



Original Article

***Malassezia* species dysbiosis in natural and allergen-induced atopic dermatitis in dogs**

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Abstract

Malassezia dermatitis and otitis are recurrent features of canine atopic dermatitis, increasing the cost of care, and contributing to a reduced quality of life for the pet. The exact pathogenesis of secondary yeast infections in allergic dogs remains unclear, but some have proposed an overgrowth of *M. pachydermatis* to be one of the flare factors. The distribution of *Malassezia* populations on healthy and allergic canine skin has not been previously investigated using culture-independent methods. Skin swabs were collected from healthy, naturally affected allergic, and experimentally sensitized atopic dogs. From the extracted DNA, fungal next-generation sequencing (NGS) targeting the ITS region with phylogenetic analysis of sequences for species level classification, and *Malassezia* species-specific quantitative real-time polymerase chain reaction (qPCR) were performed. *M. globosa* was significantly more abundant on healthy canine skin by both methods (NGS $P < .0001$, qPCR $P < .0001$). *M. restricta* was significantly more abundant on healthy skin by NGS ($P = .0023$), and *M. pachydermatis* was significantly more abundant on naturally-affected allergic skin by NGS ($P < .0001$) and on allergen-induced atopic skin lesions by qPCR ($P = .0015$). Shifts in *Malassezia* populations were not observed in correlation with the development of allergen-induced skin lesions. Differences in the lipid dependency of predominant *Malassezia* commensals between groups suggests a role of the skin lipid content in driving community composition and raises questions of whether targeting skin lipids with therapeutics could promote healthy *Malassezia* populations on canine skin.

Key words: *Malassezia*, canine atopic dermatitis, fungal microbiome, mycobiota, skin.

Introduction

Next-generation sequencing (NGS) technologies have revolutionized the study of the skin microbiota. Perturbations to the commensal microbiota (dysbiosis) are now associated with numerous cutaneous diseases.¹ Inflammatory conditions such as human and canine atopic dermatitis (AD) have been of particular interest owing to secondary infections that affect atopic individuals.² Recent studies have shown that both human³ and canine^{4,5} AD flares of inflammation coincide with increasing abundances of *Staphylococcus* and progressing lesions. Looking beyond the bacterial microbiota, a dysbiosis of the fungal mi-

crobiota (mycobiota) has also now been documented in human AD,⁶ psoriasis,⁷ chronic wounds,⁸ seborrheic dermatitis,⁹ and canine AD.¹⁰ These cutaneous diseases are often multifactorial and are thought to involve an interplay between the host, the environment, and the microbiota. However, much is still unknown regarding the exact mechanism of disease pathogenesis.^{11–14}

Many parallels exist between human and canine AD including transepidermal water loss (TEWL), ceramide alterations, and microbial dysbiosis.^{2,11} Secondary yeast infections are common in canine AD, among other conditions in both people and dogs.¹⁵ For many atopic dogs, specifically *Malassezia* dermatitis

and/or otitis are chronic and recurrent features of their disease.¹¹ *Malassezia pachydermatis* is the most common etiologic agent of secondary yeast infections in allergic dogs, and previous work has demonstrated that *M. pachydermatis* is more abundant on the skin of dogs with allergies by both culture¹⁶ and cytology.¹⁷ One proposed explanation for the prevalence of *Malassezia* infections in atopic dogs is that these dogs harbor more *M. pachydermatis* on their skin. Other research has focused on pathogen virulence traits and identified an association between the disease severity and the specific type of phospholipases produced by *M. pachydermatis* strains.^{18–21}

Our group conducted the first fungal NGS study of healthy and allergic canine skin but was unable to identify an increased relative abundance of *Malassezia* yeast (genus level) in the nonlesional skin of naturally affected allergic dogs.¹⁰ The discrepancy between our NGS results and other culture-dependent studies demonstrating increased *M. pachydermatis* are thought to be due to differences in methodology. However, an alternative explanation is that a dysbiosis might be present at the species level in allergic dogs. Findley et al. found that even though *Malassezia* was the predominant fungal commensal across all human body sites, excluding the feet, species level predilections existed between body sites.²² It has been long thought that *M. pachydermatis*, the formerly only nonlipid dependent species, is the predominant *Malassezia* commensal on canine skin. However, previous culture-dependent studies have isolated lipid-dependent *Malassezia* from both canine and feline skin,^{23–28} supporting our hypothesis that other *Malassezia* species may be present and influencing genus level abundances.

Another yet unexplored facet of canine AD is how flares of inflammation in the skin affect the mycobiota, especially *Malassezia*. An interaction between the mycobiota and the host's immune system has recently been documented, prompting more investigations into the role of the mycobiota in inflammatory conditions.^{29–33} Both atopic humans and dogs have been shown to be hypersensitive to *Malassezia* by patch testing,^{34,35} providing further evidence that *Malassezia* can interact with the host immune system in inflammatory skin conditions. Multiple longitudinal studies have shown that *Staphylococcus* bacteria increase during atopic flares in dogs,^{4,5} but these studies did not investigate changes to the mycobiota.

The first objective of this study was to measure the abundances of *Malassezia* species on healthy and nonlesional, naturally affected, allergic canine skin using a phylogenetic analysis of fungal NGS sequences and quantitative real-time polymerase chain reaction (PCR) as a confirmation. The second objective was to investigate longitudinal changes in *Malassezia* species abundances using identical methods in an allergen-induced canine model of acute AD skin lesions. We hypothesized that *Malassezia* dysbiosis exists in non-lesional allergic canine skin, and that flares of inflammation further amplifies opportunistic *M. pachydermatis* populations.

Methods

Study groups and criteria for selection

Client-owned dogs were previously enrolled into the healthy ($n = 10$) and nonlesional naturally affected allergic ($n = 8$) groups.¹⁰ The healthy dogs ranged in age from 1.5 to 11 years-old, included five spayed females and five castrated males, and were of variable breeds including Beagle (1), Boston Terrier (1), German Shepherd (1), Jack Russell Terrier (2), Mixed breed (4), and Pitbull (1). The allergic dogs ranged in age from 2 to 10 years-old, included four spayed females and four castrated males, and were of variable breeds including Australian Shepherd (1), Boston Terrier (2), King Charles Cavalier (2), Labrador Retriever (1), mixed breed (1), and Shetland Sheepdog (1). Dogs included in the allergic group were examined by a board-certified dermatologist and were diagnosed with cutaneous adverse food reactions, flea allergy dermatitis, environmental AD, and combinations thereof. Exclusion criteria included having received antibiotics or antifungals in the last month, receiving steroids in the last six (healthy) or one (allergic) month(s), having been bathed in the last week, or the presence of overt signs of skin infection at the time of sample collection. Allergic dogs were allowed to be managed with allergen immunotherapy, oclacitinib, or cyclosporine. Body sites sampled in both the healthy and nonlesional allergic skin groups included the axilla, ear canal, groin, interdigital space, lumbar, and nostril. All samples for these two study groups were collected following a protocol approved by the Texas A&M University Institutional Animal Care and Use Committee.

Purpose-bred Maltese-beagle dogs were included in the allergen induced atopic group ($n = 8$).⁴ These dogs will develop AD skin lesions after the epicutaneous application of *Dermatophagoides farinae* (Df) house dust mites (HDM). They ranged in age from 2 to 14 years-old and included four intact females, two spayed females, and two intact males. Dogs 2, 3, 5, 6, and dogs 7, 8 were closely related. They were primarily housed indoor but were allowed to be out of their runs at least twice daily with the runs being cleaned twice daily. They had not received antimicrobials, anti-inflammatory or immunosuppressive drugs in the preceding 3 months. These dogs were under the care of and scored by a board-certified veterinary dermatologist. All samples for this study group were collected following a protocol approved by the North Carolina State University Institutional Animal Care and Use Committee.

Timeline of HDM challenge

The HDM challenge was performed as previously described.⁴ The HDM solution contained 500 µg of lyophilized Df mite powder (Greer Laboratories; Lenioren, NC, USA). Twenty microliters of this solution was applied to the right groin every 24 hours for 2 (dog 6 developed severe lesions after only two applications) or

3 days. Skin swabs were taken prior to HDM application (time point 1, day 0), at lesion development (time point 2, day 2 or 3), and after lesion resolution (time point 3, 7 days post-lesion development). At 7 days post-lesion development all lesions in all dogs had resolved.

Sample collection and DNA extraction

All skin swabs included in this study had been previously collected and DNA extracted as described.^{4,10} Sterile swabs (Isohelix, Cell Projects Ltd., Kent, UK) were rubbed on the skin for a total of 20 times and placed in a sterile microcentrifuge tube that was stored at 4°C until extractions were performed. Genomic DNA was extracted from skin swabs using a commercially available kit (MoBio Power Soil DNA Isolation kit, Carlsbad, CA, USA). Negative controls included swab only and reagent only samples. Samples were stored for short term at -20°C and long term at -80°C.

NGS and bioinformatics

Fungal DNA from healthy and naturally affected allergic dogs was amplified using ITS1 fungal primers and sequenced on an Illumina Mi-Seq Instrument, as previously described.¹⁰ The fungal DNA from allergen-induced atopic lesions was amplified using ITS1 primers, with 40 cycles and sequenced on an Illumina Mi-Seq instrument (2 × 300) at the University of Minnesota Genomics Center. Mothur³⁶ and the Findley et al. 2013 databases were used to classify sequences to the genus level according to their recommended guidelines.²² Sequences were quality-filtered based on a quality score threshold of 25, chimeric sequences removed, and k-nearest neighbor algorithm used to classify sequences. Next, *Malassezia* sequences were extracted in mothur, aligned to a *Malassezia* reference tree in pplacer³⁷ using a reference package supplied by the Segre laboratory, and species level taxonomic strings exported using the guppy command. New taxonomic files were imported back into mothur for further downstream analysis including making taxa plots by body site, time point, and group. The relative abundances of *Malassezia* species are presented as relative to all fungal sequences in the main figures and used for statistical analysis. Alternatively, *Malassezia* species relative abundance is also presented in the supplementary figures as relative only to *Malassezia* genus-level sequences to minimize a potential bias introduced by variations in the copy number of the ITS gene amongst the fungal kingdom.

