THE CUTANEOUS MICROBIOTA AND CYTOKINE PROFILE IN FELINE ALLERGIC DERMATITIS

A Dissertation

by

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ABSTRACT

Feline atopic syndrome (FAS) is an allergic dermatological disease affecting cats, which has an unclear pathogenesis and clinical signs that are unique from atopic dermatitis affecting other species. Two factors thought to contribute to the disease are the microbial communities inhabiting the skin and immune dysfunction.

To address the involvement of the microbiota, the skin and oral microbiota on healthy animals was evaluated to understand what could be considered "normal". The influence of two potentially important factors, breed and environment, on the bacterial and fungal microbiota was analyzed. Environment had little to almost no impact on the skin microbiota, with differences between indoor and outdoor cats only observed in the oral cavity. Breed also had little influence, although more so than environment, which indicates this factor may be worth taking into consideration for future studies.

With the understanding breed could have an influence on the cutaneous microbial communities, the sample cohort for the second study comparing healthy and allergic feline skin was restricted to mostly domestic short-haired cats. Due to their notorious role in allergic dermatoses, staphylococcal communities on feline skin were evaluated. Species-level characterization demonstrated that feline cutaneous staphylococcal populations are diverse, with several species of substantial relative abundance identified. *S. epidermidis* was the most prevalent species identified from healthy samples, while *S. capitis* was the most prominent in allergic samples.

Finally, cutaneous and systemic immune dysfunction in allergic cats and non-affected controls was evaluated, with the hypothesis that allergic individuals would have an immune system that favored a Th2-skewed response. Immunohistochemistry targeting IL-31, an important molecule in pruritus signaling in dogs and people, and RNAscope targeting IL-31 and other Th2-associated molecules was performed on skin biopsies. Serum samples were subjected to a multiplex panel covering 19 Th1- and Th2-associated molecules and an IL-31 enzyme-linked immunosorbent assay (ELISA). One target involved in IL-31 signaling, Oncostatin M receptor subunit beta (OSMR-β) was found to have higher mRNA expression in the skin of affected cats relative to controls.

The results of this research indicate the pathomechanisms behind feline allergic dermatitis may include microbial and immune involvement. The specific immune profiles and cutaneous microbiota of allergic individuals is still unclear, however these results suggest species-level staphylococcal and OSMR signaling may be worth further investigating.

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NOMENCLATURE

cDNA	complementary DNA
CFU	Colony Forming Units
DNA	Deoxyribonucleic acid
DSH	Domestic shorthair cat
ELISA	Enzyme-linked immunosorbent assay
FAD	Feline allergic dermatitis
FAS	Feline atopic syndrome
FFPE	Formalin-fixed, paraffin-embedded
H&E	Hematoxylin and eosin
IHC	Immunohistochemistry
IL-31	Interleukin-31
IL-31RA	Interluekin-31 receptor alpha
ITS	Internal Transcribed Spacer
NGS	Next-generation sequencing
OSMR-β	Oncostatin M receptor subunit beta
OTUs	Operational Taxonomic Units
PCoA	Principal Coordinates Analysis
qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid
rRNA	Ribosomal RNA

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CHAPTER I

INTRODUCTION

Significance

Feline allergic dermatitis is one of the most common diseases affecting cats, with fleas as the most common trigger, followed by environmental allergens.[1,2] While numerous terms exist in the literature to refer to environmental allergies in cats, many have adopted the nomenclature of feline atopic syndrome (FAS) to describe the condition. Although limited information exists as to the pervasiveness of FAS, one study estimated a prevalence of 12.5%.[3] While flea allergic dermatitis can often be controlled with administration of adulticidal flea preventative, and food allergic dermatitis can be managed with identification of and feeding a diet that does not elicit an adverse reaction, therapeutic options for cats affected by allergic dermatitis due to environmental allergens (FAS) are limited.

In humans and dogs, more targeted treatments exist for managing allergic skin disease (particularly atopic dermatitis) including monoclonal antibodies[4-6] and other biologics that interrupt signaling related to allergic pruritus (itch). [7,8] Comparatively though, options for managing feline allergic dermatitis are few. This lack of options is in part due to the limited understanding of the pathomechanisms underlying FAS, particularly as it pertains to immunological dysregulation. Whereas most humans and canines affected by atopic dermatitis can be characterized by some key features such as IgE involvement and a Th2-skewed ratio of T cells in the acute phase,[9,10] the disease in

cats is less well-described and appears to be different than what has been demonstrated in other species, both clinically and mechanistically. Involvement of IgE in FAS is questionable[11,12] and there is not currently sufficient evidence to suggest a strong Th2 response.[13]

In addition to immune dysregulation, the microbiome may also play a role in disease and could represent an underappreciated area of potential for therapeutic intervention in FAS. In human atopic dermatitis, research has clearly demonstrated microbial dysbiosis in affected patients.[14,15] Certain microbes are known to exacerbate disease[16-18] and others seem to be beneficial, sparking the evaluation of topical probiotics that may ameliorate clinical signs.[19,20] In both dogs and cats, improvement of clinical signs with antimicrobial treatment have suggested a role for the microbiome in allergic dermatitis,[21,22] and recent microbiome surveys have confirmed dysbiosis associated with disease in both species.[22-27] Further research within this area may reveal options for therapy that are more effective at eliminating or reducing numbers of pathogenic microbes, and may be less harmful to the normal communities important in maintaining skin homeostasis than other currently available antimicrobial strategies.

Introduction to the microbiota

The microbiome is the collection of microorganisms (microbiota), their genes and gene products that inhabit a certain environment. These communities contain both permanent residents and transient visitors with a wide range of roles. Within the body some of these roles include aiding in digestion, teaching the immune system tolerance, and causing disease. Importantly, a single microbe can take on many roles, with their

"choice" depending on where the microbe is located, its neighbors, and the state of the host (i.e. health vs. disease).[28,29]

Before we are able to interpret what kind of shift in the microbiome, or dysbiosis, is associated with disease, we must understand what the "normal" microbiome looks like. An understanding of what is "normal" must take into account variability that may exist between individuals due to a variety of factors including, but not limited to, hygiene, geographic location, and host genetics. Research on the effect of some of these factors has been performed on various human microbiomes,[30-34] including the skin.[35-41] Only a couple studies with fairly small samples sizes have been performed for the canine skin microbiota,[42-45] and no studies evaluating the influence of these factors on the feline skin microbiota have been performed.

The skin microbiota

The skin is incredibly important in maintaining homeostasis; it is the first barrier to the environment, preventing potentially pathogenic microorganisms from entering the body, aiding in temperature regulation, and protecting a vast number of internal cells and tissues from UV damage. The microbes inhabiting our skin, like our own cells, are under pressure from these environmental factors and the conditions of the cutaneous microenvironment itself. Sebum, antimicrobial peptides, and pH of the skin can all affect which microbes will flourish and which will struggle. Not surprisingly, the distinct microhabitats across the skin can sustain different kinds of life, with some body sites being more closed-off and thus having a more humid environment, such as the axilla, and other sites being more exposed to environmental stress, like the forearm.[46,47] The

microbes present are also important determinants of the community composition, through direct interactions with other microbes or through modulating the immune system which can then subsequently influence these communities.[28,29,48] Although the skin has been recognized as an important habitat for microbes, and research of disease-associated microbiomes has become an area of current interest, studies of the cutaneous microbial communities in disease are just beginning.

Cutaneous bacterial microbiota on healthy and allergic skin

The normal microbiota inhabiting human skin has been established through multiple studies, with similar findings in terms of relative abundance of different taxa, where Proteobacteria, Actinobacteria and Firmicutes are the most prevalent phyla.[46,49] Further research has described changes in the human cutaneous microbiota associated with disease, including atopic dermatitis. Decreased diversity, in terms of the number of different taxa present and the evenness of their proportions,[14,50] has been demonstrated in affected individuals when compared to healthy individuals. Other studies have identified specific activities of certain microbes, most notably *Staphylococcus* spp.,[17,51-53] that are important in either the exacerbation or control of atopic dermatitis. Research on the microbiota in people with atopic dermatitis is now translating to clinical application of newer therapeutics; the application of *Roseomonas mucosa*[19] and coagulase-negative *Staphylococcus* species[20] to the skin of affected individuals has shown beneficial results in clinical trials with evidence of decreased *S. aureus* burden,[20] disease severity, and steroid requirement needed in atopic people.[19]

The canine cutaneous microbiota has also been researched, including that of dogs affected by allergic skin diseases. The first study utilizing next-generation sequencing (NGS) to describe the microbiota on healthy canine skin revealed similar findings to human microbiota studies, with the same predominant phyla identified (Proteobacteria, Firmicutes and Actinobacteria).[26] Additionally, this study compared the bacterial microbiota of healthy canine skin to that of nonlesional skin in dogs with allergic dermatitis and identified dysbiosis in the form of decreased alpha diversity in allergic skin and differences in taxonomic composition. Although *Staphylococcus* spp. abundance was not significantly different between these groups, there seemed to be a trend of increased relative abundances of this bacteria on allergic skin.[26] Bradley et al. further described the microbiota in dogs with allergic dermatitis, specifically in the lesions of those with current flares of atopic dermatitis, over the course of three sampling timepoints and compared their microbiota to healthy control dogs. They also identified decreased alpha diversity and significantly higher relative abundances of *Staphylococcus* spp. at baseline in affected dogs.[22] With antibiotic treatment, diversity and *Staphylococcus* spp. relative abundance became more similar to controls. Research on experimentally induced canine atopic dermatitis also demonstrated the relevance of *Staphylococcus* spp., specifically *S. pseudintermedius* (formerly *S. intermedius*),[54] in the development of lesions associated with flares of disease. [27]

Research of the feline cutaneous bacterial microbiota of cats is limited to a single study performed by our laboratory. In this study,[25] the bacterial microbiota on healthy feline skin was revealed to have a more diverse community than previously described.[55]

In comparing samples of healthy skin and nonlesional skin of allergic cats, many differences in the bacterial microbiota were described. In terms of community structure, or beta diversity, only the ear canal was different between healthy and allergic cats. Comparing beta diversity of other body sites sampled (axilla, groin, interdigital space, lumbar, nostril) between healthy and allergic cats did not result in this same observation. While there did not seem to be many differences in the diversity or structure of bacterial communities between healthy and allergic samples, multiple taxa were found to be differentially abundant. The most interesting taxon was *Staphylococcus* spp., which had elevated relative abundance in allergic samples, especially in the ear canal and interdigital space. These results provide further evidence for the involvement of the bacterial microbiota in feline allergic dermatitis, as has already been thoroughly described in the human and canine disease.

Role of *Staphylococcus* spp. in human atopic dermatitis

Staphylococcus spp. have been well studied with regards to impact on disease manifestation, specifically *S. aureus* as an important pathogen particularly in human atopic dermatitis. Some members of the genus *Staphylococcus* are also important allies to the host, playing roles in skin defense and in preventing the development or dampening flares of allergic dermatitis. Besides *S. aureus*, the most common pathogen isolated from skin infections and during flares of atopic dermatitis in people, other notable staphylococcal species in human cutaneous health include *S. epidermidis* and *S. hominis*.[56]

The mechanisms by which certain species can cause disease are numerous and elaborate. These bacteria are able to take advantage of the inherently weak skin barrier in atopic patients and enter the skin, allowing for close interactions with the hyperreactive immune system.[16] Additionally, specific strains can produce superantigens or exfoliative toxins that can further damage the skin barrier and stimulate the immune system.[57-60] For example, in humans, superantigens produced by *S. aureus* have been shown to induce Th2-like behavior from Treg cells in the skin[18] in addition to activating various inflammatory cells and inducing toxin-specific IgE production.[61]

Just as numerous as the ways *S. aureus* can cause disease are the many ways other microbial species can attenuate the pathogenicity of this bacteria.[29] Both *S. epidermidis*[62] and *S. hominis* have been found to produce antimicrobial compounds specifically targeting *S. aureus.*[20] *S. epidermidis* has also been described to interfere with the quorum sensing ability *S. aureus* uses to form biofilms.[63] Finally, some species can indirectly interfere with *S. aureus'* invasion by inducing host antimicrobial peptide production.[64,65]

Staphylococcus spp. in canine and feline cutaneous health

Staphylococcus spp. are also important cutaneous inhabitants of cats and dogs. In dogs, *S. pseudintermedius* is the predominant species on both healthy and allergic skin. Other species including *S. epidermidis*, *S. schleiferi*, and *S. aureus* can also be present on canine skin, but are typically only found in low relative abundances.[22] Unlike the staphylococci inhabiting human skin, beneficial activities of certain species have not been described on canine skin. Additionally, with the knowledge that *S.*

pseudintermedius is the predominant species regardless of host status (healthy or allergic), and is often isolated from lesions, there does not seem to be any evidence for other staphylococcal species relaying benefits to canine skin. Strain-level description of staphylococcal species on canine skin has not been performed, but perhaps would identify differences in abundance of strains that may behave differently on canine skin. It is also possible that even though there are no differences in species presence, the activity of *S. pseudintermedius* may be altered on allergic skin due to different environmental pressures; metabolomic and bacterial transcriptomic sequencing would be helpful in confirming or refuting this prospect.

Our knowledge of the staphylococcal communities on feline skin is even more limited than what is known in dogs. While we know staphylococci are present and can be isolated from lesions associated with allergic dermatitis in cats, it is unclear which species are abundant and important. Several species, including *S. felis* (formerly *S. simulans*[66]),[67] *S. aureus*,[68,69] and *S. pseudintermedius*[70-72] have been isolated from lesions. Since these species have also been identified on healthy feline skin,[73,74] it is unclear if these bacteria are relevant to the development of these lesions or simply commensals. Furthermore, while we know which species may be found on feline skin, we are not sure in what relative abundance these species are present. Studies of canine skin have employed both culture-dependent and culture-independent techniques, which have allowed confirmation of the composition of their cutaneous staphylococcal communities; only culture-dependent surveys of feline cutaneous staphylococcal communities have been performed.

In addition to our limited knowledge of the composition of the staphylococcal communities on feline skin, their contribution to disease is unclear. *Staphylococcus* spp. infections secondary to allergic dermatitis are common in dogs, but are infrequent in feline patients,[1,2,75] which some attribute to the decreased ability of these bacteria to adhere to corneocytes.[76,77] Therefore, these bacteria may not be as relevant to development or impact of feline disease, or are just not well-recognized as important to feline patients. Although *Staphylococcal* bacteria are not able to establish themselves as well on feline skin when compared to other species, perhaps they may still be able to cause disease in the short time they do interact with the cutaneous surface, especially in cats which may be hypersensitive to staphylococcal antigens.

