] *M nana* was first reported in 2002,[5] and named and described in more detail in 2004.[4] It has been isolated from the ear canals and skin of cats from Japan and Europe,[4,5,7,8,19] and from Brazilian cattle with or without otitis externa.[4] *M nana* is closely related to *M sympodialis* and has similar features in phenotypical testing, but has distinctive D1/D2 and internal transcribed spacer ITS-1 rDNA nucleotide sequences.[4,19,24] *M nana* was not reported in several recent studies of *Malassezia* species colonisation in cats where purely phenotypical methods were used in yeast identification,[15,26,29,30] whereas its presence has been confirmed by molecular techniques in our laboratory in studies of hyperthyroid cats,[8] CRC,[7] and now Sphynx cats, where it represented more than 90% of the lipophilic isolates. The more frequent occurrence of lipid-dependent *Malassezia* species in UK rather than Swedish Sphynx cats might reflect genetic or environmental differences, although the failure to identify *M nana* in the Swedish study could also reflect the phenotypical methods used in yeast identification.[4,15,19]

Malassezia species colonisation of the external ear canal was not reported in any of the 32 Sphynx cats sampled in Sweden, in marked contrast to the 11/18 Sphynx cats in the present study. Although *M nana* may be isolated from the anus, claw fold, axilla or groin, 75% of 32 isolations from cats by our laboratory originated from the external ear canal,[7,8] suggesting that this yeast is adapted to this anatomical site. The more sporadic isolation of *M slooffiae* with only two isolations in the present study, was in accordance with the single isolation in the Swedish study.[15] The claw fold has emerged as the principle location of this yeast on feline skin; 83% of the 18 isolations by our laboratory originated from this site, although it may also be found in the axilla, groin or anus.[6–8]

C albicans is infrequently isolated from the skin of healthy cats; Moriello and BeDoer isolated a *Candida* species from the haircoat of only 1/172 healthy pet cats,[31] and Mancianti et al isolated *C albicans* from either the oropharynx or haircoat of only 2/55 healthy cats.[32] There is conflicting data on whether retroviral

Table 3. Population sizes (median, maximum and interquartile range [IQR]) of *Malassezia pachydermatis* isolated from the ear, claw and anus using a swab method ($\log_{10}$[CFU swab^{-1} + 1]) and from the axilla and groin using a contact plate method (CFU cm^2) of sebborrhoeic DRC and healthy CRC, DSH cats, Sphynx and DRC.

Body	Healthy										Sebborrhoeic		
Region	Sphynx			Cornish Rex			DSH			Devon Rex			Devon Rex		
	Median	Max*	IQR	Median	Max*	IQR	Median	Max*	IQR	Median	Max*	IQR	Median	Max*	IQR
Ear	0	1.91	0	0	1.61	0		Not isolated		0	1.61	0	0	1.91	0
Claw	3.17	5.57	1.35	0[c]	3.43	0	0[c]	3.08	0	0[c]	5.18	0	3.90[a]	5.38	0.85
Anus	0	3.27	0.4	0	3.86	0	0	2.08	1.91	0	1.61	0	0	3.22	1.28
Left axilla	6.69	69.43	22.61	0[c]	2.55	0		Not isolated		0[c]	26.43	0.48	35.99[†]	79.62	75.80
Right axilla	15.61	79.62	36.94	0[c]	2.55	0		Not isolated		0[c]	17.20	0.48	38.54	79.62	76.76
Left groin	7.01	79.62	29.86	0[c]	6.37	0	0[b]	0.32	0	0[b]	66.24	0.64	79.62[a]	79.62	64.34
Right groin	13.22	79.62	32.89	0	3.18	0.16		Not isolated		0.32[†]	64.01	0.96	79.62	79.62	32.65

Comparison with Sphynx cats, [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$.
*Minimum population size for all body regions = 0 CFU cm^2 or 0 $\log_{10}$[CFU swab^{-1} + 1], unless specified otherwise.
[†]Minimum population size = 0.32 CFU cm^2.

infections favour skin and mucosal carriage of *C albicans* in cats.[12,32] *C albicans* is an extremely rare cause of skin disease in cats, although it may cause infection of the urinary tract and other organ systems in cats with local or systemic immune compromise.[33,34] The rDNA sequence homology between the Sphynx cat and human isolates is in accordance with a previous study that was unable to demonstrate host-specific genotypes and species-specific lineages amongst *C albicans*, indicating that the feline claw fold might potentially represent a reservoir for human *Candida* species infection.[35]

Further studies are required to identify the genetic, immunological and physiological factors that favour skin colonisation by *Malassezia* species in DRC and Sphynx cats.

Conflict of interest

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