Real-time quantitative PCR

M. globosa (MYA-4612) and *M. restricta* (MYA-4611) ATCC strains, and a clinical isolate of *M. pachydermatis* from a dog were used to generate standard curves. All strains were grown on modified Dixon's agar for 2–7 days at 32°C and 5% CO₂. Plates

were scraped and genomic DNA was extracted with the MoBio Power soil kit, the same used for extracting DNA from skin swabs. Concentrations of DNA were measured in duplicate using Qubit high sensitivity kit on a CFX Connect Real-Time System (Bio-Rad Laboratories Inc., Hercules, CA, USA) instrument. ITS-1 PCR and conventional sequencing was performed to confirm identification of all standards (data not included). Primers employed for the qPCR were previously published³⁸; they included a common forward primer (ITS-ANA-F), and three reverse primers specific to each species targeted (GlobR, RestrR, PachyR). These primers were chosen because they target the ITS region, which was also targeted with NGS. Targeting the same gene using both methods theoretically would minimize the potential bias introduced from having two target genes with varying copy numbers. Standard curves consisted of five-to-six serial dilutions depending on the limit of detection for each primer pair. All standards, non-template controls, and samples were run in duplicate on a CFX Connect Real-Time System (Bio-Rad Laboratories Inc., Hercules, CA, USA) instrument. Reactions consisted of 5 µl Super mix, 0.5 µl forward primer, 0.5 µl reverse primer, 2 µl water, and 2 µl standard or sample for a total volume of 10 µl. Cycle parameters were identical for all three qPCRs: an initial heating step at 98°C for 3 minutes, and 40 cycles of 95.0°C for 30 second and 60°C for 30 seconds. The concentration of DNA for all samples was calculated from the standard curve equation generated by the CFX Connect software. Next, genome equivalents were calculated using the conversion of 1 pg of DNA being equivalent to 978 Mb and the published genome sizes of *M. globosa* (8.9 Mb), *M. restricta* (7.2 Mb), and *M. pachydermatis* (8.2 Mb).³⁹

Statistical analysis

The NGS relative abundances and qPCR genome equivalents were tested for normality using JMP Pro 14 software (SAS Institute, Inc., Cary, NC, USA) and the Shapiro-Wilk test. All data were not normally-distributed and the nonparametric Kruskal-Wallis test, was chosen for the subsequent analysis of healthy and naturally-affected allergic canine skin. A Friedman test with Dunn's *post hoc* test for repeated-measures ANOVA was chosen for the analysis of timepoints for allergen-induced skin lesions. A significance value of 5% was selected for all statistical tests. Relative abundances (NGS) and genome equivalents (qPCR) were tested for significant differences between individual dogs, body sites, timepoints (allergen induced time points 1, 2 and 3), and groups (healthy, natural allergic, allergen induced). When testing for differences between the three study groups, only the groin samples of healthy and natural allergic dogs, and only the first time point of allergen induced atopic dogs were compared. A Steel-Dwas All Pairs, nonparametric, test was used for determining the significance between pairs of individuals, body sites, or groups.

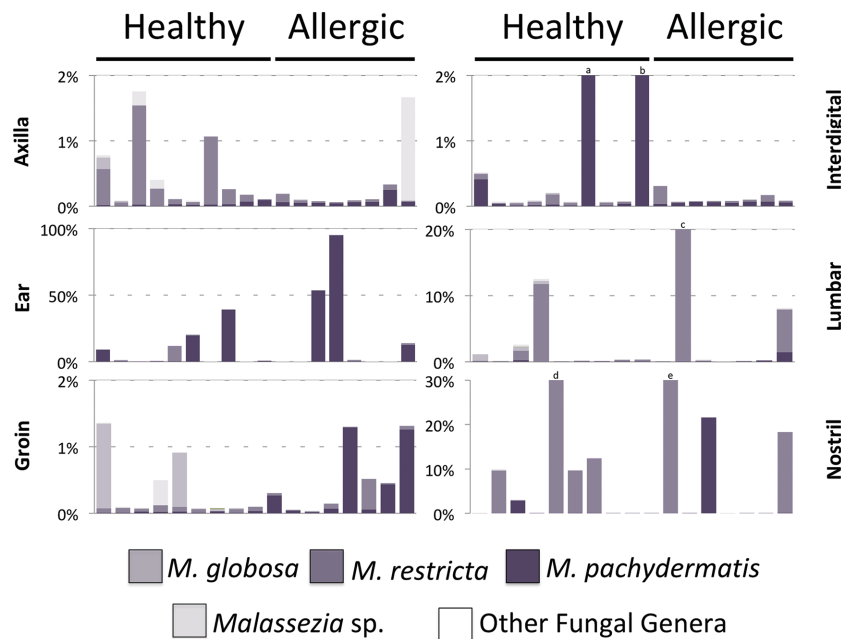


Figure 1. *Malassezia* spp. relative abundance for all healthy and naturally-affected allergic dogs by body site. Relative abundances of species are presented as relative to all fungal sequences, and colored according to the legend for each species. Each column represents one body site from one dog. Note that axes vary for better visualization and the following samples were greater than other samples but whose relative abundances are: (a) 19.3%, (b) 13.6%, (c) 31.4%, (d) 93.6%, (e) 31.0%.

Results

Malassezia populations of healthy and naturally-allergic canine skin

Next-generation sequencing

The extraction of *Malassezia* sequences from the NGS data set included 108 nonlesional skin samples from six body sites on 10 healthy and 8 naturally-affected allergic dogs yielded 151 793 sequences with an average of 1405 *Malassezia* sequences per sample. The *Malassezia* species sequenced in order of decreasing relative abundance were *M. restricta*, *M. pachydermatis*, *M. globosa*, and *M. obtusa* (Fig. 1). The Kruskal-Wallis test revealed that the relative abundance of *M. pachydermatis* (healthy range: 0–39.1, mean: 21.8; allergic range: 0–95, mean: 44.2; $P < .0001$) was significantly increased on non-lesional allergic skin, while *M. restricta* (healthy range: 0–94, mean: 32.7; allergic range: 0–31.4, mean: 2; $P = .0023$) and *M. globosa* (healthy range: 0–1.3, mean: 0.08; allergic range: 0–0.2, mean: 0; $P < .0001$) were significantly increased on healthy skin (Fig. 2). The body site was not an influencing factor in either healthy or allergic dogs; however, the population of *M. globosa* ($P = 0.0025$) varied significantly between healthy dogs and *M. pachydermatis* ($P = .0450$) between allergic dogs (Table 1). The relative abundances for all samples are presented in supplementary table S1. Relative abundances normalized by the number of *Malassezia* genus level sequences are presented in supplementary figures S1 and S2, as well as supplementary table S2.

Quantitative real time polymerase chain reaction

Species-specific qPCRs were performed for *M. pachydermatis*, *M. restricta* and *M. globosa* on all healthy and naturally-affected allergic dog skin samples. Genome equivalents were calculated using culture-derived standards and the genome size of these three species. Including all samples *M. restricta* genome equivalents (range: 0–3876, mean: 75) was the most abundant by this method, followed by *M. pachydermatis* (range: 0–244, mean: 5) and lastly *M. globosa* (range: 0–213, mean: 3). Kruskal-Wallis testing confirmed that *M. globosa* genome equivalents was significantly increased on healthy skin compared to that of allergic skin samples ($P < .0001$, Fig. 3). Genome equivalents of *M. restricta* were increased on healthy skin but this finding was non-significant, and genome equivalents of *M. pachydermatis* did not differ between groups. In addition to health status, *M. restricta* genome equivalents were found to significantly vary by body site in both healthy and allergic dog skin samples (Fig. 4). Body site was not an influencing factor on genome equivalents of *M. pachydermatis* or *M. globosa* (Table 1). The individual dog was identified as an influencing factor on genome equivalents of *M. restricta* for healthy skin samples, and *M. pachydermatis* for both healthy and allergic skin samples (Table 1). By Kruskal-Wallis testing, the individual was identified as the strongest influencing factor, followed by group, and lastly by body site. Correlation between NGS and qPCR are highlighted in Table 1. Genome equivalents for all healthy and nonlesional allergic dog samples are presented in the supplementary table S3.

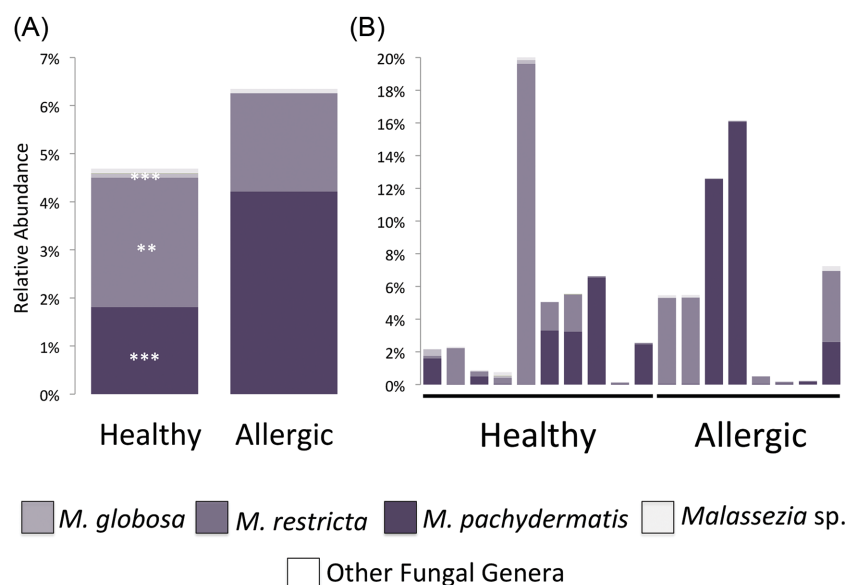


Figure 2. *Malassezia* spp. relative abundances by health status. Relative abundances of species are presented as relative to all fungal sequences and are averaged (A) across all samples in each group, or (B) across all body sites in an individual dog. Kruskal-wallis testing was performed on the average relative abundance of *Malassezia* species and *p*-values denoted in the figure with asterisks. **P* < .05, ***P* < .01, ****P* < .001.