Cutaneous fungal microbiota on healthy and allergic skin

Relative to the bacterial microbiota, the cutaneous fungal microbiota, or mycobiota, is understudied. However, this is certainly not due to a lack of fungal communities on the skin or insignificance of fungi in cutaneous disease. Fungi are not only important opportunistic pathogens in atopic dermatitis, but some genera such as *Alternaria*, *Aspergillus*, and *Fusarium*, can be allergenic.[78]

Surveys of the human skin mycobiome have found the genus *Malassezia* to predominate with average relative abundance ranging from 50% to almost 100% in most body sites of healthy adults.[7,15,79,80] Interestingly, fungal communities are more diverse on the skin of children, where *Malassezia* spp. is still highly abundant but in addition to several other abundant taxa including *Aspergillus* spp. and *Epicoccum* spp.[81] On the skin of individuals affected by atopic dermatitis, there is increased relative abundance of

some opportunistic pathogens including *Candida albicans* and *Cryptococcus* spp. Additionally, fungal communities on affected individuals are different from those on healthy individuals, with communities influenced by severity of disease.[15]

Like the fungal communities on the skin of children, canine[24] and feline[23] cutaneous fungal communities are more diverse than adult human skin. Their communities are composed of fungi primarily from the phyla Dothideomycetes and Sorderiomycetes, with *Malassezia* spp. having an average relative abundance of <20% in all body sites of healthy animals. *Alternaria* spp. and *Cladosporium* spp. are some of the genera with the highest relative abundance on dogs, while cats have a substantial relative abundance of *Acremonium* spp. and more variability between individuals. Comparing samples from allergic individuals to healthy counterparts have identified a dysbiosis in cats[23] and dogs[24] with allergic dermatitis, with communities that cluster by health status. Further research may identify microorganisms that are of particular relevance to allergic dermatitis affecting dogs and cats. Currently, despite its relatively low abundance, *Malassezia* spp. has garnered much attention due to its role as an allergen and cause of secondary infections in humans affected by atopic dermatitis[60] as well as its role as a common isolate in secondary infections in atopic animals. Like secondary bacterial infections however, secondary fungal infections are considered less common in cats and therefore have been given less attention within feline research.[21,82,83]

Immune response in allergic dermatitis

Allergic disorders are inherently due to an abnormal immune response. In humans[9,84,85] and dogs,[86,87] the response in allergic dermatitis is described as biphasic,

with predominantly Th2/Th22 activity in the acute phase, followed by an increase in Th1 activity in the chronic phase. The acute Th2 response is associated with the release of cytokines that trigger inflammation and IgE production by B cells.[88] The process is evident in the skin, where sections stained with H&E show an inflammatory infiltrate composed primarily of T-lymphocytes, antigen-presenting cells, mast cells[89] and eosinophils.[90] Some of the cytokines associated with this reaction are IL-4, IL-5, IL-6, L-13, and IL-31.[91]

As mentioned previously, cats affected by allergic dermatitis do not appear to exhibit this typical Th2 response. While increased numbers of T cells have been described in samples from allergic cats with various clinical presentations,[89] the importance of Th2 cytokines has not been clearly demonstrated. In cats with miliary dermatitis, a predominance of CD4+ cells[92] with a Th2 phenotype have been identified from skin samples.[93] Only a single study has evaluated cytokine profiles in the skin of allergic cats; in the evaluation of mRNA expression of 13 cytokines, they did not identify increased levels of any of those associated with a Th2 response.[13] Although no other studies regarding cytokine expression in feline allergic dermatitis have been published, one abstract did find a single cytokine, IL-31, in higher circulating levels in cats with a presumed diagnosis of atopic dermatitis.[94]

Involvement of IL-31 in allergic dermatitis

Among the many soluble mediators found to contribute to allergic dermatitis is interleukin-31 (IL-31). This cytokine is relatively new, discovered in 2004 by researchers searching the human genome for novel type I cytokine receptors. It is part of

the IL-6 family of cytokines and is primarily produced by Th2 cells, in addition to other cells such as eosinophils, mast cells and keratinocytes. Further work to characterize this cytokine led them to demonstrate that injecting IL-31 into mice induced pruritus.[95] IL-31 is able to elicit this response and others upon binding to the heterodimeric receptor composed of oncostatin M receptor subunit beta (OSMR-β) and IL-31 receptor alpha (IL-31RA). This leads to signaling via the janus kinase (JAK)/signal transducer and activator of transcription (STAT), phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT), and various mitogen-activated protein pathways to induce production of proinflammatory cytokines, keratinocyte differentiation, and, most notably, pruritus.[96,97] Since its discovery, studies have found IL-31 to be relevant in human[98] and canine[99,100] atopic dermatitis, where its expression is increased both systemically and cutaneously in affected individuals. It has become a drug target in human and canine medicine through the use of monoclonal antibodies against IL-31[101-105] and a subunit of its receptor,[5,106] and indirectly via JAK/STAT inhibitors.[107] It is just now beginning to be explored as a potential target in feline medicine.

In cats with a presumed diagnosis of allergic dermatitis, circulating IL-31 levels were found to be elevated in a single study.[94] Another study demonstrated that injection of recombinant IL-31 induced pruritus in cats, which could be attenuated with a JAK/STAT inhibitor (oclacitinib).[108] While the success of the JAK/STAT inhibitor could not directly confirm the success of targeting IL-31, since many other inflammatory cytokines signal through this pathway, this finding provides evidence for further investigation into IL-31's relevance for feline allergy. Other studies have also shown that

the use of oclacitinib reduces pruritus in cats with naturally-occurring allergic dermatitis.[109,110] Work to develop a monoclonal antibody against feline IL-31 has begun and so far seems to be successful in reducing IL-31 induced pruritus in cats.[111]

CHAPTER II

THE EFFECT OF BREED AND ENVIRONMENT ON THE FELINE CUTANEOUS AND ORAL MICROBIOTA*

Introduction

Until recently, the feline skin microbiota had not been described using next-generation sequencing. We now know that feline skin is inhabited by bacterial communities that are distinct to each body site[25] and fungal communities more unique to the individual cat.[23] Additionally, the composition of the feline cutaneous microbiota is more diverse than previously described in culture-based studies.[55] Like canine[26,27,43] and human[46,49,79,112] skin, the primary bacterial phyla present on cats are Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes, although in different proportions. Unlike human skin which is primarily colonized by *Malassezia* spp.,[15,79,113] canine[24] and feline[23] skin are colonized by a more diverse fungal mycobiota, with Dothideomycetes (mainly *Cladosporium* spp., *Alternaria* spp., *Epicoccum* spp.), a class of many environmental fungi, being the predominant one found.

The feline oral cavity also has a diverse and unique microbiota. Due to the prevalence of oral disease[114-118] and cat bite infection,[119-121] which are known to be associated with bacteria, much of the feline oral microbiota researched has focused on

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the bacterial populations present; however the fungal oral microbiota has been described in a previous study focused on the cutaneous microbiota.[23] The feline oral bacterial communities are similar to what has been described on the skin, but with increased abundances of Bacteroidetes,[25] a phylum containing many bacteria typically found in microbiota surveys of the oral cavity of cats[40,116,118,122,123] and other species.[124-126]

Microbial communities inhabiting the body are shaped through a variety of intrinsic and extrinsic factors, two of which are the host's genotype and environment.[47] Human microbiome research has indicated that genetic variation can affect the microbiota, through comparing monozygotic and dizygotic twins[31,34] or by associating microbiota factors with specific genetic diseases.[127-129] Studies have found evidence for genotype affecting the diversity of the microbial communities found, with respect to the number of species present, as well as the taxonomic composition of the communities.[30-34,127] There are even taxa that have been associated with genetic diseases, such as increased abundances of *Clostridium difficile*[127] and *Enterobacteriaceae*[128] in patients with NOD2 genotypes associated with increased risk of inflammatory bowel disease.

Research on the microbiota of humans[42,130] and animals[131,132] has revealed that the environment can also shape microbial communities. This has been described in multiple studies assessing the cutaneous microbiota of humans living in different environments; individuals living in more urbanized habitats tend to have a microbiota with decreased diversity,[39,130,133] which has been associated with an increased risk of developing allergies.[130,134] Additionally, the presence of animals in a home has been

demonstrated to alter the home microbiota,[135] so it is not surprising that direct contact with animals, including cohabitation with[36,40,42] or working with animals, [35,37,41] can also have a considerable effect on the diversity and composition on the human skin microbiota.

Evaluation of the cutaneous microbiota in various animal species, including cats, is still in its infancy, and many factors influencing the skin microbiome in animals are still unknown and should be further researched. With respect to cats, several breeds are at higher risk for certain cutaneous infectious diseases, such as Persian cats with dermatophytosis[136-138] or Devon Rex cats with *Malassezia* dermatitis;[139,140] perhaps some of this increased risk could be related to the microbiota. With the known effect of the environment on the human skin microbiota, including a potential role in the development of allergies, the effect of environment on the feline skin microbiota should be elucidated.

Therefore, the objectives of this study were to evaluate how genotype and environment can influence the bacterial and fungal microbiota of feline skin. With the grooming habits of cats likely playing a role in microbial community composition of the skin, the oral cavity is also of interest and thus was sampled. In order to assess the effect of genotype, purebred cats of five different breeds were sampled. These cats are selectively bred to have a specific hair phenotype.[141-143] The different hair phenotypes seen may provide an altered habitat in terms of other features (e.g. lipid content, hydration, etc.), which could affect the composition and diversity of the microbiota. With respect to environment, we characterized the microbiotas of mixed genetic background cats kept strictly indoors or strictly outdoors. We hypothesized that different

feline breeds would vary in their microbial communities due to the differences in genotype, resulting in phenotypic characteristics affecting the development and maintenance of the microbiota. Furthermore, we hypothesized outdoor cats would have more diverse microbial communities of a different composition relative to indoor cats, due to their exposure to a greater diversity of microbes and less stable environmental conditions.

Materials and methods

This study was approved by the Texas A&M University (TAMU) Institutional Animal Care and Use Committee and in accordance with the relevant guidelines. Informed consent was obtained for all cats enrolled in the study.

Sample collection

Sixty-nine cats were enrolled in this study: 11 Bengals, 10 Cornish Rexes, 4 Devon Rexes, 6 Siberians, 13 Sphynxes, 13 indoor Domestic short/medium/long hairs, and 12 outdoor Domestic shorthairs (Table S1). Samples were taken from the axilla, dorsum, ear canal, nostril and oral cavity by rubbing each side of two Isohelix buccal swabs (Cell Projects Ltd., Kent, UK) 10 times. Both swabs were placed in a MO BIO PowerBead tube (MoBio Laboratories, Carlsbad, CA). DNA was extracted using a modified protocol with the MoBio PowerSoil DNA Extraction Kit and stored at -80^{o}C until used. Extracted DNA from the samples and from controls (swab only and reagent only) was sent to MR DNA Lab in Shallowater, TX for sequencing on an Illumina MiSeq (Illumina, San Diego, CA). The V4 region of the 16S rRNA gene was sequenced using primers 515F: GTGYCAGCMGCCGCGGTAA and 806R:

GGACTACNVGGGTWTCTAAT. The internal transcribed spacer 1 (ITS-1) region between the 18S and 5.8S rRNA genes was sequenced using primers ITS1-F: CTTGGTCATTTAGAGGAAGTAA and ITS2: GCTGCGTTCTTCATCGATGC. The sequences analyzed are available in the NCBI sequence read archive under BioProject ID PRJNA473778.

Sequence processing

The resulting sequences were processed using QIIME 1.9.[144] Sequences were demultiplexed and open-reference OTU picking was performed with uclust.[145] For the 16s sequences, the Greengenes database (13_8 release)[146] was used with a 97% threshold of identity, and for the ITS sequences, the Findley database[79] was used. Taxa presumed to be contaminants were removed as previously described.[40]

Prior to diversity analyses, samples were rarefied to 21000 bacterial and 3800 fungal sequences in order to account for unequal sequencing depth. To evaluate alpha diversity, the Chao1, Observed OTUs, and Shannon metrics were used. Good's coverage was used to assess sampling depth. For beta diversity the Bray Curtis, weighted UniFrac, and unweighted UniFrac metrics were used for the 16s sequences and the Bray Curtis, Abundance Jaccard and Pearson correlation metrics were used for the ITS sequences.[147]

Species-level classification of Malassezia sequences

To obtain species-level assignments for the *Malassezia* spp. sequences, the raw fungal sequences were processed using mothur[148] where they were classified with the k-nearest Neighbor algorithm and blasted against the Findley database. *Malassezia* spp. sequences were then extracted and aligned to a reference alignment of *Malassezia* spp.

sequences. Species level assignments were determined using pplacer[149] and a *Malassezia* reference package.[79]

Quantitative PCR (qPCR)

The extracted DNA was also used for qPCRs targeting *Malassezia* spp. and *Propionibacterium* spp. For the *Malassezia* spp. qPCR, primers ITSANA-F (CGAAACGCGATAGGTAATGTG) and ITSANA-R (CAAATGACGTATCATGCCATGC)[150] were used with reactions containing 5 uL of iTaq Univeral SYBR Green Supermix (Bio-Rad Laboratories, Hercules, California), 2 uL Invitrogen UltraPure water (Invitrogen, Carlsbad, CA), 0.5 uL of each primer (Integrated DNA Technologies, Coralville, IA), and 2 uL of sample. After 3 minutes at 95°C, 39 cycles of 30 seconds at 95°C and 30 seconds at 60°C were performed, followed by a melt curve from 65°C to 95°C.

For the *Propionibacterium* spp. qPCR 20 uL reactions consisted of 10 uL iTaq Universal Probes Supermix, 5 uL Invitrogren UltraPure water, 1 uL each of oligos EUB519F (CAGCAGCCGCGGTRATA), U785R (GGACTACCVGGGTATCTAAKCC), and Prop_P ([FAM]CTTTCGATACGGGTTGACTT[BHQ-1]) (Sigma-Aldrich, St. Louis, MO) using the thermocycler conditions previously published.[7]

PCRs were run on a Bio-Rad CFX Connect™ Real-Time PCR Detection System, results were analyzed using Bio-Rad CFX Manager™ and data were normalized based

on DNA concentration, as determined using the Qubit high sensitivity dsDNA assay (Qubit, London, UK).

Statistical analysis

Statistical significance of alpha diversity results was analyzed using the Kruskal-Wallis test for overall significance and the Wilcoxon test for pairwise tests in JMP Pro 12 (SAS Institute, Cary, NC). For beta diversity results, ANOSIM on the resulting distance matrices in PRIMER 6 (PRIMER-E, Albany, New Zealand) or using the vegan package in R was used. Kruskal-Wallis tests, followed by Wilcoxon pairwise tests and Benjamini-Hochberg procedure for p-value correction[151] where appropriate, and LEfSe (with a $p<0.01$ considered significant) were used to analyze differential taxa abundance. For Kruskal-Wallis tests on relative taxa abundances, only taxa present at greater than 1% in at least 10 samples were tested.

Results

To evaluate differences in the microbiota between different cat breeds, samples from Bengal, Cornish Rex, Devon Rex, Siberian, Sphynx, and indoor domestic (mixed genotype) cats were analyzed. The environment analyses included samples from indoor and outdoor domestic cats. The average Good's coverage estimate for bacterial sequences was 0.971 and for fungal sequences 0.986.

Bacteria

Cat breeds were significantly different in terms of alpha diversity (Chao1,

observed OTUs, and Shannon diversity index $p<0.0001$; Fig 1a), which measures the number of different unique taxa identified and, with some metrics, how evenly abundant they are. Devon Rex cats had the lowest medians for alpha diversity, indicating relatively low diversity, and Bengal cats had the highest. With regards to body site, the most pronounced differences were observed for the dorsum and ear canal (Table 1). When only the dorsum was evaluated, Sphynx cats had the most diverse microbial populations (Fig S1). Comparison of all cats based on hair length did not reveal significant differences. Alpha diversity was also not significantly different between indoor and outdoor cats, regardless of metric used or body site analyzed (Table 1; Fig 1b).

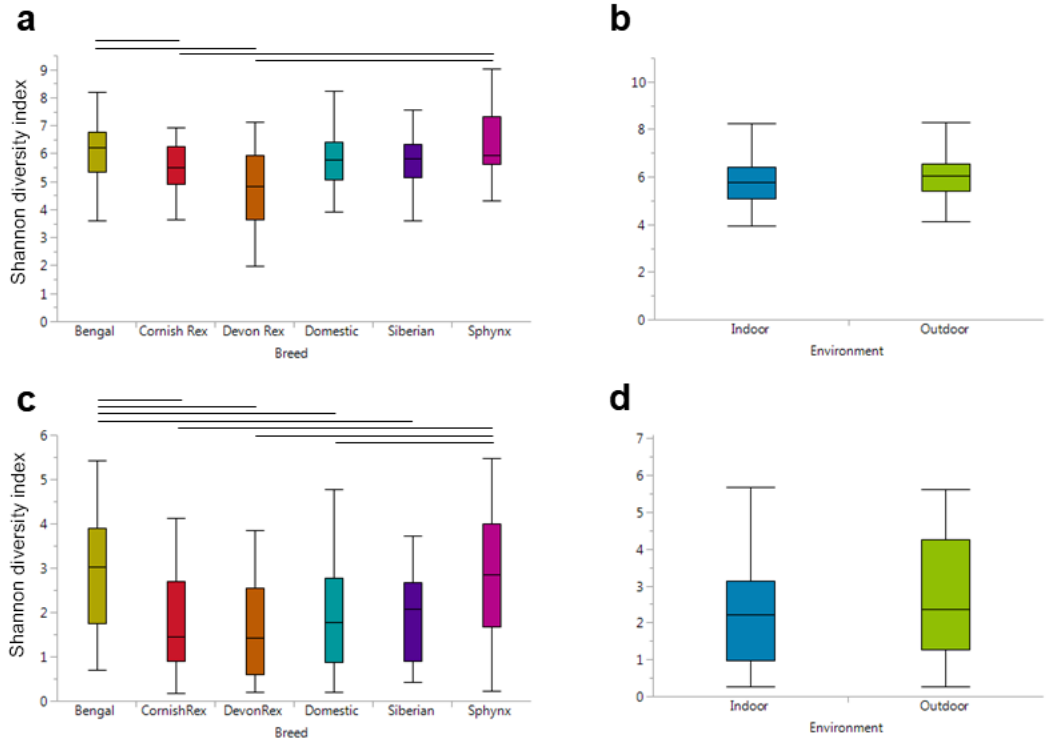

Figure 1. Comparing alpha diversity as measured by the Shannon diversity index between cat breeds and indoor and outdoor cats.
Comparing alpha diversity of (a) bacterial communities between cat breeds ($p<0.0001$), (b) bacterial communities between indoor and outdoor cats ($p=0.2509$), (c) fungal communities between cat breeds ($p<0.0001$), and (d) fungal communities between indoor and outdoor cats ($p=0.8340$) using the Shannon diversity metric. Lines show significant pairwise tests where $p<0.01$. Sample sizes (bacterial sequencing, fungal sequencing): Bengal (54, 54), Cornish Rex (45, 45), Devon Rex(19, 19), Domestic/Indoor (55, 59), Siberian (29, 30), Sphynx (70, 69), and Outdoor (60, 58).

Table 1. Statistical analysis of alpha diversity results.
P-values from Kruskal-Wallis test comparing alpha diversity results across body sites with respect to breed and environment for bacterial and fungal microbiota. P<0.05 are bolded.

	Bacteria			Fungi		
Breed	Chao1	Observed OTUs	Shannon	Chao1	Observed OTUs	Shannon
All	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Dorsum	**0.0018**	**0.0007**	**0.0026**	**0.0006**	**0.0011**	**0.0013**
Ear canal	**0.0259**	**0.0043**	**0.0060**	**0.0274**	**0.0267**	**0.0165**
Groin	0.1205	0.0502	**0.0297**	0.0639	0.2155	0.1178
Nostril	0.3831	0.2762	0.8045	**0.0120**	**0.0010**	**0.0038**
Oral	0.4665	0.8634	0.5689	0.7634	0.5888	0.6466
Environment						
All	0.5269	0.2212	0.0836	0.7768	0.32908	0.2780
Dorsum	0.7290	0.6649	0.7728	0.4529	0.8625	0.6861
Ear canal	0.0479	0.0250	0.0210	0.4529	0.4189	0.2482
Groin	0.7416	0.8951	0.8951	0.6666	0.7119	0.5796
Nostril	0.4984	0.1567	0.6225	0.1872	0.0805	0.0559
Oral	0.1659	0.1123	0.9081	0.8703	0.5676	0.6831

Beta diversity analysis revealed significant differences in the dorsum samples between cat breeds (R=0.247 and p=0.001, Fig 2a). The Bray-Curtis and weighted UniFrac metrics were significant, while the unweighted UniFrac was not, indicating the dissimilarity in communities is attributed to differential abundance, regardless of phylogenetic relationships between taxa. Significant differences in beta diversity between indoor and outdoor cats were only seen in the oral cavity (Table 2), with the Bray-Curtis (R=0.321 and p=0.001, Fig 2c) and weighted UniFrac (R=0.416 and p=0.001, Fig 2d) metrics.

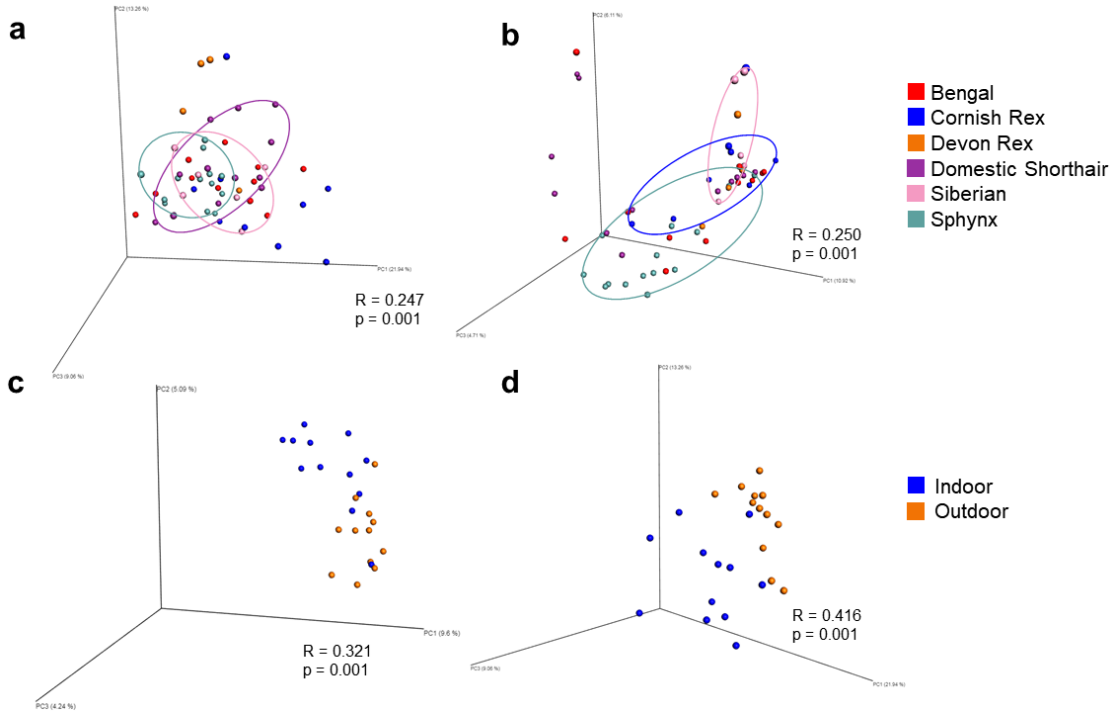

Figure 2. Principle coordinate analysis (PCoA) plots of beta diversity distance matrices comparing different cat breeds and indoor and outdoor cats.
Comparing beta diversity of (a) bacterial communities on the dorsum between cat breeds with the weighted UniFrac metric, (b) fungal communities on the dorsum between cat breeds with the Bray-Curtis metric, (c) bacterial communities in the oral cavity between indoor and outdoor cats with the Bray-Curtis metric, and (d) bacterial communities in the oral cavity between indoor and outdoor cats with the weighted UniFrac metric. R and p-values are from analysis of similarities (ANOSIM) test of beta diversity distance matrices.

Table 2. Results from ANOSIM tests on bacterial beta diversity results.
Results from ANOSIM on distance matrices comparing structure of bacterial communities. R value, p-value. Results with R>0.150 and P=0.001 are bolded.

Breed	Bray-Curtis	Weighted UniFrac	Unweighted UniFrac
All	0.099873, 0.001	0.077965, 0.001	0.046498, 0.002
Dorsum	**0.204435, 0.001**	**0.247425, 0.001**	0.16356, 0.001
Ear Canal	0.086077, 0.028	0.144358, 0.003	0.023431, 0.287
Groin	0.168733, 0.001	0.126788, 0.004	0.044658, 0.166
Nostril	0.12199, 0.005	0.093338, 0.023	0.040002, 0.179
Oral	0.11469, 0.011	0.11641, 0.011	0.060964, 0.089
Environment			
All	0.03111, 0.012	0.032157, 0.023	0.019495, 0.057
Dorsum	-0.06634, 0.981	-0.01042, 0.458	-0.07344, 0.970
Ear Canal	0.082282, 0.100	0.031982, 0.281	0.08228, 0.125
Groin	0.014865, 0.369	-0.00586, 0.466	-0.02462, 0.603
Nostril	0.05303, 0.154	0.079264, 0.088	-0.01415, 0.571
Oral	**0.32097, 0.001**	**0.416351, 0.001**	**0.19939, 0.004**

The average relative abundance of bacterial taxa by sample type is shown in Fig 3. The main phyla identified were Proteobacteria (mean relative abundance = 44.03%), Firmicutes (21.04%), Bacteroidetes (16.65%), and Actinobacteria (10.38%). Some of the most abundant taxa included bacteria within the family *Pasteurellaceae* (11.14%) and from the genera *Porphyromonas* (7.40%) and *Staphylococcus* (4.79%). *Veillonellaceae*, a family of bacteria typically found in the gastrointestinal microbiota of humans and animals,[152,153] and in lesser abundances in the human[154] and animal[25,43] skin microbiota, was found to have significantly different relative abundances between cat breeds (Kruskal-Wallis p=0.0004) when considering all body sites, with greater relative abundances in the ear canal of Sphynx cats (LEfSe LDA score>4.0). Additionally, other

taxa such as *Porphyromonas* spp. (p=0.0003) and *Lactobacillus* spp. (p<0.0001) were differentially abundant across cat breeds (Table S3).

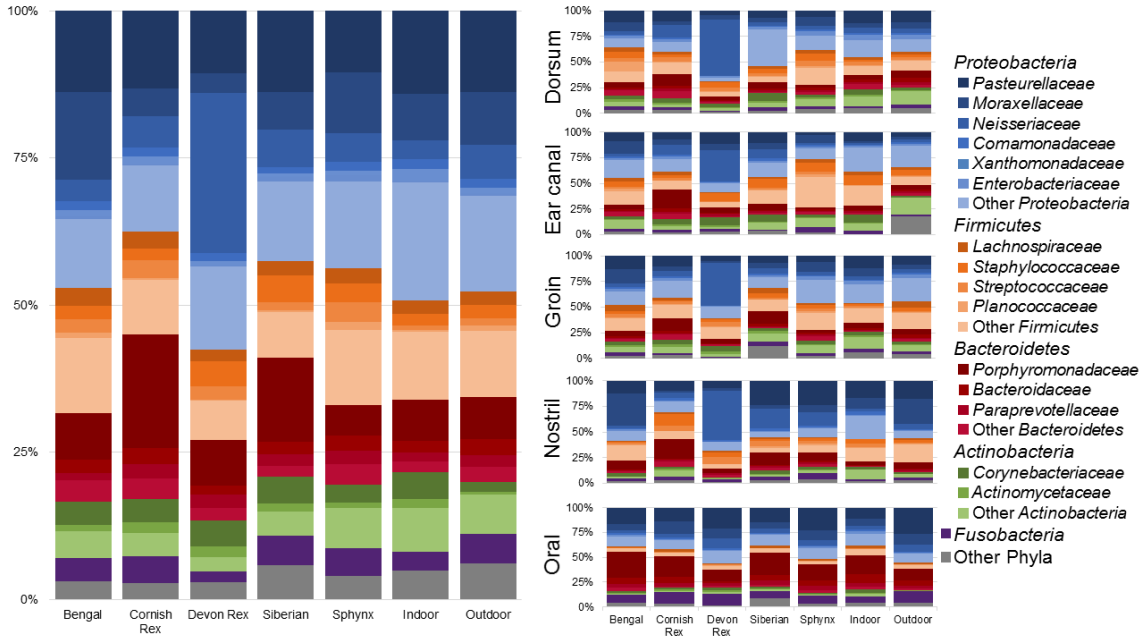

Figure 3. Average relative abundance of bacterial taxa.
The average relative abundance of bacterial taxa across the different cat breeds and outdoor cats including all body sites and separated by body site.