Table 1. Level of significance for differences in abundances of *M. restricta*, *M. globosa* and *M. pachydermatis* between body sites, individuals, and study groups.

	<i>M. restricta</i>		<i>M. globosa</i>		<i>M. pachydermatis</i>	
	NGS	qPCR	NGS	qPCR	NGS	qPCR
Healthy v. allergic	**	—	***	***	***	—
Healthy body sites	—	**	—	—	—	—
Allergic body sites	—	*	—	—	—	—
Healthy individual	—	*	*	—	—	**
Allergic individual	—	—	—	—	*	***

P* < .05; *P* < .01; ****P* < .001. NGS, next-generation sequencing; qPCR, real-time quantitative polymerase chain reaction.

Malassezia populations during atopic dermatitis flares after challenge of sensitized dogs

Next-generation sequencing

Fungal NGS was performed on DNA extracted from skin swabs of a colony of allergen-challenged dogs, and 24 samples (8 dogs, 3 time points) yielded 2 108 463 fungal sequences. Kruskal-Wallis testing demonstrated that the relative abundances of *Malassezia* genus (range: 9.5–99.7, mean: 40.8) were significantly different between individual dogs (*P* = .0267), but not between time points (*P* = .8332).

Extraction of *Malassezia* sequences from the NGS data set including 24 samples (8 dogs, 3 time points) yielded 845 038 *Malassezia* sequences with an average of 31 971 per sample. The *Malassezia* species represented in order of decreasing relative abundance were *M. pachydermatis*, *M. restricta*, *M. obtusa*, *M. furfur*, *M. globosa*, *M. slooffiae* and *M. sympodialis* (Fig. 5). *M. pachydermatis* and *M. restricta* were considered the most abundant species, while the mean relative abundance of all other

species was less than 5%. Kruskal-Wallis testing demonstrated that species relative abundance significantly varied by individual but not time point (Table 2, Supplementary table S5). Relative abundances are found in Supplementary table S1 (relative to all fungal sequences) and Supplementary table S2 (relative to *Malassezia* genus level sequences).

Quantitative real-time polymerase chain reaction

Species-specific qPCRs were performed for *M. restricta* (range: 6–160, mean: 55) and *M. pachydermatis* (range: 0–252, mean: 39) on all 24 allergen-challenged skin swab samples (8 dogs, 3 time points). Neither species were found to vary significantly between time points by this method. The individual was a significant influencing factor on genome equivalents of *M. pachydermatis* (Fig. 6, Table 2, Supplementary table S6). Genome equivalents for all allergen challenged dog samples are presented in supplementary table S4.

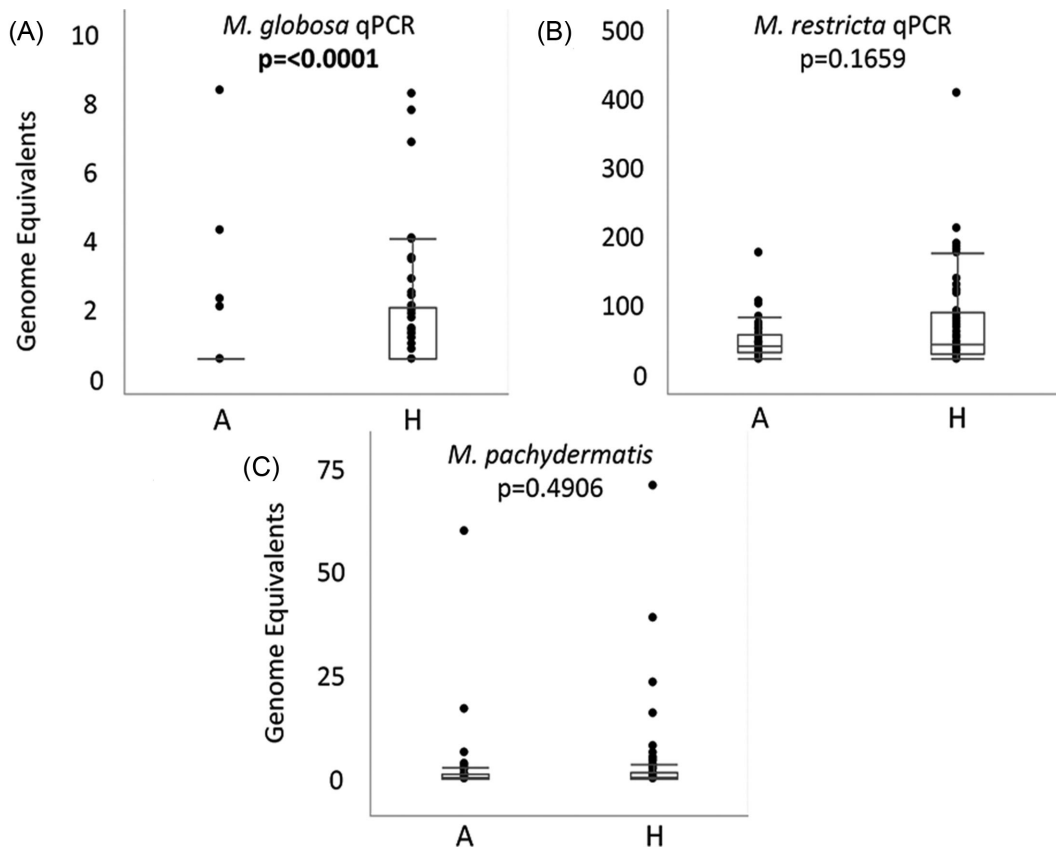


Figure 3. Genome equivalents of *M. globosa*, *M. restricta*, and *M. pachydermatis* by health status. All samples were included for comparison between healthy (H) and naturally affected allergic (A) dogs. Box plots represent the interquartile range. Kruskal-wallis testing was performed on the average genome equivalents of all three *Malassezia* species. *P*-values are reported as text within the figure. *P*-values less than 0.05 were considered significant.

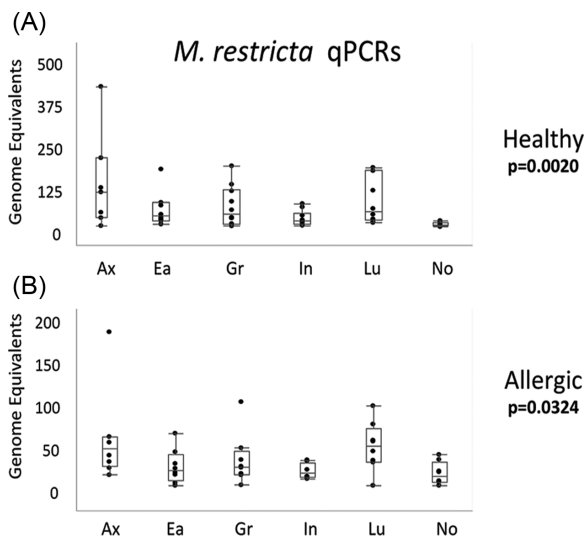


Figure 4. Genome equivalents of *M. restricta* vary by body site in both healthy and nonlesional allergic dogs. All samples were included for comparison between body sites of (A) healthy and (B) naturally affected allergic dogs. Ax, Axilla; Ea, Ear; Gr, Groin; In, Interdigital; Lu, Lumbar; and No, Nostril. Box plots represent the interquartile range.

Evaluation of baseline *Malassezia* populations between all three study groups

Direct comparisons using statistical analysis of NGS relative abundances between the three study groups were not performed due to the NGS being performed at different sequencing facilities and on different days. Comparison of genome equivalents, which was performed on the same plates, between all three study groups (healthy groin, naturally affected allergic groin, pre-HDM challenged atopic groin) demonstrated that *M. pachydermatis* was significantly increased by eightfold on dogs with experimental AD (Fig. 7, $P = .0015$). *M. restricta* was not significantly different between the three study groups.

Discussion

For the first time, we have examined cutaneous *Malassezia* species populations in dogs using a combination of culture-independent methods (NGS and qPCR). One of the most intriguing findings from this study is that healthy canine skin is dominated by the lipid-dependent yeast, *M. restricta* and *M. globosa*. The veterinary literature tends to focus on *M. pachydermatis* with fewer investigations of these other yeast. Cafarchia et al. found that lipid-dependent yeast were more commonly isolated

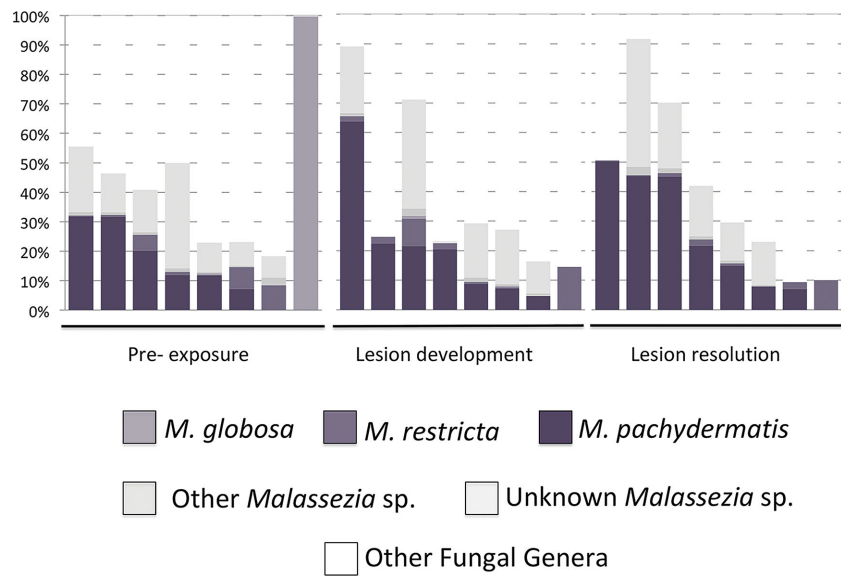


Figure 5. *Malassezia* spp. relative abundances during atopic lesion development and resolution of HDM-sensitized laboratory dogs. Relative abundances of species are presented as relative to all fungal sequences, and colored according to the legend for each species. Each column represents on dog at one time point. The columns are ordered by decreasing relative abundance of *M. pachydermatis*.