Many bacterial taxa were also found to be differentially abundant between indoor and outdoor cats when considering all body sites (Fig 4 and Fig S2a, without oral cavity), and when considering only the nostril samples (Fig S2c). One bacterial genus identified to be differentially abundant when considering all body sites was *Corynebacterium* spp.; greater relative abundance was identified in samples from indoor cats (average relative abundance of 5.7% in indoor cats and 1.9% in outdoor; LEfSe |LDA score| > 3.5; Wilcoxon test p=0.0043).

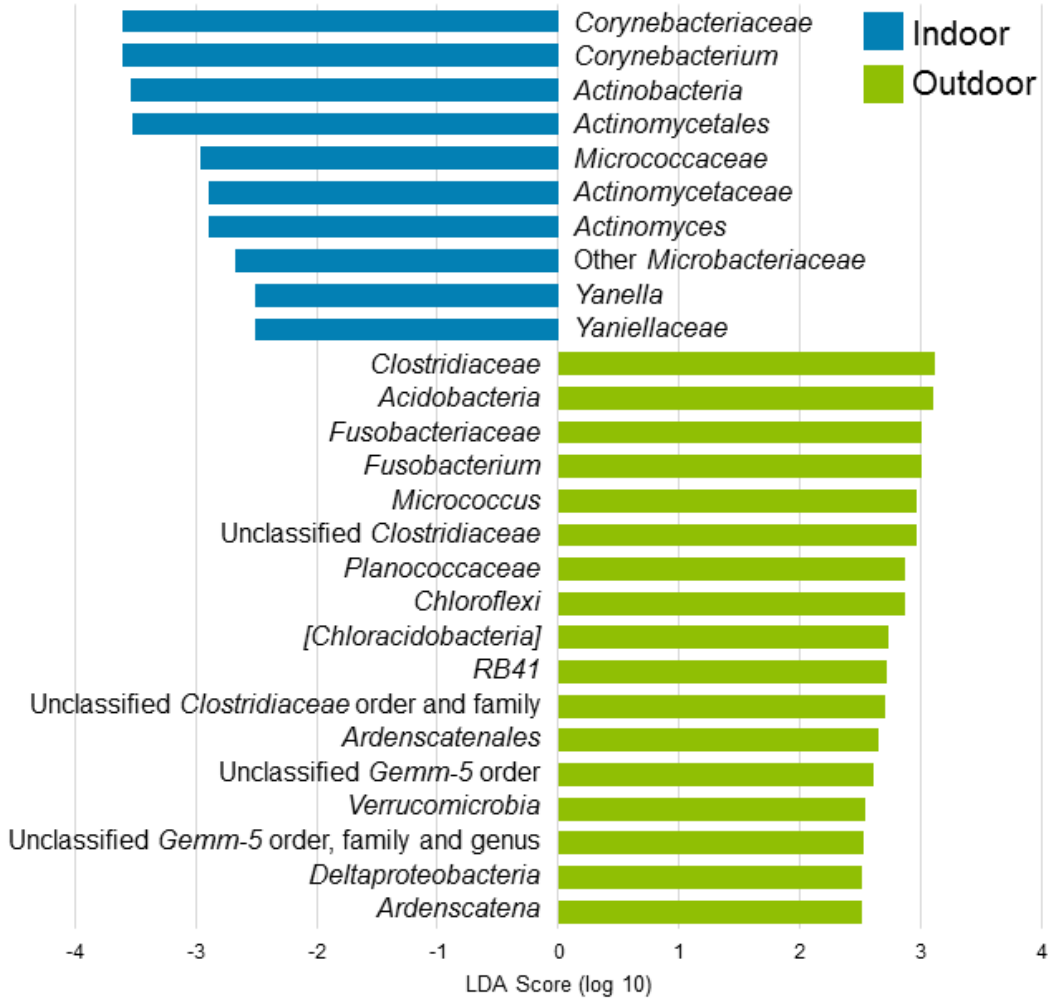

Figure 4. Bacteria found to be differentially abundant between indoor and outdoor cats as determined by LEfSe.
When comparing all body sites, many taxa were identified as differentially abundant between indoor and outdoor cats.

Because of the relevance of *Propionibacterium* spp. in the cutaneous microbiota of humans and the known inability of the sequencing primers used in this study to target this genus accurately,[155,156] a qPCR for the genus was performed to investigate its abundance on feline skin. No significant differences in *Propionibacterium* spp.

abundance between the different cat breeds or between indoor and outdoor cats were found (Fig S3a).

Fungi

As was found with the bacterial microbiota, alpha diversity of fungal communities was significantly different between different cat breeds when considering all body sites, with Sphynx and Bengal cats having the highest diversity ($p<0.0001$, across all 3 metrics) (Fig 1c). Furthermore, with regards to body site, cat breeds were significantly different on the dorsum, ear canal and nostril (Table 1). Similar to the bacterial data, no significant difference in alpha diversity was found in the fungal sequences between indoor and outdoor cats (Table 1; Fig 1d). When hair length was analyzed, significant differences were observed when evaluating all body sites together and when analyzing only the dorsum with all alpha diversity metrics (all $p<0.01$). Breeds with short (DSH) and very short hair coats (Cornish Rex, Devon Rex, and Sphynx cats) seemed to have more diverse communities relative to cats with long or medium hair (Fig S7).

Both the dorsum (Bray-Curtis $R=0.250$, $p=0.001$; Pearson $R=0.221$ and $p=0.001$; Table 3 and Fig 2b) and groin (Bray-Curtis $R=0.244$, $p=0.001$) were body sites where significant differences in beta diversity were found between cat breeds. Regardless of metric used or body sites analyzed, no significant clustering was found between indoor and outdoor cat samples (Table 3).

Table 3. Results from ANOSIM tests on fungal beta diversity results.
Results from ANOSIM on distance matrices comparing structure of fungal communities. R value, p-value.

Breed	Bray-Curtis	Pearson	Jaccard
All	0152456, 0.001	0.125037, 0.001	0.074513, 0.001
Dorsum	**0.250435, 0.001**	**0.221035, 0.001**	0.03777, 0.198
Ear Canal	0.178865, 0.001	0.135659, 0.001	0.067723, 0.059
Groin	**0.243501, 0.001**	0.170137, 0.001	0.092201, 0.022
Nostril	0.039623, 0.197	0.005078, 0.457	-0.03487, 0.77
Oral	-0.04238, 0.834	-0.01625, 0.662	-0.02459, 0.707
Environment			
All	0.011423, 0.079	0.014186, 0.028	-0.00166, 0.513
Dorsum	-0.04267, 0.889	-0.04682, 0.947	-0.03809, 0.795
Ear Canal	0.015152, 0.254	0.017677, 0.21	-0.03388, 0.725
Groin	0.014525, 0.312	0.018908, 0.285	0.022414, 0.322
Nostril	-0.056765, 0.946	-0.01471, 0.591	0.003754, 0.454
Oral	0.016049, 0.333	0.051852, 0.111	-0.01289, 0.537

Fig 5 shows a summary of the most abundant fungal taxa. Relative to the bacterial microbiota, the composition of the fungal communities was more variable between cat breeds. Some of the most abundant genera included *Cladosporium* spp. and *Malassezia* spp. When comparing all samples across cat breeds many taxa were differentially abundant (Table S4), including *Alternaria* spp. (Kruskal-Wallis p=0.0064), *Aspergillus* spp. (p=0.0026), and *Malassezia* spp. (p=0.0026). Looking at differences in taxa abundance at the body site level, the dorsum had the most significant changes at different taxonomic levels, followed by the nostril and groin. This was particularly evident in the relative abundance of *Malassezia* spp. (p=0.0096) and *Alternaria* spp. (p=0.0078) in samples collected from the dorsum.

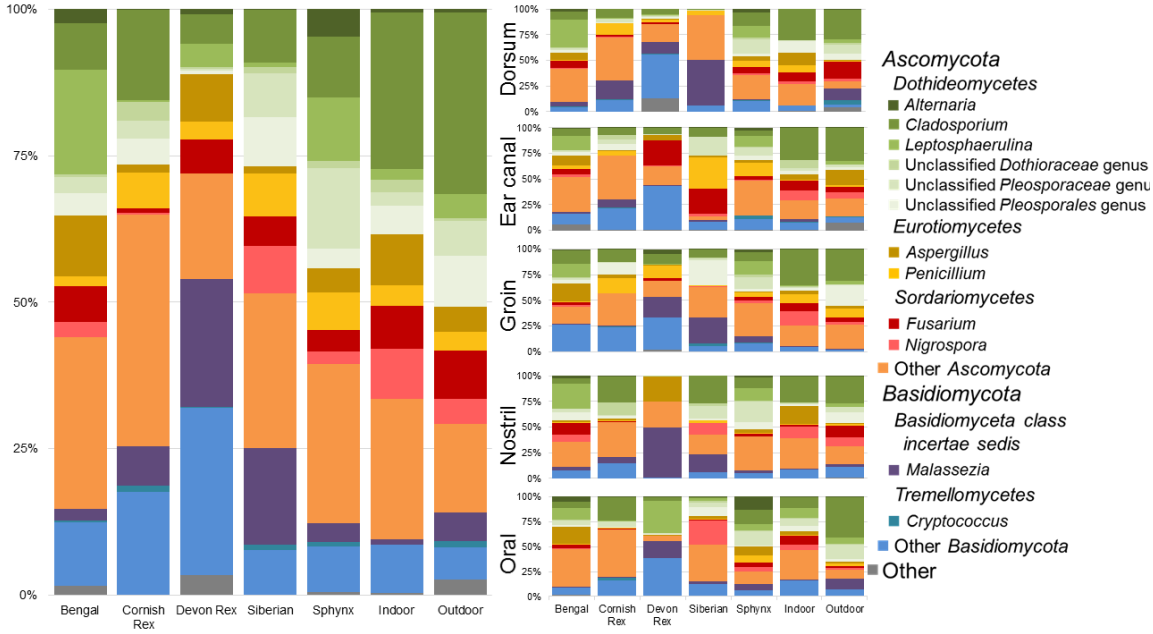

Figure 5. Average relative abundance of fungal taxa.
The average relative abundance of fungal taxa across the different cat breeds and outdoor cats including all body sites and separated by body site.

In comparing indoor and outdoor cats, taxa that were differentially abundant were found when including all sites except the oral cavity (Fig S2b), and when considering the dorsum (Fig S2d) and the nostril samples (Fig S2e) separately. Ustilaginomycetes and Ustilaginales,[157] taxa containing primarily plant pathogens, were found to be increased on outdoor cats in the analyses including all body sites except oral cavity, and in samples from the dorsum (<0.0001% in indoor cats, 1.09% in outdoor cats). Two of three phyla were identified to be differentially abundant in the nostril samples: Ascomycota (Wilcoxon test p=0.0303, higher in indoor) and Basidiomycota (p=0.01934, higher in outdoor), in addition to sequences that were unassigned to any phylum (p=0.0224).

Malassezia sequence analysis

Due to the significant findings in regards to *Malassezia* spp. abundance and previous research into their differential abundance between cat breeds[140], species-level classification of these sequences was performed. *M. restricta* and *M. globosa* were the most prevalent species with average relative abundances (relative to total *Malassezia* spp. sequences) of 37.0% and 23.9%, respectively, across all samples. Sequences also matched *M .slooffiae, M. furfur, M. nana, M. pachydermatis, M. dermatis, M. sympodialis, M. japonica, M. obtusa,* and *M. yamatoensis,* with average relative abundances <8.6% (S5 Table), and with an average of 17.0% of the *Malassezia* sequences not classified to the species level. Although *M. slooffiae* accounted for 25.4% of the total *Malassezia* spp. sequences, five samples had the majority of these sequences (sequence range: 13260 to 103995), which were from various body sites from two non-cohabiting Cornish Rex cats (6 and 16). As shown in S4 Fig, *Malassezia* spp. abundance is significantly different between cat breeds ($p=0.0026$), with Devon Rex cats having the highest abundance. No significant difference in abundance of any specific *Malassezia* species were found between the different cat breeds or when comparing indoor and outdoor cats.

Quantitative PCR targeting *Malassezia* spp. revealed significant differences in abundance between the different cat breeds ($p<0.0001$), but not the indoor and outdoor cats (S3b Fig). *Malassezia* spp. were significantly more abundant in the domestic shorthair cats relative to all other feline breeds ($p<0.0016$).

Other factors affecting the microbiota

Samples from the domestic (mixed genotype) indoor cats were assessed for influence of age group and sex. Females were found to have more diverse fungal communities in the oral cavity (Wilcoxon on Chao1 p=0.0201 and Shannon diversity index p=0.0201) and on the skin (Chao1 p=0.0153, observed OTUs p=0.0443). Additionally, senior cats (7+ years) had more diverse bacterial (observed OTUs p=0.0327) and fungal (Chao1 p=0.0416) communities on the skin compared to adult cats (1-7 years). Only the oral cavity was affected by either of these factors in terms of beta diversity, with bacterial communities being influenced by age group (ANOSIM on Bray-Curtis dissimilarity R=0.2332, p=0.037) and the fungal communities by sex (Pearson correlation R=-0.2602, p=0.986). Some taxa were found to be differentially abundant on the skin with respect to these factors (LEfSe, $p<0.01$), however many had relatively low LDA scores indicating minor impacts on differences between the groups and/or are not of known importance in the oral cavity or skin microbiota (Table S6).

Discussion

This is the first study evaluating the effect of breed and environment on the feline skin and oral microbiota. Many of the cat breeds that are recognized today were developed through selection of specific hair coats. Mutations that contribute to the different hair coats have been identified and can result in a reduced coat, almost to the point of being considered ”hairless”, such as in the Sphynx, or short wavy hair, such as in the Cornish Rex.[141-143] The difference in hair coats, and perhaps variation in other features of the skin (lipid production, water content), may be responsible for altered

microhabitats that could support different microbial communities. For example, Devon Rex cats are thought to develop seborrheic dermatitis involving the lipophilic yeast *Malassezia* spp. more often than other breeds.[139,140] Currently this has not been further investigated but perhaps genetic mutations that affect lipid content or epidermal maturation in Devon Rex cats could explain this. In the results presented, the different cat breeds sampled showed differences with respect to the diversity of their bacterial and fungal communities (Fig 1) and showed that individual cats did cluster with others of their same breed (Fig 2).

One aspect of the cat breeds we thought would contribute to the microbiome was the length of the hair coat. However, when cats were grouped based on this factor (Cornish Rex, Devon Rex, and Sphynx="very short", Bengals and DSH="short", DMH="medium", Siberian and DLH ="long") significant differences were only found in evaluating fungal alpha diversity (Fig S7) and in terms of some differentially abundant taxa in specific body sites (Table S7). Considering we observed many other differences between cat breeds, there are likely other physiologic differences, likely influenced by genetics, which play a role. If hair length were the only influencing difference in physiology that contributes to the microbiome, we would expect the short-haired Cornish Rex, Devon Rex cats, and almost hairless Sphynx cats to harbor a different microbiome from long haired Siberian cats. In the results presented however, this is not observed; comparing alpha diversity showed that Sphynx cats had higher Shannon diversity than all three of these other breeds, with significantly more diverse communities compared to the Cornish and Devon Rex cats, but not the Siberian cats (Fig

1a and 1c). To our knowledge, no studies have evaluated the cutaneous microhabitat in terms of pH, hydration, lipid composition, etc. of cats or comparing between cat breeds that would allow for a clear understanding of which features may be responsible for the microbiome differences observed. Once these data are available, it would be possible to re-analyze the data in the present study, in the context of these physiological differences.

The environment also has a role in shaping the microbiota. In terms of composition, many taxa were found to be differentially abundant. For example, outdoor cats harbored higher relative abundances of two fungal plant pathogen taxa, Ustilaginomycetes and Ustilaginales (Fig S2b and S2d). Bacterial taxa were also found to have significant differences in relative abundance (Fig S2a and S2e), however many of those were present in relatively low abundances, so their impact is not clear at this point. One bacterial taxon with differential abundance was *Corynebacterium* spp.; this microbe is found in relatively high abundances on human skin,[158] so its higher abundance on indoor cats could be due to their closer contact with human microbiota. Interestingly, environment also affected beta diversity of bacterial communities, but only in the oral cavity (Table 2). Perhaps this could be attributed partially to diet, since outdoor cats have access to a greater diversity of food sources. Contrary to what we hypothesized, outdoor cats did not have a more diverse skin microbiota than indoor cats in terms of the number of different taxa found (Fig 1b and 1d). Considering the sharing of microbiota that we know to exist between cohabiting humans and animals[36,40,41] and humans and the environment,[42,130] maybe larger differences between indoor and outdoor cats were not seen because indoor-only cats also come into regular contact with

environment-associated microbes through the microbial communities that are carried in the air or on their owners. The grooming habits of cats also likely contributes to these findings; perhaps the oral cavity acts as a collection site, collecting the microbes from the environment that are only transiently associated with the skin. Alternatively, maybe the microbiota exchange occurs in the opposite direction, with the oral cavity microbes being transplanted to the skin and potentially stabilizing the cutaneous communities.

While environment, and to a lesser degree breed, had an effect on the oral bacterial microbiota, there were no significant differences in the oral mycobiota between either indoor and outdoor cats or the different cat breeds. As mentioned above, diet likely also has a role in influencing the oral cavity microbiome. Indoor cats are most often fed a commercial diet, whereas outdoor cats may receive a commercial diet, but also have access to small mammals, insects, plants, etc. Another study has found diet can affect the feline oral microbiome; cats fed a dry food diet had more diverse oral communities relative to cats fed a wet food diet, which could be attributed to the higher relative abundance of several taxa.[123] Within our study, we were not able to obtain information regarding diet for all cats, especially outdoor cats, preventing us from analyzing the influence of diet.

One particularly interesting finding across the mycobiota of different cat breeds was the relative abundance of *Malassezia* spp. In our NGS data, we had similar results to those of Bond et al., with Devon Rex cats having the highest abundance of *Malassezia* spp. ($p=0.0003$) compared to the other cat breeds sampled (Fig S4).[140] In our qPCR data, while *Malassezia* spp. were not significantly more abundant in the Devon Rex cats

compared to the other breeds, these cats did have the highest median abundance (Fig S3b). This lack of agreement may be due to amplification bias, meaning the two primer pairs do not equally amplify all species.[159] In addition to further investigating differential *Malassezia* spp. abundance across cat breeds, we were also interested in describing the abundance of different *Malassezia* species. Previously, *M. pachydermatis* was found to be the most abundant on feline skin,[140] however in the presented NGS data, *M. restricta* and *M. globosa* were the most abundant species across all cat breeds and both indoor and outdoor cats (Table S5). The previous study utilized a culture-based technique to describe the *Malassezia* populations on feline skin which likely contributes to the different findings, due to the fastidious nature of some *Malassezia* species.[160] These findings further support differential *Malassezia* spp. abundance across breeds and warrant further research into this yeast's role on feline skin.

With next generation sequencing studies, the bias introduced by primer pair choice should be considered and primer sets that best capture the microbiota of interest should be used when possible.[156,161] With the bacterial primer set used in the presented study, *Propionibacterium* spp. abundance is not accurately represented.[155] However, previous studies have indicated *Propionibacterium* spp. may not be as prominent in the skin microbiota of cats and dogs,[25,26] so the lack of *Propionibacterium* spp. sequences may not be as impactful as in human studies. Perhaps there is a lack of *Propionibacterium* spp. on canine and feline skin, which could be attributed to physiological differences of their skin relative to human skin, however more research describing the normal microhabitat of companion animal skin is needed to provide better

support for this.[162,163] In order to describe the *Propionibacterium* spp. populations on feline skin, we used a quantitative PCR, but did not find differences between the cat breeds or indoor and outdoor cats (Fig S3a). Although studies have focused on optimizing primers for human skin studies, this has not yet been done for cats or dogs. Future sequencing projects utilizing other primers sets and larger cohorts would add to the existing characterization of the feline cutaneous microbiota. Additionally, since we know their communities and skin habitats are different from humans, studies identifying optimal sequencing primers for animal skin microbiota surveys should be performed.

Additional studies looking at other breeds as well as other influencing factors should be performed to better understand the importance of the findings presented. In this study, analysis of only the indoor domestic cats indicated some significant differences with respect to age and sex, however the sample numbers used to perform these comparisons were low and no differences of seemingly biological significance were observed; further studies focused on these factors, while controlling for others, would be more informative on their impact. In addition to considering what differences may exist, we also need to understand why these differences exist and the impact of their effects. For example, perhaps some of the differences with environment are only transient and simply due to exposure to a more varied microbial habitat outdoors; longitudinal studies would help discern this. Additionally, this study included cats from a relatively small area; surveys encompassing other geographic ranges of different climates and types of outdoor environments would add to our knowledge of the environment's influence on the feline skin microbiota. Lastly, studies sequencing the

host genome along with the skin microbiota, would allow for clearer associations between the feline genotype and the microbial communities inhabiting their skin.

Conclusion

Our findings demonstrate that the breed and, to a lesser degree, the environment, play a significant role in shaping the feline cutaneous microbiota. The many differences in the microbiota of different cat breeds are likely due to innate features of the different cat breeds, such as hair coat, that may support growth of different microbial communities. Grooming is likely an important influence on the feline skin microbiota, and may overshadow other factors known to be relevant for humans and other animals; research into how grooming shapes the microbiota may allow us to better understand the importance of other factors.

CHAPTER III

CHARACTERIZATION OF STAPHYLOCOCCAL COMMUNITIES ON HEALTHY AND ALLERGIC FELINE SKIN*

Introduction

Staphylococcus spp., although often thought of as pathogens, are known cutaneous commensals. In humans, *Staphylococcus epidermidis* is the most abundant species on healthy skin, and is outgrown by *S. aureus* on the skin of individuals affected by atopic dermatits.[14] In atopic dermatitis, *S. aureus* can take advantage of the barrier dysfunction and increase its numbers, gaining deeper access into the skin, resulting in secondary bacterial infections.[16] Besides the skin barrier and various components of the cutaneous ecosystem that have roles in preventing this, some microbes, such as *S. epidermidis,* are also important in host defense.[28,29] These bacteria can prevent overgrowth of *S. aureus* through a variety of mechanisms and can modulate the host immune system. Therefore, the genus *Staphylococcus* is of great importance in allergic dermatitis. This impact however is not strictly limited to humans.

In dogs, *S. pseudintermedius* has been recognized as the predominant staphylococcal species on both healthy and allergic canine skin with both culture-dependent and culture-independent studies.[164] In one study, *S. pseudintermedius* accounted for 59.4% of the total *Staphylococcus* spp. in all canine samples (healthy and

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atopic).[22] In lesions of canine pyoderma secondary to allergic disease, the overall *Staphylococcus* spp. abundance was found to be increased in affected samples relative to healthy canine samples and *S. pseudintermedius* was the predominant species in both sample types.[165] Collectively, these results suggest that unlike human skin, there does not seem to be a difference in the relative abundance of specific *Staphylococcus* species between healthy and allergic skin in dogs.

In feline patients, our knowledge of the cutaneous staphylococcal communities is very limited. Research on the cutaneous feline staphylococcal communities has been performed, however most reports have focused solely on isolates from lesions. Those that have included samples from healthy cats have found higher abundances of coagulase-negative as opposed to coagulase-positive staphylococcal species.[67,74,166] Studies that have determined the specific coagulase-negative species present have described a predominance of *S. felis* (previously thought to be *S. simulans*[66]).[73,74] Within the coagulase-positive species identified on healthy skin, *S. pseudintermedius* (previously classified as *S. intermedius*[167]) seems to be most abundant, followed closely behind by *S. aureus*.[73,166]

On lesional feline skin, two studies have described higher abundances of coagulase-positive isolates, relative to the numbers found on healthy skin[67] and relative to the number of coagulase-negative isolates.[70] Many species, including *S. felis, S. aureus,* and *S. pseudintermedius*[72] have been isolated from lesions. Given the fact that

these species are also found on healthy skin, it is unclear whether these organisms are indeed pathogens or simply commensals.

These previously reported studies have relied on culture-based methods, which might not offer growth conditions that are equally favorable to all species and therefore may not provide a broad image of the relative abundances of these staphylococcal species. Molecular-based methods are able to get around the issue of cultivability and can allow for species-level identification. Therefore, these methods can complement data from existing culture-based studies. While increased *Staphylococcus* spp. on non-lesional allergic feline skin has been described,[25] the importance of specific staphylococcal species in feline allergic skin disease has not been evaluated. In this study, we sought to confirm previous findings of increased *Staphylococcus* spp. on allergic feline skin and delve deeper into the staphylococcal communities with molecular techniques that may give a more comprehensive view of these communities.

Methods

Sample collection

All cats were evaluated by a board-certified dermatologist and only sampled if fitting within the previously described inclusion criteria.[25] Swabs of the ear canal and groin were obtained from 11 healthy and 10 allergic cats (Table S7). Swab samples were taken by rubbing two Isohelix buccal swabs (Cell Projects Ltd., Kent, UK) 10 times per side of each swab. Hair was parted with gloved hands to ensure that swabs were applied to the skin and not the fur. The two swabs were then stored in a single MO BIO

PowerBead tube (MOBIO Laboratories, Carlsbad, CA) and stored at 4°C (for no longer than 7 days) until DNA extraction with the MO BIO PowerSoil DNA Isolation Kit. Along with skin swab samples, DNA was extracted from an empty PowerBead tube and a PowerBead tube containing a sterile swab as controls.

Next-generation sequencing and sequence processing

DNA was sent to the University of Minnesota Genomics Center for next-generation sequencing of the V1-3 region of the 16s rRNA gene (V1_27F: AGAGTTTGATCMTGGCTCAG, V3_534R: ATTACCGCGGCTGCTGG) on an Illumina MiSeq (Illumina, San Diego, CA). Data were analyzed in mothur v.1.39.5,[148] where chimeras were removed with uchime,[168] sequences were aligned to the greengenes(13_8_99) database,[169] and classified with the RDP classifier (version 16).[170] Staphylococcal sequences were extracted and aligned to a reference alignment available in a previously published package trimmed to the sequencing region.[171] Staphylococcal sequences having less than 99% similarity with sequences in the staphylococcal alignment were considered unclassified to the species level.

For diversity analyses, data were rarefied to 15,066 sequences for pan-bacterial data and 497 sequences for staphylococcal data. Alpha diversity was analyzed using the observed OTUs, Chao1, Shannon diversity index, and Faith's phylogenetic diversity metrics. Beta diversity was analyzed using the Bray-Curtis, Jaccard, weighted UniFrac and unweighted UniFrac methods.

Statistical analysis

Based on the non-normal distribution of data, as determined with the Shapiro-Wilks test in JMP Pro 14 (SAS Institute Inc., Cary, NC), alpha diversity and differential taxa relative abundance were analyzed using nonparametric Wilcoxon tests in JMP Pro 14. Differential relative abundance of taxa was also analyzed using the Linear Discriminant Analyze Effect Size (LEfSe) algorithm on the Huttenhower lab Galaxy (http://huttenhower.sph.harvard.edu/galaxy/). Distance matrices for beta diversity were analyzed with the analysis of similarities (ANOSIM) test in R with the vegan package.