Table 2. Level of significance for differences in abundance between individual and time points in allergen challenged dogs.

	<i>Malassezia</i> NGS	<i>M. pachydermatis</i>		<i>M. restricta</i>	
		NGS	qPCR	NGS	qPCR
Individual	*	—	**	*	—
Time	—	—	—	—	—

P* < .05; *P* < .01; ****P* < .001. NGS, next-generation sequencing; qPCR, real-time quantitative polymerase chain reaction.

from healthy compared to allergic canine skin, but the species identification was not carried out.²⁶ A more recent culture-dependent investigation isolated both *M. restricta* and *M. globosa* from healthy canine skin at greater rates compared to *M. pachydermatis*.²⁸ Increased abundances of *M. globosa* on healthy canine skin are of particular interest because researchers have shown recently that this yeast appears to play a beneficial role to human hosts.⁴⁰ These authors found that *M. globosa* isolated from human skin secretes an aspartyl protease with antibiofilm activities against *Staphylococcus aureus*, a known opportunistic pathogen in human atopic dermatitis flares. It is not known whether *M. globosa* has a similar action on *S. pseudintermedius*, the opportunistic pathogen in canine AD.

The skin lipid content is known to be reduced in canine AD^{41,42} and this might explain why *M. globosa*, which has a very strict lipid dependency, thrives on healthy but not atopic canine skin. This particular dysbiosis could provide a niche for *M. pachydermatis* with a more versatile ability to metabolize a broader range of lipids⁴³ and contribute to its overgrowth on atopic canine skin. A recent lipidomics assay demonstrated that

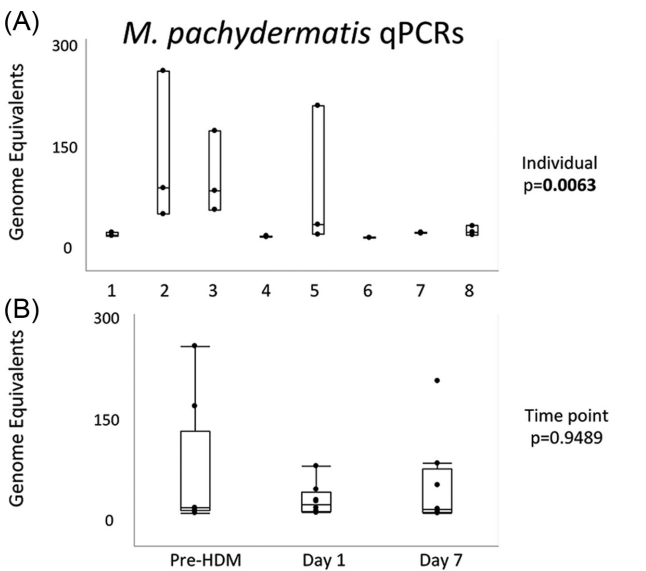


Figure 6. Genome equivalents of *M. pachydermatis* on HDM-sensitized dogs, before and during lesion development and resolution. All samples are grouped by (A) individual dog, or (B) time point where Pre-HDM is the baseline measurement prior to exposure to HDM, Day 1 is the first day of lesion development, and day 7 is at lesion resolution. Box plots represent the interquartile range.

triglycerides are significantly more abundant on the forehead of healthy human individuals where *Malassezia* predominates as a fungal commensal, as compared to the feet that has a lower triglyceride content and significantly less *Malassezia* yeast.⁴⁴ Future studies performing simultaneous analysis of skin lipidomics and fungal microbiota in healthy and allergic dogs may validate the hypothesis that the skin lipid content influences which yeast are the abundant colonizers.

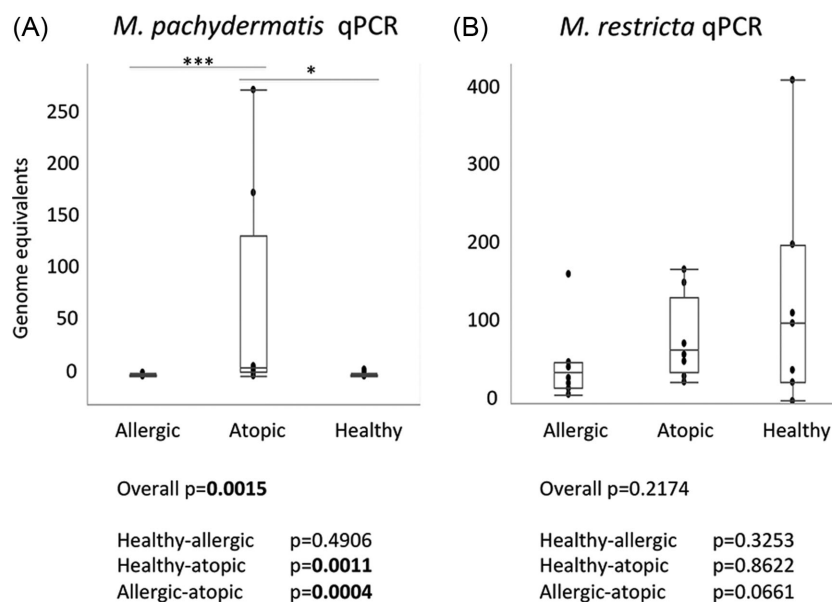


Figure 7. Baseline genome equivalents of *M. pachydermatis* and *M. restricta* across three study groups. Only the groin samples from healthy and naturally affected allergic (Allergic) dogs, and the Pre-HDM (day 0) samples from experimental atopic (Atopic) dogs were included. The overall P -value was generated with the Kruskal-Wallis statistical test, and pair-wise P -values with the Steel-Dwass all pairs test. Box plots represent the interquartile range.

We previously identified *Malassezia* as representing on average 6% of the skin mycobiota in healthy and non-lesional naturally affected allergic dogs but presently found that laboratory dogs with experimental AD have on average 41% relative abundance of *Malassezia* on their skin by NGS. A variety of factors could contribute to this phenomenon. One potential cause for smaller abundances of *Malassezia* on the client-owned allergic dogs could be that 5/8 of them were on immunomodulatory medications that theoretically were reducing the amount of inflammation in the skin compared to the colony dogs that were not receiving any allergy medications. This colony of dogs is highly genetically-related, and recent work has shown that the breed or genetic makeup of an animal has an influence on their skin microbiota.^{45,46} Half of the laboratory dogs (2, 3, 4, and 5) in this study tended to have more *Malassezia* and *M. pachydermatis* by both NGS and qPCR. These dogs were also more closely related. Additionally, these laboratory dogs are kept mostly indoors with increased humidity and greater environmental hygiene compared to client-owned animals. It has been proposed that hygiene could play a significant factor in why people harbor more *Malassezia* on their skin compared to dogs and cats that have greater exposure to the environment and decreased hygiene practices.⁴⁷ Lastly, as part of this colony's animal welfare regimen, these dogs are fed a diet enriched in fatty acids that have been shown to enhance the skin barrier in atopic dogs. Another recent study showed that *Malassezia* abundances by qPCR increased with the application of a topical essential oil and fatty acid-rich product⁴⁸ further supporting the idea that lipid supplementation could be influencing the abundance of lipid metabolizing yeast on the skin.

Our previous work on the mycobiota of dogs and cats found that the mycobiota was more strongly influenced by the individ-

ual than by body site in healthy animals compared to allergic animals. Interestingly we found the same trend at the genus and species levels for *Malassezia* in this study. We previously proposed that the mycobiota tends to be more unique to the individual when there is lack of skin disease. Conversely, in allergic individuals this unique signature is lost, likely due to inflammatory mediators, shifting the mycobiota to appear more influenced by body sites where allergic skin disease is manifested. Body site was only an influencing factor for *M. restricta* abundance; however, this was the most abundant species across all study groups.

There were clear alterations to the abundance of *Malassezia* species in client-owned naturally affected allergic dogs, which may indicate a widespread predisposition to *M. pachydermatis* dermatitis in allergic dogs. However, changes to *Malassezia* during flares of inflammation as assessed by the longitudinal study of allergen challenged dogs were less clear. We identified two trends following exposure to HDM allergens in these sensitized dogs: increasing or decreasing abundances of *M. pachydermatis*. These trends could be explained by some allergic dogs being more prone to *Malassezia* dermatitis than others. Canine AD can have variable presentations, which have been attributed to its multifactorial pathogenesis.¹¹ The exact prevalence rate of *Staphylococcus* pyoderma or *Malassezia* dermatitis in allergic dogs is not currently known. For some dogs these will occur simultaneously, but in other dogs their disease will be predominated by one or the other.

Advantages and limitations exist for both culture-dependent and -independent techniques. Studies are underway to determine the exact lipid preferences of each *Malassezia* species and may lead to development of more robust culture methodologies. Currently with such vast differences in the ability of *Malassezia* spp.

to grow in culture, quantitatively comparing the culture derived abundances of *Malassezia* species between samples introduces bias. Molecular methods offer an advantage for mycobiota studies, however, we still do not know the exact biases introduced by copy number variation in the ITS region, primer efficiencies or contaminating fungal DNA since culture-independent methods cannot distinguish between dead and alive yeast.⁴⁹ Upon publishing complete *Malassezia* genomes, Dawson and colleagues were able to show that some reports of *Malassezia* in metagenomic data sets was likely inaccurate.³⁹ Future studies using metagenomics might better confirm the results presented in this study; however, corroboration between NGS and qPCR currently supports our conclusions.

Currently, veterinary clinical microbiology laboratories do not routinely use lipid-supplemented media when performing fungal isolation. This could lead to false negative results and may have contributed to the conception that lipid-dependent yeast are not important in veterinary specimens. It was previously thought that *M. pachydermatis* was the most predominant commensal and the main pathogenic *Malassezia* yeast in dogs and cats. The findings of this study demonstrate that lipid dependent species predominate on healthy canine skin in our study population and are still present but in lower numbers on atopic canine skin. The more routine use of lipid-supplemented media in fungal isolation from skin may contribute increased knowledge regarding the epidemiology and pathogenicity of these yeast in our veterinary patients.