Results

A total of 1,402,395 sequences (median=33,477) from 42 samples remained after quality filtering. Sequence files are available in the NCBI sequence read archive under BioProject ID PRJNA580000. In terms of composition, this study revealed similar results to what has previously been described in cats,[25,172] with primarily Proteobacteria (average relative abundance=52.29%), Firmicutes (17.94%), Actinobacteria (13.99%) and Bacteroidetes (11.87%) as the predominant phlya. Analysis of the entire bacterial microbiota did not reveal differences in diversity between healthy and allergic cats, nor when analyzing the effect of individual or body site within health groups. However, numerous taxa were found to be differentially abundant between healthy and allergic cats (Figure 6). Among the many differentially abundant taxa was the opportunistic pathogen *Streptococcus* spp., which was present in higher relative abundances in samples from allergic cats (LDA=3.8208, $p<0.05$)

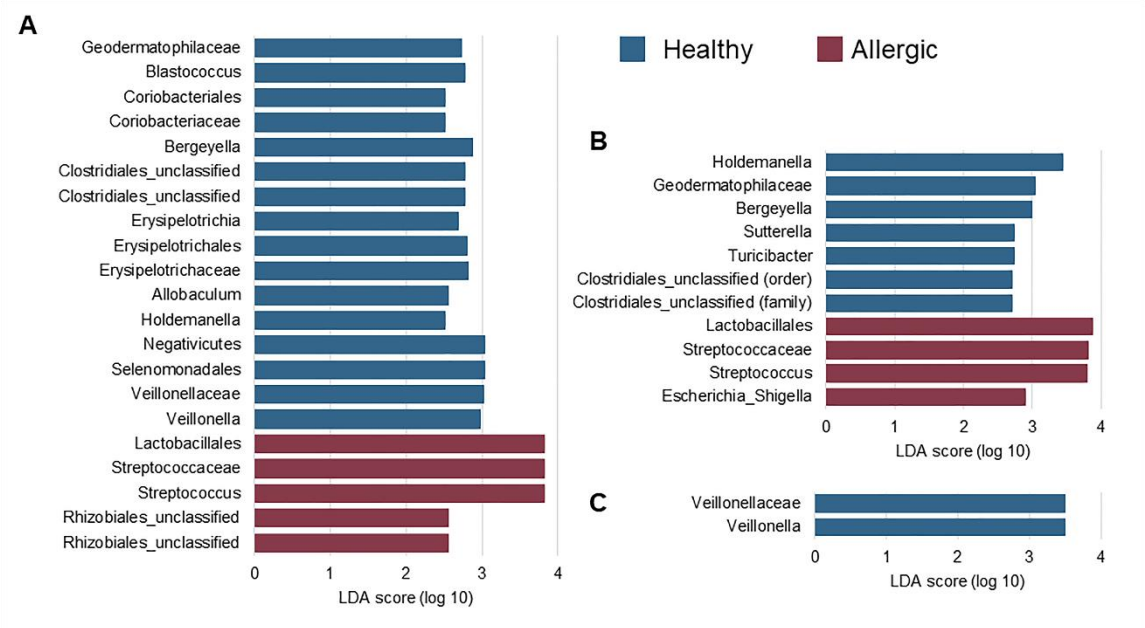

Figure 6: Linear discriminant analysis effect size (LEfSe) analysis of bacterial relative abundance between healthy and allergic cat samples.
Various taxa were found to be differentially abundant when analyzing (a) both body sites, or the (b) ear canal and (c) groin samples only.

Overall, relative abundance of *Staphylococcus* spp. was low, with average relative abundances of 4.34% in healthy and 3.61% in allergic samples (Figure 7). Of the 42 samples analyzed, four healthy and three allergic samples had no *Staphylococcus* spp. sequences (Figure 8). In healthy samples (23,440 staphylococcal sequences), 40.3% and 26.9% of the *Staphylococcus* sequences were classified to *S. epidermidis* and *S. pseudintermedius*, respectively (Figure 9). When looking at the staphylococcal communities in terms of average relative abundance, *S. epidermidis* was the most abundant (average relative abundance=29.4%), followed by *S. capitis* (15.3%) and *S. felis* (6.61%).

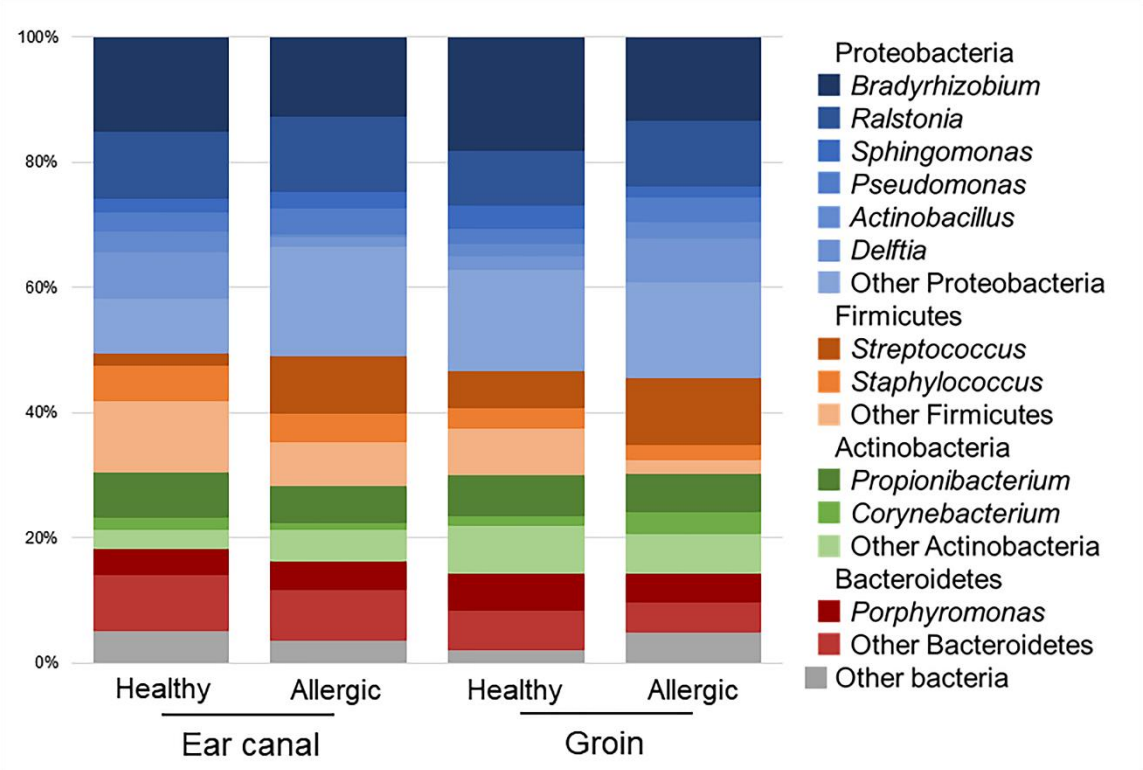

Figure 7: Average relative abundance of taxa.
Within these samples, Proteobacteria was the phyla with the highest average relative abundance followed by Firmicutes, Actinobacteria and Bacteroidetes, with some differences between the two body sites samples.

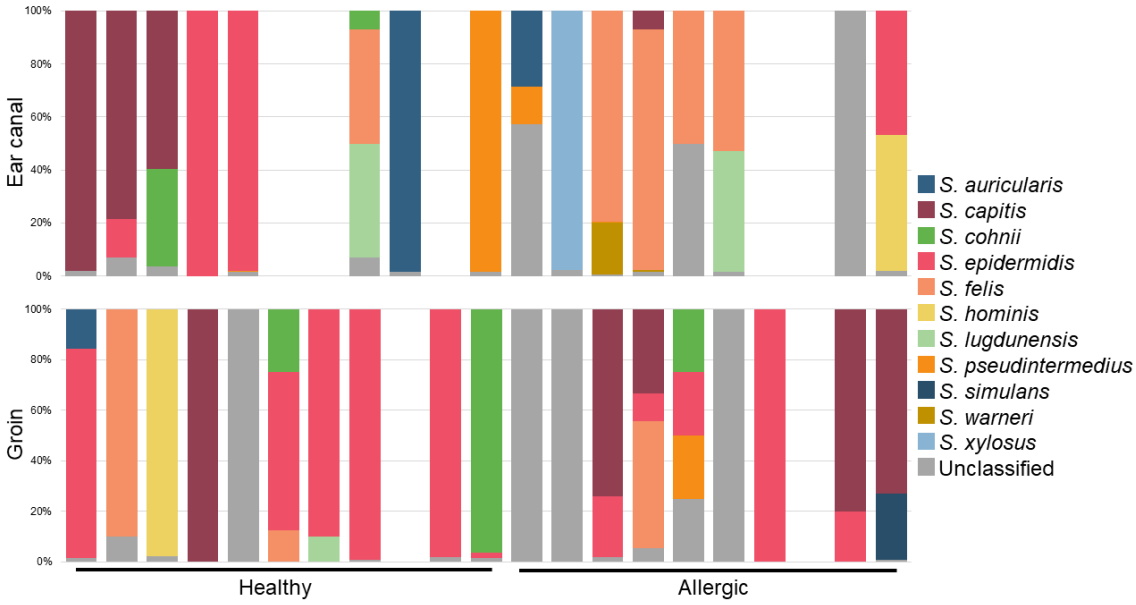

Figure 8: Relative abundance of staphylococcal species in each sample. Staphylococcal community composition was variable across individual cats and body site.

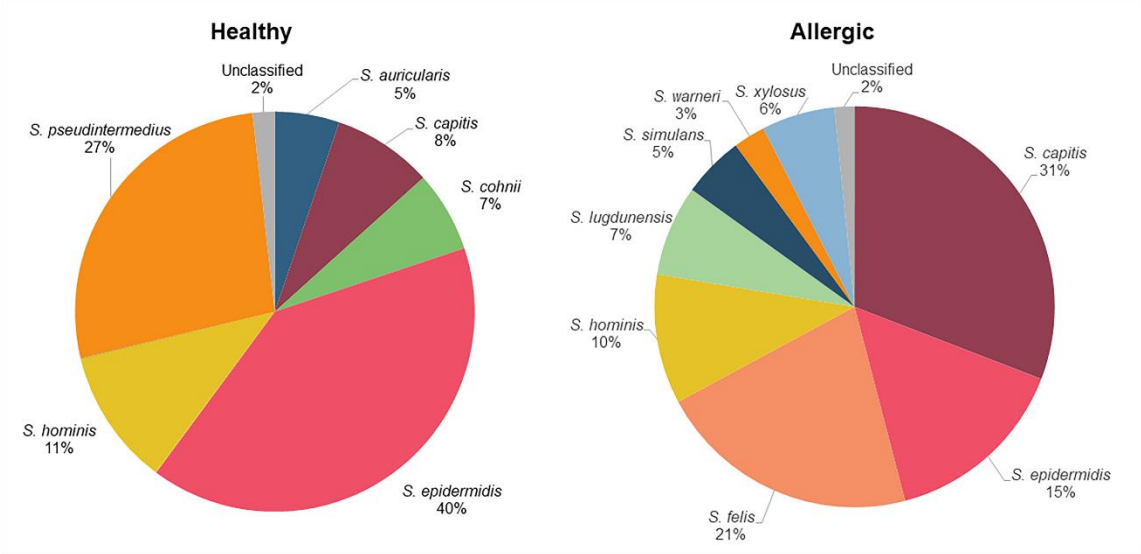

Figure 9: Average relative abundance of staphylococcal species in healthy and allergic samples.
Regardless of health status, staphylococcal communities on feline skin are diverse, containing multiple species of substantial relative abundance. Healthy cats are predominately colonized by *S. epidermidis* and *S. pseudintermedius,* while on allergic cats, *S. capitis* and *S. felis* are the most prevalent staphylococcal species present.

In allergic samples (19,139 staphylococcal sequences), *S. capitis* accounted for 30.8% and *S. felis* accounted for 21.2% of sequences. In terms of average relative abundance, *S. felis* (16.2%) and *S. capitis (*13.4%) were the most abundant. No species was found to be significantly differentially abundant between healthy and allergic cat samples (Figure S1: all samples).

Discussion

The presented study is the first to describe the species-level staphylococcal communities on feline skin. Previous studies have indicated *S. aureus, S. intermedius*

(likely *S. pseudintermedius*, which was first described after these studies[54]), *S. felis*, and *S. simulans* (likely *S. felis*, which was first described after these studies[66]) as some of the most prevalent staphylococci present on healthy feline skin.[73,167,173] In the results presented from healthy skin samples, *S. epidermidis* accounted for the highest number of sequences and had the highest average relative abundance. Due to the high variability in the abundance of *Staphylococcus* species among cats, the definition of a core staphylococcal community on feline skin is still not clear. However, when looking at individual healthy cat samples, *S. epidermidis* was the most abundant species in 7/22 samples and *S. capitis* was most abundant in 4/22 samples. All other staphylococcal species were only most abundant in at most a single sample. Various species have been identified as pathogens in cats, including *S. aureus, S. felis*,[174] and *S. intermedius* (more likely *S. pseudintermedius*).[72] *S. capitis* and *S. felis* accounted for the majority of staphylococcal sequences and were the species with the highest average relative abundance from allergic samples in the cats evaluated. These results suggest that in addition to *S. felis*, which is typically associated with feline skin, *S. epidermidis* and *S. capitis* may also be important species that have previously not received much attention with respect to feline cutaneous staphylococcal communities.

Given the importance of *Staphylococcus* spp. in human and canine allergic dermatitis, we hypothesized *Staphylococcus* spp. may also have roles in feline allergic dermatitis. This genus of bacteria has previously been demonstrated to be increased in abundance on both allergic human and canine[22] skin, compared to healthy counterparts. Their relative abundance has also been found to be significantly increased on the skin of

allergic cats,[25] particularly in the ear canal and interdigital space. Additionally, human staphylococcal communities are quite dynamic in terms of the specific species present. *S. epidermidis* predominates on healthy skin, but *S. aureus* bypasses *S. epidermidis'* protective roles and becomes highly abundant on allergic skin. This often leads or contributes to secondary infections during allergic flares.

Although not statistically significant, our results indicate there may be differences in the species-level composition of the staphylococcal communities inhabiting healthy and allergic feline skin, including increased abundances of *S. epidermidis* in healthy samples. As previously mentioned, this species has beneficial roles on human skin; the organisms can produce antimicrobials targeted to more pathogenic bacterial species and secrete compounds that can enhance the host immune system.[28,29] Similar activities of *S. epidermidis* and other staphylococcal species have not yet been demonstrated on canine and feline skin, but may provide an avenue for therapeutic intervention.

Secondary infections are also known to occur in allergic feline patients, however the true occurrence is unknown and likely underestimated. Although *Staphylococcus* seems to be present on cats in similar relative abundances as canine skin, based on the current and previous study,[25] perhaps the inability of many staphylococcal species to adhere strongly to feline corneocytes[76,175] is responsible for the seemingly lower occurrence of bacterial skin infection reported in feline patients. However, this potential lack of secondary infections does not negate the importance of this bacteria in feline allergic dermatitis. Perhaps in cats, staphylococci are not as important in terms of

causing infection when compared to dogs, but may instead be primarily influential through modulating inflammation.

Interestingly, some of the staphylococcal species observed within these feline samples, such as *S. hominis*, and *S. capitis*, are often found on human skin.[56] It is uncertain whether these particular staphylococcal species are stable residents of feline skin, or whether they are transient organisms transferred via contact with humans. While there have been a few studies evaluating the microbiome of humans and their pets,[36,40,158] the direction and impact of microbiome sharing between species that cohabitate remains unclear.

Studies describing the staphylococcal communities on lesional skin should be performed to better understand which species may be more relevant during flares of allergic skin disease. Within our own study, some staphylococcal species were found to have higher relative abundance in allergic skin, but these did not reach significance, perhaps due to the small sample size. While next-generation sequencing does allow us to see a broader picture of the entire staphylococcal communities present, like culture-based techniques, it can be biased towards specific species and, given the short length of amplicons, also has the potential for species-level misclassification. Therefore, the use of other techniques to complement existing studies is desired. When samples from another cohort of healthy and allergic cats were subjected to qPCR targeting *Staphylococcus* spp., the bacteria was only detected in a small number of samples with low abundances (unpublished data). Perhaps other methods would be more beneficial for further describing the staphylococcal communities inhabiting healthy and allergic feline skin.

Conclusion

While its relative abundance on feline skin is low, *Staphylococcus* spp. may have an important role in skin homeostasis and during flares in feline allergic skin disease. Although statistically significant differences were not demonstrated in the present study, there are appreciable differences in the species-level composition of these communities between healthy and allergic cats, and between individual cats. Unlike their canine counterparts, and more similar to the dynamic communities on human skin, there was not a single predominating species in all samples, regardless of health status. Further research of the staphylococcal communities inhabiting lesional feline skin may identify species that are associated with disease and may represent new targets for therapy.

CHAPTER IV

CUTANEOUS AND SYSTEMIC CYTOKINE EXPRESSION IN FELINE ALLERGIC DERMATITIS

Introduction

Feline allergies have long been a difficult health issue to handle in the veterinary community. The difficulty in diagnosis and treatment stems from a lack of understanding the pathogenesis of allergic diseases affecting cats. Oftentimes, our understanding of feline diseases is speculated based on what we know from similar diseases that occur in humans or dogs. However, not surprisingly, cats do not always suffer from the same diseases as their human and canine counterparts. Allergic dermatitis affecting cats is unique, with regards to both clinical signs and pathomechanisms.

Atopic dermatitis is well-documented in humans and dogs, with similarities between the two species in terms of various clinical parameters including lesion types, IgE involvement and immune response. In cats, "atopic dermatitis" is not a well-accepted term for the cutaneous hypersensitivity reaction to environmental allergens due to the distinct clinical patterns, potential lack of IgE involvement, and differing immunological profile compared to what is seen in people and dogs. The disease affecting cats is more appropriately referred to as "atopic-like dermatitis" or "non-flea, non-food hypersensitivity dermatitis" (NFNFHD).[75,176] Part of the reason that feline environmental allergies is not identified as akin to atopic dermatitis in dogs and people, is the lack of sufficient research in the realm of feline allergic skin disease.

In allergic disease, the immune system is thought to be skewed towards Th2 activity, characterized by increased production of cytokines such as IL-4, IL-5, and IL-13 which result in high numbers of infiltrating eosinophils and mast cells, class switching of antibody production to IgE, and propagation of this inflammatory response.[177] Although a similar cellular infiltrate has been demosntrated in allergic feline skin in several studies,[89,90,92,93] an increase in IgE production is not well supported[11,12,178] and the only study to evaluate expression of these cytokines did not find any differences in expression of Th2-associated cytokines between healthy and allergic cats.[13]

Given the lack of understanding of FAD, treatment options are limited. While people and dogs can now benefit from host-specific biologics, no feline-specific biologics are available. Feline patients affected by allergic dermatitis are primarily limited to glucocorticoids, antihistamines and/or cyclosporine.[176] Unfortunately, these medications are not always effective and can lead to adverse effects when administered long-term.[179] Clearly, cats would greatly benefit from alternative therapies.

For human and canine allergic dermatitis patients, some of the biologics available are JAK inhibitors, for example oclacitinib (Apoquel), which is used in dogs to block the JAK/STAT signaling pathway associated with multiple pro-inflammatory cytokines;[107] others are monoclonals, such as dupilumab which can specifically block IL-4/IL-13 signaling in humans.[4] Based on previous studies,[95,99,107,179-181] IL-31 has become a particularly popular target for treatment. In canine medicine, lokivetmab (Cytopoint), a monoclonal antibody against canine IL-31, has been successful in reducing pruritus associated with canine atopic dermatitis.[101,182] Nemolizumab, a

humanized antibody against IL-31RA, was recently shown to decrease pruritus in adults affected by moderate-to-severe atopic dermatitis.[5] Besides IL-31, the blocking of several other molecules with monoclonals in humans is being evaluated in clinical trials, including IL-4, IL-5, IL-13, IgE, IL-31 and IL-33.[8]

A few studies have already begun to describe a role for IL-31 in feline allergic skin disease. Injection of recombinant feline IL-31 in healthy cats resulted in pruritus, and when oclacitinib was given 1 hour prior to injection, pruritus was noticeably reduced.[108] In a single study, cats with a presumptive diagnosis of allergic dermatitis demonstrated higher circulating levels of IL-31 in serum when compared to healthy controls.[94] Additionally, feline IL-31 has exhibited similar activity to canine IL-31 in cell based assays, where it was shown to utilize the JAK/STAT pathway for signaling.[183]

Although research has demonstrated a potential role for IL-31 in feline allergic dermatitis in a small number of studies, no further work has been published and no other promising targets have been proposed for cats. In an effort to further understand the pathomechanisms associated with feline allergic dermatitis, we sought to evaluate the levels of IL-31 and other Th2 cytokines in the skin and serum of cats affected by allergic dermatitis when compared to healthy cats.

Materials and methods

This study was approved by the Texas A&M University Institutional Animal Care and Use Committee.

Formalin-fixed, paraffin-embedded tissue sample collection

Formalin-fixed, paraffin-embedded tissue (FFPE) samples from 22 cats with allergic skin disease were retrieved from the Dermatopathology Service archive in the College of Veterinary Medicine and Biomedical Sciences at Texas A&M University. All cats were over 1 year of age at the time of sample collection. Biopsies showed histopathological changes compatible with solely allergic skin, as determined by examination of H&E stained slides by a board-certified anatomic pathologist. Formalin-fixed, paraffin-embedded tissue samples from 21 cats without allergic skin disease were obtained from the Texas A&M University Dermatopathology Service and surgical pathology archives. These were also determined appropriate upon examination of H&E stained slides by a board-certified anatomic pathologist and included marginal normal skin from non-inflammatory neoplastic tumors.

RNA extraction and qPCR

From each case, RNA was extracted from 50 uM of FFPE tissue. RNA extraction was performed with the MO BIO FFPE Tissue RNA Isolation Kit (formerly MO BIO, now Qiagen, Hilden, Germany) according to the manufacturer's protocol. Following extraction, RNA quantification was performed using the Qubit High Sensitivity RNA kit (Life Technologies Corporation, Carlsbad, CA). cDNA was synthesized using the iScript Reverse Transcription Supermix for RT-qPCR (Bio-Rad Laboratories, Hercules, CA), following the manufacturer's protocol and using enough RNA to get as close to 1ng of RNA as possible. Quantitative PCRs were performed using the iTaq Universal SYBR

Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA) or iTaq Universal Probes Supermix (Bio-Rad Laboratories, Inc.). PCRs targeting IL-31 and IL-31RA, in addition to ribosomal protein S7 (RPS7) and TATA-box-binding protein 1 (TBP1) were performed in duplicate using the oligos shown in table S8. qPCR parameters included: 95°C for 30 seconds; 40 cycles of 95°C for 5 seconds, 57°C (RPS7, IL-31, and IL-31RA) or 60°C (TBP1) for 30 seconds, and a plate read; and a melt curve for the RPS7 and TBP1 assays. Resulting data were acquired in and exported from CFX Manager (Bio-Rad Laboratories, Inc.). Mean Cqs were obtained for each sample from all 4 assays. The data from the qPCR assays targeting IL-31 and IL-31RA were normalized to the expression of both of the normalizing genes, RPS7 and TBP1.

Immunohistochemistry

From the FFPE tissue blocks, 4 uM sections were cut and adhered to charged slides. After deparaffinization, antigen retrieval was performed using a pressure cooker and sodium citrate buffer (pH 7.6). All steps were performed at room temperature with Tris-buffered saline as the wash buffer. Blocking was performed first with 0.3% H2O2 for 5 minutes followed by incubation with Dako serum-free protein block (Agilent Technologies, Santa Clara, CA) for 5 minutes. Tissues were incubated with a 1:1000 dilution of a monoclonal antibody targeting feline IL-31 provided by Zoetis Inc.[184] for 30 minutes. Next, the slides were incubated with Novolink post-primary (Leica Biosystems) for 10 minutes, followed by Novolink polymer for 10 minutes. Staining was detected with the ImmPACT NovaRed Peroxidase (HRP) Substrate (Vector Laboratories

Inc., Burlingame, Ca), and Modified Mayer's hematoxylin (Richard-Allan Scientific Co., San Diego, CA) as a counterstain. Intensity of epidermal staining was scored where 0=no staining, 1=weak staining, 2=moderate staining, and 3=strong staining. Epidermal hyperplasia was also scored with 0=no hyperplasia, 1= mild, 2=moderate, and 3=severe.

RNAScope

Slides were first baked at 60°C for 15 minutes then subjected to RNAScope using probes (Advanced Cell Diagnostics, Inc., Newark, CA) targeting IL-31, IL-31RA, OSMR, IL-33, IL-5, and PPIB on a Leica BOND RX Fully Automated Research Stainer (Leica Biosystems, Wetzlar, Germany). Staining for each target was graded in the epidermis using the ACD scoring system (Advanced Cell Diagnostics, Inc.).

Serum sample collection

Serum was collected from 17 healthy cats and 18 cats with allergic skin disease from Texas A&M and collaborating clinics within Houston and Austin, Texas. Healthy cats included those over 1 year of age, with no history of chronic disease and no medication, besides flea, tick or heartworm preventative within the last 2 months. For affected cats, only those over 1 year of age, with 14 days since last administration of oral steroids or cyclosporine, 1 month since injectable steroids, 2 months since Depo-Medrol and 7 days since topical steroid use were included. Cats fitting into the allergic skin disease group included those with a history of pruritus/over-grooming and/or that showed typical cutaneous reactions patterns associated with feline cutaneous

hypersensitivity disease (eosinophilic skin lesions including plaques, granulomas, and indolent ulcers, miliary dermatitis, self-induced alopecia, and/or head and neck pruritus), where all infectious and parasitic causes of pruritus were ruled out.[25]

Serum testing

Serum samples were subjected to measurement of IL-31 with a Simoa HD-1 Analyzer (Quanterix Corporation, Billerica, MA) and used for the MILLIPLEX MAP feline cytokine/chemokine magnetic bead panel (MilliporeSigma, Burlington, MA) on a Luminex FLEXMAP 3D (Luminex Corporation, Austin, TX). The IL-31 ELISA was done with 80 uL of neat serum with each sample in duplicate. The MILLIPLEX MAP kit was used according to the manufacturer's protocol, with 10 uL of neat serum used from each sample in duplicate.

Statistical analysis

Data were tested for normality using the Shapiro-Wilkes test in JMP Pro 14 (SAS Institute, Cary, NC), followed by Kruskal-Wallis or Wilcoxon tests where appropriate. For ELISA data, samples with quantities below the limit of detection (LoD) were set to LoD/2 before being subjected to Kruskal-Wallis or Wilcoxon tests.

Results

RNAScope

Expression of mRNA for PPIB (positive control) and OSMR-β (Supplemental figure S9) could easily be visualized at 40X magnification, however IL-31RA (Supplemental figure S10) and IL-33 mRNA expression could only occasionally be observed at 200X magnification. IL-5 and IL-31(Supplemental figure S11) staining was rare, often not visible even at 400X magnification. Cytoplasmic and nuclear staining for all targets was primarily in the epidermis and follicular epithelial cells in the hair bulb and outer root sheath, and occasionally observed in sebaceous and apocrine glands, muscle, and infiltrating inflammatory cells. Significant differences in staining for IL-5 ($p=0.0285$) and IL-33 ($p=0.042$) were found, with samples from allergic cats having higher scores for both targets. However, average scores for both groups were low (<1) and the differences between them were low in magnitude. OSMR-β staining was also significantly higher in allergic animals ($p<0.0001$). Unlike IL-5 and IL-33, this target had substantial difference in scores between allergic and nonaffected animals (Table 4).

Table 4: RNAscope scores expressed as mean + standard deviation.
P-values are from Wilcoxon tests.

Target	Control (n=20)	Allergic (n=21)	P-value
IL-31	0.075 ± 0.183	0.227 ± 0.329	0.1599
IL-31RA	1.025 ± 0.499	0.929 ± 0.350	0.2689
IL-33	0.632 ± 0.436	0.909 ± 0.464	0.0420
IL-5	0	0.114 ± 0.179	0.0285
OSMR	0.944 ± 0.539	2.545 ± 0.890	<0.0001

qPCR

The normalizing genes RPS7 and TBP1 were quantified in the skin of both allergic and control cats with an average Cq (cycle quantification value) of 29.36 for RPS7 and 33.99 for TBP1 assays. IL-31 and IL-31RA could only be detected in a small number of samples (n=16 for IL-31 and n=8 for IL-31RA), and only in very low quantities, with average Cqs of 38.05 for IL-31 and 38.64 for IL-31RA. Cats with allergic dermatitis seemed to have higher IL-31 and IL-31RA mRNA expression, particularly when data were normalized to TBP1 expression (Figure 10). Although expression of IL-31 was not significantly different between allergic and control samples (relative to RPS7 P=0.2328, andTBP1=0.5698), expression of IL-31RA was significantly higher in samples from allergic cats (relative to RPS7 P=0.0455, and TBP1 P=0.0455).