There are several limitations of the study design that could have introduced bias into the results of this study including sample size, breeds of dogs included in the three study groups, and the use of clinical isolates for real-time-PCR characterization. The sample size of this study was small (26 dogs), and future studies with larger numbers of animals would be needed to clarify the phenomena presented in this discussion; studies also would benefit from the simultaneous investigation of the bacterial microbiota and other host factors such as transepidermal water loss, skin lipid content, and a history of *Malassezia* dermatitis. The client-owned healthy and allergic groups consisted of dogs from various breeds, but the allergen-induced group consisted of only one breed. Evidence is accumulating that genetic makeup can influence the composition of the microbiota.⁵⁰ This is likely even more pronounced when comparing breeds of animals that have a reduced genetic diversity due to in-breeding.⁵¹ Future studies should either include only one breed for comparisons or have a diversified background in both groups. Lastly, the use of a clinical strain of *M. pachydermatis* may have introduced bias during the characterization of the qPCR and future studies should include the reference strain for further characterization. However, it remains unclear how genetically diversified are strains of *Malassezia* isolated from the canine host, and whether our reference strains accurately represent the natural milieu of canine skin microbiota.

The results in our study clearly show *Malassezia* spp. dysbiosis occurs in allergic dogs that are more predisposed to develop *Malassezia* infections and redefine our existing knowledge of healthy canine *Malassezia* populations. Harnessing this canine model of AD, we have an animal model to further investigate interactions between the skin microbiota, such as between *Malassezia* and *Staphylococcus*. Finally, this canine model could serve as an opportunity to study how different therapeutic interventions affect the mycobiota in correlation with clinical outcomes.

Supplementary material

Supplementary material are available at [MMYCOL](https://mmymcol.com) online.

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Declaration of interest

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

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Supplementary Table S1. NGS abundances of *Malassezia* species relative to all fungal genera for allergen challenged dogs.

Group	Body Site	Time point	Dog	<i>M. pachy</i>	<i>M. restricta</i>	<i>M. globosa</i>	<i>M. obtusa</i>	<i>M. furfur</i>	<i>M. slooffiae</i>	<i>M. sympo</i>	<i>M. yama</i>	<i>Mal sp. unknown</i>	Other Fungal
Allergen challenged	Groin	1	1	0.0012	0.0828	0.0000	0.0001	0.0001	0.0000	0.0271	0.0000	0.0717	0.8170
Allergen challenged	Groin	1	2	0.3175	0.0057	0.0005	0.0055	0.0023	0.0001	0.0000	0.0000	0.1328	0.5354
Allergen challenged	Groin	1	3	0.3185	0.0026	0.0011	0.0101	0.0012	0.0000	0.0000	0.0000	0.2214	0.4451
Allergen challenged	Groin	1	4	0.2014	0.0545	0.0003	0.0060	0.0023	0.0001	0.0000	0.0000	0.1440	0.5913
Allergen challenged	Groin	1	5	0.0009	0.0000	0.9959	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.0029
Allergen challenged	Groin	1	6	0.0722	0.0744	0.0008	0.0028	0.0011	0.0000	0.0001	0.0000	0.0794	0.7692
Allergen challenged	Groin	1	7	0.1186	0.0001	0.0065	0.0040	0.0007	0.0000	0.0000	0.0000	0.0994	0.7708
Allergen challenged	Groin	1	8	0.1198	0.0100	0.0012	0.0082	0.0028	0.0001	0.0001	0.0000	0.3559	0.5018
Allergen challenged	Groin	2	1	0.2239	0.0233	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0022	0.7506
Allergen challenged	Groin	2	2	0.6370	0.0173	0.0001	0.0100	0.0014	0.0000	0.0000	0.0000	0.2246	0.1096
Allergen challenged	Groin	2	3	0.0875	0.0065	0.0014	0.0097	0.0037	0.0001	0.0001	0.0000	0.1840	0.7070
Allergen challenged	Groin	2	4	0.2153	0.0933	0.0090	0.0176	0.0068	0.0004	0.0004	0.0000	0.3681	0.2892
Allergen challenged	Groin	2	5	0.2044	0.0212	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0064	0.7680
Allergen challenged	Groin	2	6	0.0001	0.1452	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.8545
Allergen challenged	Groin	2	7	0.0465	0.0000	0.0002	0.0064	0.0020	0.0001	0.0000	0.0000	0.1086	0.8361
Allergen challenged	Groin	2	8	0.0720	0.0042	0.0045	0.0045	0.0014	0.0001	0.0000	0.0000	0.1844	0.7289
Allergen challenged	Groin	3	1	0.0700	0.0233	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0019	0.9047
Allergen challenged	Groin	3	2	0.4505	0.0119	0.0011	0.0130	0.0027	0.0001	0.0000	0.0000	0.2216	0.2991
Allergen challenged	Groin	3	3	0.2162	0.0214	0.0004	0.0076	0.0026	0.0001	0.0001	0.0000	0.1713	0.5804
Allergen challenged	Groin	3	4	0.4529	0.0025	0.0010	0.0203	0.0071	0.0003	0.0002	0.0000	0.4325	0.0834
Allergen challenged	Groin	3	5	0.5021	0.0035	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0016	0.4926
Allergen challenged	Groin	3	6	0.0010	0.0993	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0029	0.8968
Allergen challenged	Groin	3	7	0.1493	0.0073	0.0015	0.0059	0.0023	0.0001	0.0001	0.0000	0.1295	0.7042
Allergen challenged	Groin	3	8	0.0791	0.0001	0.0006	0.0033	0.0014	0.0001	0.0000	0.0000	0.1460	0.7694
Allergic	Axilla	-	11	0.0006	0.0013	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9980
Allergic	Axilla	-	12	0.0006	0.0003	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9990
Allergic	Axilla	-	13	0.0006	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9992
Allergic	Axilla	-	14	0.0005	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9993
Allergic	Axilla	-	15	0.0007	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9990

Group	Body Site	Time point	Dog	<i>M. pachy</i>	<i>M. restricta</i>	<i>M. globosa</i>	<i>M. obtusa</i>	<i>M. furfur</i>	<i>M. slooffiae</i>	<i>M. sympo</i>	<i>M. yama</i>	<i>Mal sp. unknown</i>	Other Fungal
Allergic	Axilla	-	16	0.0007	0.0004	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9989
Allergic	Axilla	-	17	0.0025	0.0008	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9966
Allergic	Axilla	-	18	0.0007	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0158	0.9833
Allergic	Ear	-	11	0.0004	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9995
Allergic	Ear	-	12	0.0004	0.0003	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9993
Allergic	Ear	-	13	0.5351	0.0003	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.4645
Allergic	Ear	-	14	0.9496	0.0024	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0476
Allergic	Ear	-	15	0.0007	0.0125	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9867
Allergic	Ear	-	16	0.0007	0.0005	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9987
Allergic	Ear	-	17	0.0004	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9995
Allergic	Ear	-	18	0.1269	0.0127	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.8603
Allergic	Groin	-	11	0.0004	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9995
Allergic	Groin	-	12	0.0002	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9997
Allergic	Groin	-	13	0.0007	0.0007	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9985
Allergic	Groin	-	14	0.0129	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9869
Allergic	Groin	-	16	0.0006	0.0045	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9948
Allergic	Groin	-	17	0.0044	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9955
Allergic	Groin	-	18	0.0126	0.0005	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9868
Allergic	Interd	-	11	0.0004	0.0027	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9969
Allergic	Interd	-	12	0.0005	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9993
Allergic	Interd	-	13	0.0007	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9992
Allergic	Interd	-	14	0.0006	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9992
Allergic	Interd	-	15	0.0005	0.0003	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9992
Allergic	Interd	-	16	0.0007	0.0003	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9990
Allergic	Interd	-	17	0.0007	0.0010	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9982
Allergic	Interd	-	18	0.0006	0.0003	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9991
Allergic	Lumb	-	11	0.0005	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9993
Allergic	Lumb	-	12	0.0008	0.3144	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0108	0.6740
Allergic	Lumb	-	13	0.0007	0.0001	0.0021	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9971
Allergic	Lumb	-	14	0.0003	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9996
Allergic	Lumb	-	16	0.0006	0.0004	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0006	0.9984

Group	Body Site	Time point	Dog	<i>M. pacy</i>	<i>M. restricta</i>	<i>M. globosa</i>	<i>M. obtusa</i>	<i>M. furfur</i>	<i>M. slooffiae</i>	<i>M. sympo</i>	<i>M. yama</i>	<i>Mal sp. unknown</i>	Other Fungal
Allergic	Lumb	-	17	0.0019	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9978
Allergic	Lumb	-	18	0.0144	0.0646	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0021	0.9189
Allergic	Nostril	-	11	0.0007	0.3103	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0099	0.6791
Allergic	Nostril	-	12	0.0004	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9995
Allergic	Nostril	-	13	0.2159	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.7839
Allergic	Nostril	-	14	0.0003	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9997
Allergic	Nostril	-	16	0.0008	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9992
Allergic	Nostril	-	17	0.0005	0.0004	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9990
Allergic	Nostril	-	18	0.0012	0.1820	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.8166
Healthy	Axilla	-	1	0.0002	0.0055	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.9922
Healthy	Axilla	-	2	0.0001	0.0005	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9991
Healthy	Axilla	-	3	0.0003	0.0151	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0021	0.9824
Healthy	Axilla	-	4	0.0000	0.0027	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0013	0.9960
Healthy	Axilla	-	5	0.0003	0.0007	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9988
Healthy	Axilla	-	6	0.0002	0.0004	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.9991
Healthy	Axilla	-	7	0.0003	0.0104	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9893
Healthy	Axilla	-	8	0.0003	0.0023	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9973
Healthy	Axilla	-	9	0.0007	0.0010	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9982
Healthy	Axilla	-	10	0.0010	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9988
Healthy	Ear	-	1	0.0915	0.0006	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.9077
Healthy	Ear	-	2	0.0004	0.0127	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0005	0.9864
Healthy	Ear	-	3	0.0003	0.0013	0.0008	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9976
Healthy	Ear	-	4	0.0002	0.0033	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0053	0.9911
Healthy	Ear	-	5	0.0002	0.1154	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0037	0.8806
Healthy	Ear	-	6	0.1977	0.0056	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.7968
Healthy	Ear	-	7	0.0003	0.0003	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9992
Healthy	Ear	-	8	0.3909	0.0004	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.6085
Healthy	Ear	-	9	0.0004	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9994
Healthy	Ear	-	10	0.0072	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9927
Healthy	Groin	-	1	0.0001	0.0007	0.0127	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.9864

Group	Body Site	Time point	Dog	<i>M. pachy</i>	<i>M. restricta</i>	<i>M. globosa</i>	<i>M. obtusa</i>	<i>M. furfur</i>	<i>M. slooffiae</i>	<i>M. sympo</i>	<i>M. yama</i>	<i>Mal sp. unknown</i>	Other Fungal
Healthy	Groin	-	2	0.0001	0.0007	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9991
Healthy	Groin	-	3	0.0003	0.0004	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9992
Healthy	Groin	-	4	0.0002	0.0009	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0038	0.9950
Healthy	Groin	-	5	0.0003	0.0007	0.0082	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9909
Healthy	Groin	-	6	0.0001	0.0005	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9992
Healthy	Groin	-	7	0.0003	0.0000	0.0003	0.0001	0.0000	0.0000	0.0000	0.0000	0.0001	0.9992
Healthy	Groin	-	8	0.0001	0.0005	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9992
Healthy	Groin	-	9	0.0004	0.0006	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9989
Healthy	Groin	-	10	0.0027	0.0004	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9969
Healthy	Interd	-	1	0.0041	0.0008	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9948
Healthy	Interd	-	2	0.0004	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.9993
Healthy	Interd	-	3	0.0001	0.0003	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.9993
Healthy	Interd	-	4	0.0002	0.0005	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.9991
Healthy	Interd	-	5	0.0003	0.0015	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9980
Healthy	Interd	-	6	0.0001	0.0004	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9993
Healthy	Interd	-	7	0.1929	0.0008	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.8060
Healthy	Interd	-	8	0.0001	0.0004	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9993
Healthy	Interd	-	9	0.0004	0.0003	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9993
Healthy	Interd	-	10	0.1362	0.0011	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.8626
Healthy	Lumb	-	1	0.0002	0.0014	0.0098	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9885
Healthy	Lumb	-	3	0.0002	0.0004	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0006	0.9988
Healthy	Lumb	-	4	0.0027	0.0138	0.0059	0.0003	0.0000	0.0000	0.0000	0.0000	0.0034	0.9739
Healthy	Lumb	-	5	0.0005	0.1170	0.0044	0.0000	0.0000	0.0000	0.0000	0.0000	0.0032	0.8750
Healthy	Lumb	-	6	0.0002	0.0005	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9992
Healthy	Lumb	-	7	0.0002	0.0012	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9983
Healthy	Lumb	-	8	0.0006	0.0005	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9988
Healthy	Lumb	-	9	0.0007	0.0023	0.0003	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9967
Healthy	Lumb	-	10	0.0007	0.0027	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9965
Healthy	Nostril	-	1	0.0001	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9997
Healthy	Nostril	-	2	0.0004	0.0962	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0032	0.9002

[illegible]

Study Group	Dog	Body Site	Time point	<i>M. pachy</i>	<i>M. restricta</i>	<i>M. globosa</i>	<i>M. fufur</i>	<i>M. obtusa</i>	<i>M. slooffiae</i>	<i>M. sympo</i>	<i>M. yama</i>	<i>Mal sp. unknown</i>
Allergic	12	Interdi	-	0.7143	0.2143	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0714
Allergic	13	Interdi	-	0.8182	0.1364	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0455
Allergic	14	Interdi	-	0.7500	0.2500	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Allergic	15	Interdi	-	0.6364	0.3333	0.0303	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Allergic	16	Interdi	-	0.6909	0.2727	0.0182	0.0000	0.0000	0.0000	0.0000	0.0000	0.0182
Allergic	17	Interdi	-	0.4054	0.5811	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0135
Allergic	18	Interdi	-	0.6667	0.3333	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Allergic	11	Lumbar	-	0.6957	0.2609	0.0217	0.0000	0.0000	0.0000	0.0000	0.0000	0.0217
Allergic	12	Lumbar	-	0.0025	0.9645	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0330
Allergic	13	Lumbar	-	0.2391	0.0435	0.7174	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Allergic	14	Lumbar	-	0.7500	0.0000	0.2500	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Allergic	16	Lumbar	-	0.3684	0.2368	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3947
Allergic	17	Lumbar	-	0.8889	0.1026	0.0085	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Allergic	18	Lumbar	-	0.1771	0.7964	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0265
Allergic	11	Nostril	-	0.0022	0.9670	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0309
Allergic	12	Nostril	-	0.6667	0.3333	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Allergic	13	Nostril	-	0.9990	0.0004	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0006
Allergic	14	Nostril	-	0.7500	0.2500	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Allergic	16	Nostril	-	0.9286	0.0714	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Allergic	17	Nostril	-	0.5625	0.4375	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Allergic	18	Nostril	-	0.0065	0.9924	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0011
Healthy	1	Axilla	-	0.0239	0.7050	0.2256	0.0000	0.0000	0.0000	0.0000	0.0000	0.0456
Healthy	2	Axilla	-	0.1364	0.5455	0.2727	0.0000	0.0000	0.0000	0.0000	0.0000	0.0455
Healthy	3	Axilla	-	0.0146	0.8599	0.0058	0.0000	0.0000	0.0000	0.0000	0.0000	0.1197
Healthy	4	Axilla	-	0.0000	0.6667	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3333
Healthy	5	Axilla	-	0.2727	0.6364	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0909
Healthy	6	Axilla	-	0.2759	0.4483	0.0690	0.0000	0.0000	0.0000	0.0000	0.0000	0.2069
Healthy	7	Axilla	-	0.0249	0.9652	0.0025	0.0000	0.0000	0.0000	0.0000	0.0000	0.0075

Study Group	Dog	Body Site	Time point	<i>M. pachy</i>	<i>M. restricta</i>	<i>M. globosa</i>	<i>M. fufur</i>	<i>M. obtusa</i>	<i>M. slooffiae</i>	<i>M. sympo</i>	<i>M. yama</i>	<i>Mal sp. unknown</i>
Healthy	8	Axilla	-	0.1190	0.8571	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0238
Healthy	9	Axilla	-	0.4000	0.5500	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0500
Healthy	10	Axilla	-	0.8276	0.1207	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0517
Healthy	1	Ear	-	0.9909	0.0064	0.0009	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018
Healthy	2	Ear	-	0.0296	0.9370	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0333
Healthy	3	Ear	-	0.1064	0.5213	0.3404	0.0000	0.0000	0.0000	0.0000	0.0000	0.0319
Healthy	4	Ear	-	0.0199	0.3722	0.0174	0.0000	0.0000	0.0000	0.0000	0.0000	0.5906
Healthy	5	Ear	-	0.0019	0.9661	0.0010	0.0000	0.0000	0.0000	0.0000	0.0000	0.0310
Healthy	6	Ear	-	0.9726	0.0274	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Healthy	7	Ear	-	0.4000	0.4000	0.2000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Healthy	8	Ear	-	0.9985	0.0010	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004
Healthy	9	Ear	-	0.6786	0.1429	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1786
Healthy	10	Ear	-	0.9908	0.0092	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Healthy	1	Groin	-	0.0056	0.0503	0.9330	0.0000	0.0000	0.0000	0.0000	0.0000	0.0112
Healthy	2	Groin	-	0.1111	0.7222	0.0556	0.0000	0.0000	0.0000	0.0000	0.0000	0.1111
Healthy	3	Groin	-	0.3333	0.4848	0.1212	0.0000	0.0000	0.0000	0.0000	0.0000	0.0606
Healthy	4	Groin	-	0.0442	0.1905	0.0102	0.0000	0.0000	0.0000	0.0000	0.0000	0.7551
Healthy	5	Groin	-	0.0311	0.0745	0.8944	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Healthy	6	Groin	-	0.1154	0.6538	0.1154	0.0000	0.0000	0.0000	0.0000	0.0000	0.1154
Healthy	7	Groin	-	0.3750	0.0000	0.3750	0.0000	0.1250	0.0000	0.0000	0.0000	0.1250
Healthy	8	Groin	-	0.1067	0.6133	0.1867	0.0000	0.0000	0.0000	0.0000	0.0000	0.0933
Healthy	9	Groin	-	0.3529	0.5882	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0588
Healthy	10	Groin	-	0.8788	0.1212	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Healthy	1	Interdi	-	0.8000	0.1563	0.0188	0.0000	0.0000	0.0000	0.0000	0.0000	0.0250
Healthy	2	Interdi	-	0.5000	0.2000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3000
Healthy	3	Interdi	-	0.2105	0.4474	0.1053	0.0000	0.0000	0.0000	0.0000	0.0000	0.2368
Healthy	4	Interdi	-	0.1905	0.5238	0.0952	0.0000	0.0000	0.0000	0.0000	0.0000	0.1905
Healthy	5	Interdi	-	0.1333	0.7556	0.1111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Study Group	Dog	Body Site	Time point	<i>M. pachy</i>	<i>M. restricta</i>	<i>M. globosa</i>	<i>M. fufur</i>	<i>M. obtusa</i>	<i>M. slooffiae</i>	<i>M. sympo</i>	<i>M. yama</i>	<i>Mal sp. unknown</i>
Healthy	6	Interdi	-	0.2000	0.5200	0.1600	0.0000	0.0000	0.0000	0.0000	0.0000	0.1200
Healthy	7	Interdi	-	0.9943	0.0039	0.0008	0.0000	0.0000	0.0000	0.0000	0.0000	0.0010
Healthy	8	Interdi	-	0.1750	0.6000	0.1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1250
Healthy	9	Interdi	-	0.5909	0.4091	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Healthy	10	Interdi	-	0.9908	0.0081	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0011
Healthy	1	Lumbar	-	0.0133	0.1244	0.8533	0.0000	0.0000	0.0000	0.0000	0.0000	0.0089
Healthy	3	Lumbar	-	0.1702	0.3191	0.0426	0.0000	0.0000	0.0000	0.0000	0.0000	0.4681
Healthy	4	Lumbar	-	0.1029	0.5287	0.2261	0.0000	0.0108	0.0000	0.0000	0.0000	0.1316
Healthy	5	Lumbar	-	0.0038	0.9360	0.0350	0.0000	0.0000	0.0000	0.0000	0.0000	0.0252
Healthy	6	Lumbar	-	0.2188	0.6875	0.0313	0.0000	0.0000	0.0000	0.0000	0.0000	0.0625
Healthy	7	Lumbar	-	0.1094	0.7344	0.1094	0.0000	0.0000	0.0000	0.0000	0.0000	0.0469
Healthy	8	Lumbar	-	0.5476	0.4286	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0238
Healthy	9	Lumbar	-	0.2095	0.6959	0.0878	0.0000	0.0000	0.0000	0.0000	0.0000	0.0068
Healthy	10	Lumbar	-	0.2128	0.7660	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0213
Healthy	1	Nostril	-	0.3333	0.5556	0.1111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Healthy	2	Nostril	-	0.0043	0.9634	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0323
Healthy	3	Nostril	-	0.9621	0.0276	0.0069	0.0000	0.0000	0.0000	0.0000	0.0000	0.0034
Healthy	4	Nostril	-	0.2000	0.6000	0.0667	0.0000	0.0000	0.0000	0.0000	0.0000	0.1333
Healthy	5	Nostril	-	0.0008	0.9696	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0294
Healthy	6	Nostril	-	0.0013	0.9825	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0162
Healthy	7	Nostril	-	0.0018	0.9781	0.0009	0.0000	0.0000	0.0000	0.0000	0.0000	0.0193
Healthy	8	Nostril	-	0.9000	0.1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Healthy	9	Nostril	-	0.7568	0.2432	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Healthy	10	Nostril	-	0.7059	0.2353	0.0588	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Allergen challenged	1	Groin	1	0.0066	0.4524	0.0002	0.0003	0.0005	0.0001	0.1481	0.0000	0.3918
Allergen challenged	2	Groin	1	0.6834	0.0124	0.0011	0.0050	0.0119	0.0002	0.0001	0.0001	0.2859
Allergen challenged	3	Groin	1	0.5740	0.0046	0.0020	0.0022	0.0183	0.0000	0.0000	0.0000	0.3990
Allergen challenged	4	Groin	1	0.4928	0.1333	0.0008	0.0057	0.0146	0.0003	0.0001	0.0000	0.3523

Study Group	Dog	Body Site	Time point	<i>M. pachy</i>	<i>M. restricta</i>	<i>M. globosa</i>	<i>M. fufur</i>	<i>M. obtusa</i>	<i>M. slooffiae</i>	<i>M. sympo</i>	<i>M. yama</i>	<i>Mal sp. unknown</i>
Allergen challenged	5	Groin	1	0.0009	0.0000	0.9987	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002
Allergen challenged	6	Groin	1	0.3127	0.3224	0.0033	0.0049	0.0123	0.0001	0.0002	0.0000	0.3440
Allergen challenged	7	Groin	1	0.5173	0.0005	0.0282	0.0029	0.0174	0.0000	0.0000	0.0000	0.4337
Allergen challenged	8	Groin	1	0.2405	0.0201	0.0024	0.0056	0.0165	0.0003	0.0002	0.0000	0.7144
Allergen challenged	1	Groin	2	0.8975	0.0935	0.0000	0.0002	0.0001	0.0001	0.0000	0.0000	0.0086
Allergen challenged	2	Groin	2	0.7155	0.0195	0.0001	0.0015	0.0112	0.0000	0.0000	0.0000	0.2522
Allergen challenged	3	Groin	2	0.2988	0.0222	0.0046	0.0126	0.0330	0.0004	0.0004	0.0001	0.6279
Allergen challenged	4	Groin	2	0.3029	0.1312	0.0126	0.0095	0.0247	0.0005	0.0005	0.0001	0.5179
Allergen challenged	5	Groin	2	0.8810	0.0913	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0276
Allergen challenged	6	Groin	2	0.0008	0.9977	0.0000	0.0000	0.0000	0.0002	0.0001	0.0000	0.0012
Allergen challenged	7	Groin	2	0.2837	0.0001	0.0012	0.0122	0.0393	0.0006	0.0003	0.0000	0.6626
Allergen challenged	8	Groin	2	0.2655	0.0156	0.0165	0.0052	0.0165	0.0003	0.0001	0.0001	0.6802
Allergen challenged	1	Groin	3	0.7346	0.2447	0.0001	0.0001	0.0005	0.0002	0.0000	0.0000	0.0197
Allergen challenged	2	Groin	3	0.6427	0.0169	0.0015	0.0039	0.0186	0.0002	0.0000	0.0000	0.3162
Allergen challenged	3	Groin	3	0.5153	0.0510	0.0009	0.0062	0.0180	0.0002	0.0002	0.0001	0.4081
Allergen challenged	4	Groin	3	0.4940	0.0027	0.0011	0.0077	0.0221	0.0003	0.0002	0.0000	0.4718
Allergen challenged	5	Groin	3	0.9897	0.0068	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0032
Allergen challenged	6	Groin	3	0.0094	0.9622	0.0000	0.0001	0.0002	0.0002	0.0001	0.0000	0.0277
Allergen challenged	7	Groin	3	0.5046	0.0245	0.0051	0.0078	0.0199	0.0002	0.0002	0.0000	0.4376
Allergen challenged	8	Groin	3	0.3428	0.0003	0.0027	0.0062	0.0144	0.0003	0.0002	0.0000	0.6331

Supplementary Table S3. Genome equivalents from qPCR for healthy and non-lesional naturally affected allergic dogs.

Dog	Body Site	Study Group	<i>M. pachydermatis</i>	<i>M. restricta</i>	<i>M. globosa</i>
1	Axilla	Healthy	4.65	n/a	n/a
2	Axilla	Healthy	0.05	n/a	n/a
3	Axilla	Healthy	0.40	187.36	1.53
4	Axilla	Healthy	0.57	n/a	n/a
5	Axilla	Healthy	0.00	39.13	0.00
6	Axilla	Healthy	0.18	2.64	0.00
7	Axilla	Healthy	5.61	106.46	0.00
8	Axilla	Healthy	0.41	381.14	7.12
9	Axilla	Healthy	0.01	94.37	3.44
10	Axilla	Healthy	3.09	24.68	0.00
11	Axilla	Allergic	0.67	152.34	0.00
12	Axilla	Allergic	0.21	48.43	0.00
13	Axilla	Allergic	0.62	48.62	0.00
14	Axilla	Allergic	1.13	10.55	0.00
15	Axilla	Allergic	0.01	29.95	0.00
16	Axilla	Allergic	0.02	17.51	0.00
17	Axilla	Allergic	2.94	42.78	0.00
18	Axilla	Allergic	3.24	23.62	0.00
1	Ear	Healthy	1.34	25.06	n/a
2	Ear	Healthy	0.01	17.56	n/a
3	Ear	Healthy	0.93	156.62	6.21
4	Ear	Healthy	0.91	66.13	2.90
5	Ear	Healthy	0.01	66.27	0.72
6	Ear	Healthy	14.14	6.99	0.00
7	Ear	Healthy	1.00	33.71	0.00
8	Ear	Healthy	63.35	15.83	0.00
9	Ear	Healthy	0.04	58.20	0.00
10	Ear	Healthy	5.61	20.53	0.00
11	Ear	Allergic	0.00	33.35	0.00
12	Ear	Allergic	1.14	2.64	0.00
13	Ear	Allergic	15.07	51.73	0.00
14	Ear	Allergic	53.51	10.86	0.00
15	Ear	Allergic	0.07	17.03	0.00
16	Ear	Allergic	0.10	12.87	0.00
17	Ear	Allergic	0.08	21.75	0.00
18	Ear	Allergic	0.00	0.00	0.00
1	Groin	Healthy	0.85	97.41	n/a

Dog	Body Site	Study Group	<i>M. pachydermatis</i>	<i>M. restricta</i>	<i>M. globosa</i>
	2 Groin	Healthy	0.05	5.52	0.01
	3 Groin	Healthy	0.70	25.81	0.28
	4 Groin	Healthy	0.25	23.25	0.00
	5 Groin	Healthy	0.02	45.82	0.00
	6 Groin	Healthy	0.02	2.01	0.00
	7 Groin	Healthy	3.79	68.86	0.00
	8 Groin	Healthy	2.52	165.45	3.46
	9 Groin	Healthy	0.19	115.29	0.60
	10 Groin	Healthy	2.07	8.45	0.00
	11 Groin	Allergic	0.01	0.56	0.00
	12 Groin	Allergic	0.39	83.01	0.00
	13 Groin	Allergic	0.69	11.88	0.00
	14 Groin	Allergic	1.94	10.43	1.50
	15 Groin	Allergic	0.03	37.29	0.00
	16 Groin	Allergic	0.02	19.01	0.00
	17 Groin	Allergic	5.67	25.61	3.69
	18 Groin	Allergic	2.47	17.89	0.00
	1 Interdigital	Healthy	7.06	6.73	n/a
	2 Interdigital	Healthy	0.04	12.74	n/a
	3 Interdigital	Healthy	0.58	30.84	1.90
	4 Interdigital	Healthy	0.15	16.61	0.00
	5 Interdigital	Healthy	0.02	62.83	1.39
	6 Interdigital	Healthy	0.35	3.48	0.75
	7 Interdigital	Healthy	34.78	15.43	1.82
	8 Interdigital	Healthy	0.01	53.70	0.88
	9 Interdigital	Healthy	0.01	4.73	2.29
	10 Interdigital	Healthy	244.10	18.82	0.00
	11 Interdigital	Allergic	0.05	25.38	0.00
	12 Interdigital	Allergic	0.18	6.71	0.00
	13 Interdigital	Allergic	0.27	24.23	0.00
	14 Interdigital	Allergic	0.37	9.19	0.00
	15 Interdigital	Allergic	0.01	8.16	0.00
	16 Interdigital	Allergic	0.03	15.82	0.00
	17 Interdigital	Allergic	3.38	16.14	7.70
	18 Interdigital	Allergic	0.97	9.27	0.00
	1 Lumbar	Healthy	0.49	33.61	1.18
	2 Lumbar	Healthy	0.03	19.17	0.44
	3 Lumbar	Healthy	0.14	21.10	0.00
	4 Lumbar	Healthy	20.80	3876.15	213.14
	5 Lumbar	Healthy	0.01	98.95	2.86

Dog	Body Site	Study Group	<i>M. pachydermatis</i>	<i>M. restricta</i>	<i>M. globosa</i>
6	Lumbar	Healthy	0.79	13.32	1.30
7	Lumbar	Healthy	4.23	161.07	7.60
8	Lumbar	Healthy	0.28	49.52	0.00
9	Lumbar	Healthy	0.09	152.40	0.84
10	Lumbar	Healthy	0.02	11.29	0.00
11	Lumbar	Allergic	0.08	44.89	0.00
12	Lumbar	Allergic	0.12	25.04	0.00
13	Lumbar	Allergic	0.95	23.03	0.00
14	Lumbar	Allergic	0.30	43.83	0.00
15	Lumbar	Allergic	0.01	34.08	0.00
16	Lumbar	Allergic	0.07	0.00	0.00
17	Lumbar	Allergic	1.97	79.05	0.00
18	Lumbar	Allergic	1.21	60.84	0.00
1	Nostril	Healthy	0.03	2.24	n/a
2	Nostril	Healthy	0.00	7.71	n/a
3	Nostril	Healthy	0.21	5.66	0.00
4	Nostril	Healthy	0.23	13.76	0.00
5	Nostril	Healthy	0.19	n/a	n/a
6	Nostril	Healthy	0.02	6.01	0.00
7	Nostril	Healthy	0.10	16.46	0.00
8	Nostril	Healthy	0.10	0.00	0.00
9	Nostril	Healthy	0.06	6.00	0.00
10	Nostril	Healthy	0.58	2.25	0.00
11	Nostril	Allergic	0.06	30.65	0.00
12	Nostril	Allergic	0.01	3.29	0.00
13	Nostril	Allergic	0.32	13.44	0.00
14	Nostril	Allergic	0.01	4.91	0.00
15	Nostril	Allergic	0.04	14.35	0.00
16	Nostril	Allergic	0.01	0.00	1.72
17	Nostril	Allergic	0.22	4.65	0.00
18	Nostril	Allergic	0.17	25.77	0.00

Supplementary Table S4. Genome equivalents from qPCR for allergen challenged dogs.

Dog	Time point	Group	<i>M.</i> <i>pachydermatis</i>	<i>M.</i> <i>restricta</i>
	1	1 Allergen Challenged	8.04	70.50
	2	1 Allergen Challenged	252.29	142.31
	3	1 Allergen Challenged	161.59	157.75
	4	1 Allergen Challenged	2.80	49.47
	5	1 Allergen Challenged	5.02	31.93
	6	1 Allergen Challenged	0.20	25.26
	7	1 Allergen Challenged	7.21	57.42
	8	1 Allergen Challenged	8.69	71.19
1	2	2 Allergen Challenged	3.61	14.90
2	2	2 Allergen Challenged	35.81	37.54
3	2	2 Allergen Challenged	71.06	125.27
4	2	2 Allergen Challenged	1.31	14.74
5	2	2 Allergen Challenged	19.94	46.60
6	2	2 Allergen Challenged	0.74	27.54
7	2	2 Allergen Challenged	8.10	44.44
8	2	2 Allergen Challenged	17.85	159.87
1	3	3 Allergen Challenged	3.05	6.01
2	3	3 Allergen Challenged	75.18	31.21
3	3	3 Allergen Challenged	42.45	42.06
4	3	3 Allergen Challenged	1.23	15.38
5	3	3 Allergen Challenged	199.72	36.17
6	3	3 Allergen Challenged	0.02	24.48
7	3	3 Allergen Challenged	6.51	48.14
8	3	3 Allergen Challenged	4.29	44.72

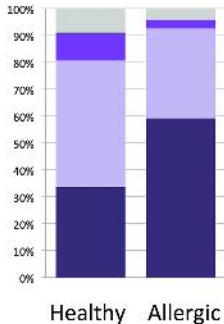
Supplementary Table 5. Kruskal-wallis p-values for *Malassezia* species relative abundances.
in atopic allergen challenged dogs.

Taxonomic level	Individual	Time point
<i>Malassezia</i> sp.	0.0070	0.9608
<i>M. furfur</i>	0.0153	0.9115
<i>M. globosa</i>	0.1853	0.3390
<i>M. obtusa</i>	0.0095	0.934
<i>M. pachydermatis</i>	0.2546	0.4317
<i>M. restricta</i>	0.0437	0.8208
<i>M. slooffiae</i>	0.1425	0.3432
<i>M. sympodialis</i>	0.2914	0.9763
<i>M. yamatoensis</i>	0.0251	0.9536

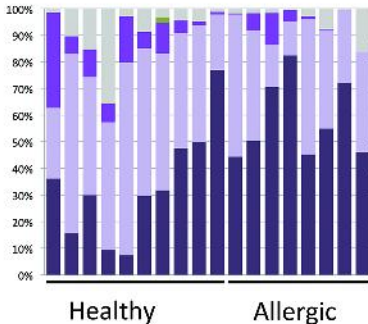
Supplementary Table 6. Kruskal-wallis p-values for *Malassezia* species genome equivalents in atopic allergen challenged dogs.

Taxonomic level	Individual	Time point
<i>M. pachydermatis</i>	0.0063	0.9489
<i>M. restricta</i>	0.1307	0.0750

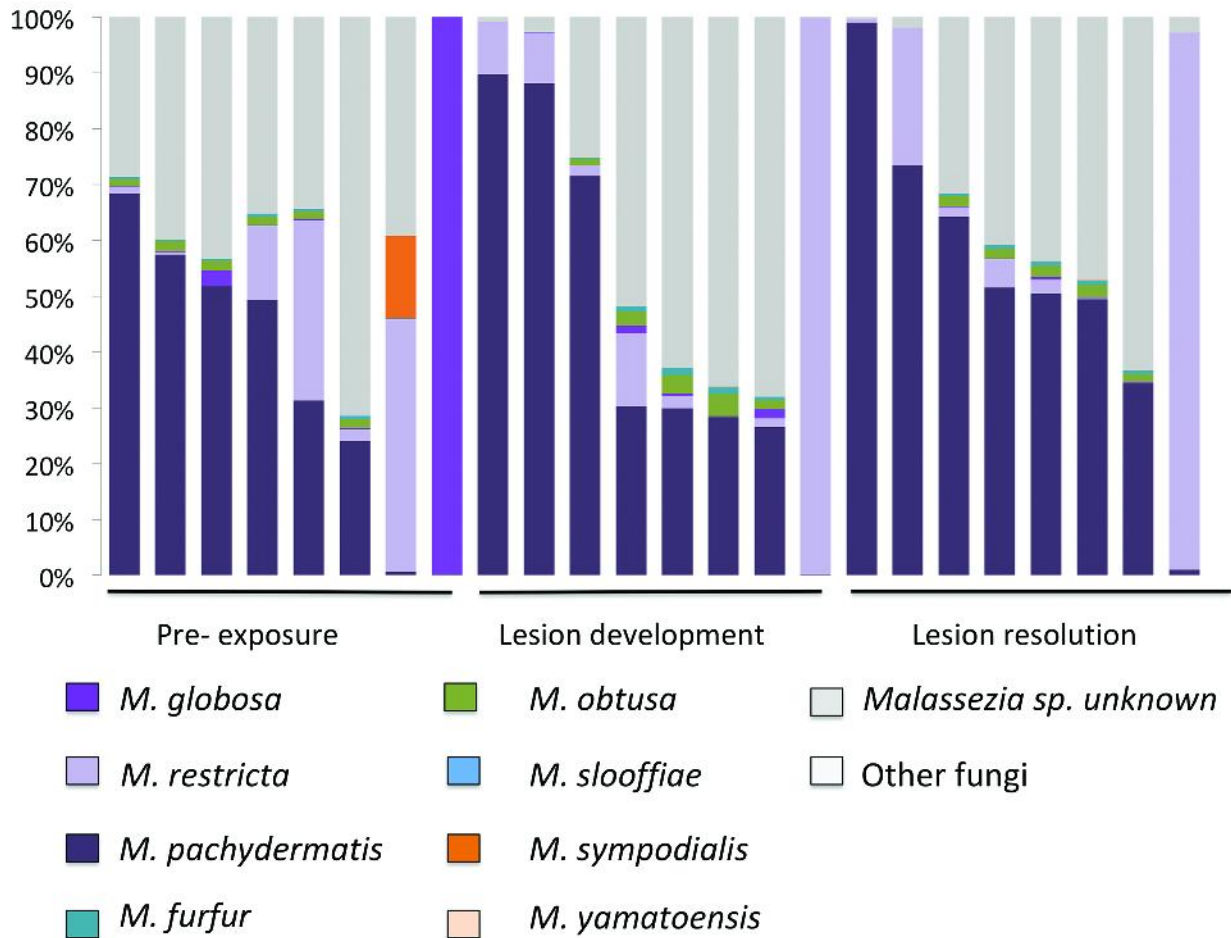
A.



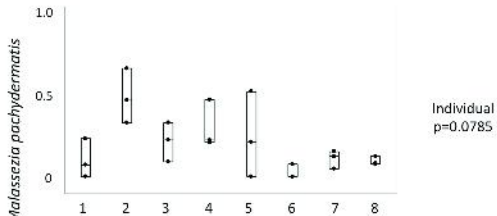
B.



M. globosa
 M. restricta
 M. pachydermatis
 M. obtusa
 Malassezia sp.



A.



B.