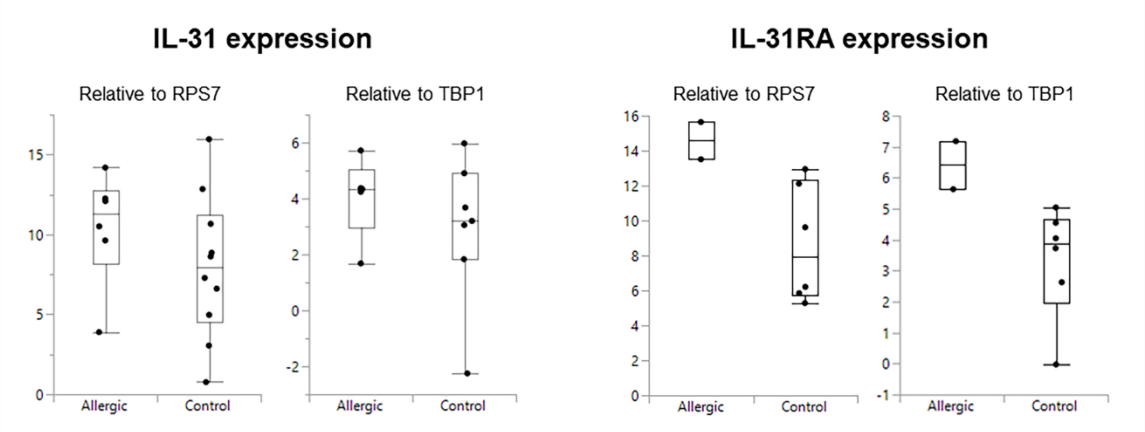

Figure 10: Results of IL-31 and IL-31RA qPCR assays.
Results shown are from samples where at least one replicate was positive. Expression is shown relative to expression of normalizing genes RPS7 and TBP1.

IHC

Immunohistochemistry demonstrated positive staining for IL-31 protein expression in all samples. Staining could be observed in the cytoplasm of keratinocytes, follicular epithelial cells in the outer root sheath and hair bulb, muscle, sebaceous glands and occasionally in infiltrating inflammatory cells (Figure 11). The strongest staining could be observed in the epidermis and around hair follicles. Comparing staining scores between controls (average=2.05 $\pm$ 0.776) and allergic (2.114 $\pm$ 0.7389) samples did not reveal statistically significant differences (p=0.6588). Although staining intensity was not different, allergic samples clearly had more cells capable of producing IL-31 due to epidermal hyperplasia and abundant inflammatory cells infiltrating the dermis (Figure 12).

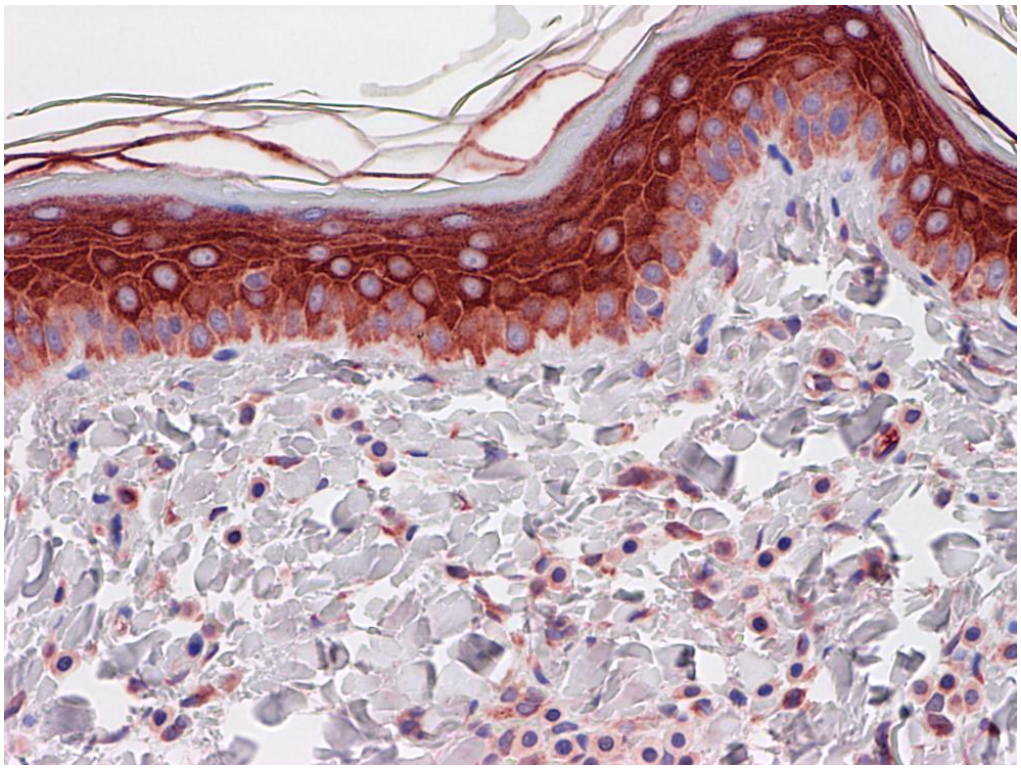

Figure 11: IL-31 staining in feline skin.
Besides prominent staining in keratinocytes, weaker staining also could be observed in infiltrating cells (most likely mast cells).

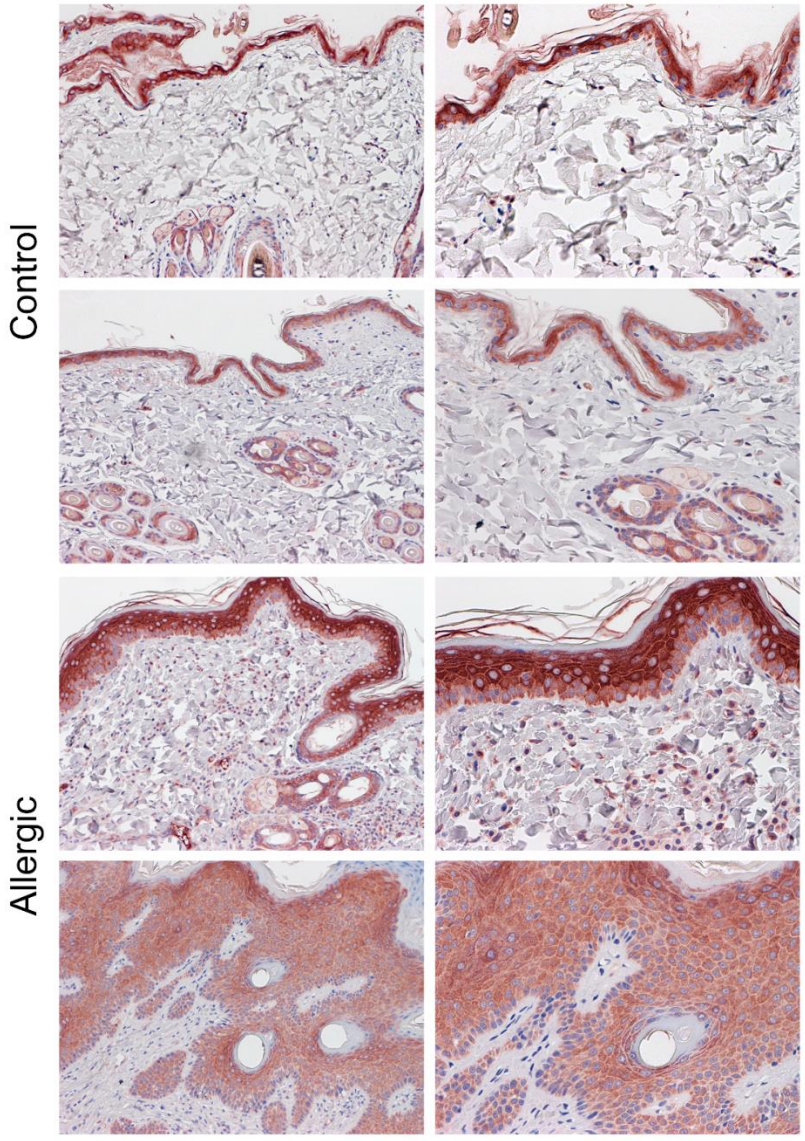

Figure 12: Immunostaining of IL-31 in representative healthy and allergic feline skin samples at 100X and 200X.
Staining intensity did not differ between samples from allergic and nonaffected animals. Epidermal hyperplasia and inflammatory infiltrates, which exhibited weak staining, were evident in many samples from allergic animals.

ELISA

Within the multiplex assay quantifying 19 different cytokines/chemokines, 5 cytokines (IL-12, IL-8, SDF1, RANTES, SCF) were successfully measured in all samples (Table 5). IL-31 was found at detectable levels in only 15/35 samples and was the cytokine with the lowest detected quantities. TNF-α was another cytokine that was not detectable in a substantial number of samples; it was detected in roughly half of both healthy and allergic samples. No cytokines were found to have significantly different levels between the groups, but there were notable differences in the number of samples where IL-31 could be measured. While 10/18 allergic dermatitis had detectable quantities, only 4/17 healthy samples could be quantified. Additionally, MCP-1 levels were below the limit of quantification for 4/18 allergic dermatitis but quantifiable in all healthy samples.

Table 5: Cytokine measurements from feline serum samples.
Data are presented as mean levels (pg/ml) + SD, and number of samples with quantified levels greater than the limit of detection, presented as number of samples, (percentage of samples).

Cytokine	Mean levels (pg/ml) + SD		Samples with levels > LOD	
	Healthy	Allergic	Healthy (n=17)	Allergic dermatitis (n=18)
sFAS	129.547 ± 161.703	138.369 ± 240.938	16 (94%)	16 (88%)
Flt-3 ligand	150.015 ± 76.918	168.969 ± 90.268	16 (94%)	18 (100%)
GM-CSF	31.190 ± 40.199	28.839 ± 50.320	10 (59%)	11 (61%)
IFN-γ	575.703 ± 723.762	416.617 ± 872.032	16 (94%)	17 (94%)
IL-1β	136.473 ± 143.471	85.728 ± 140.256	15 (88%)	12 (67%)
IL-2	85.548 ± 95.229	65.844 ± 128.755	15 (88%)	13 (72%)
IL-4	2727.485 ± 3288.596	2005.093 ± 3961.016	16 (94%)	17 (94%)

Table 5 Continued

Cytokine	Mean levels (pg/ml) + SD		Samples with levels > LOD	
	Healthy	Allergic	Healthy (n=17)	Allergic dermatitis (n=18)
IL-6	1052.306 ± 1232.638	708.602 ± 1300.651	16 (94%)	17 (94%)
IL-8	96.005 ± 78.500	71.748 ± 88.688	17 (100%)	18 (100%)
IL-12 p40	433.304 ± 296.188	581.991 ± 627.330	17 (100%)	18 (100%)
IL-13	66.478 ± 58.374	60.919 ± 68.404	16 (94%)	18 (100%)
IL-18	1609.112 ± 1642.229	1127.636 ± 1819.762	16 (94%)	16 (88%)
IL-31	0.731 ± 1.945	1.501 ± 3.136	5 (20%)	10 (56%)
KC	16.614 ± 26.058	36.905 ± 110.352	14 (82%)	15 (83%)
MCP-1	6890.386 ± 5640.698	5109.124 ± 5782.252	17 (100%)	14 (78%)
PDGF-BB	1560.812 ± 2558.865	843.805 ± 1368.123	16 (94%)	14 (78%)
RANTES	94.904 ± 71.493	104.054 ± 74.474	17 (100%)	18 (100%)
SCF	338.531 ± 258.434	277.151 ± 295.540	17 (100%)	18 (100%)
SDF-1	3459.988 ± 1685.020	2797.614 ± 1516.317	17 (100%)	18 (100%)
TNF-α	488.482 ± 644.455	307.263 ± 687.215	9 (53%)	9 (50%)

Discussion

Our results did not demonstrate differences in systemic or cutaneous IL-31 expression between cats affected by allergic dermatitis and controls. IL-31 mRNA and protein levels were similar in control (average=2.05 ± 0.776) and allergic (2.114 ± 0.7389) samples, which contradicted our original hypothesis that cats with allergic dermatitis would have higher levels of IL-31 either in terms of production per cell. Although it seems as though IL-31 production may be consistent regardless of health status, thus leading to a larger number of IL-31-positive cells. Like mRNA expression,

systemic levels were low and could not be detected in a majority of samples. Our results did not corroborate those of a previous study, which identified increased expression in allergic cats relative to controls,[185] however we were able to quantify IL-31 in a higher number of samples from allergic cats than healthy cats. Our results do not reject the hypothesis that IL-31 contributes to pruritus in feline allergic disease; however, it does not provide strong evidence this may be a useful biomarker for disease or therapeutic target in the feline species.

Within the present study, IL-31 staining was predominately observed in keratinocytes within the epidermis, follicular epithelial cells in the outer root sheath and hair bulb, arrector pili muscle, and less so in sebaceous glands. While some cells within the inflammatory infiltrate demonstrated staining, it was often weaker than the staining observed within the epidermis and less frequent. A majority of these cells appeared to be mast cells, which is fitting with the important role of mast cells in allergic disease. Detecting mRNA expression for IL-31 was difficult, with only a small fraction of samples have detectable levels by qPCR and very rare expression observed with RNAScope. These results may suggest IL-31 mRNA expression is low or tightly controlled via quick degradation after transcription.

Even if IL-31 expression is not increased in allergic animals, it's possible that upregulation in the expression of molecules that interact with IL-31 may occur and be important in the disease process. For this reason, we were interested in evaluating the expression of the two subunits of the heterodimeric IL-31 receptor, IL-31RA and OSMR-β. Within our results, RNAScope and qPCR were not strongly suggestive of

upregulation of IL-31RA mRNA expression in the skin. Although we could demonstrate a significantly higher IL-31RA mRNA expression in allergic samples, this was based on a comparison of 2 allergic and 6 control samples, all of which had low expression. RNAScope results showed significantly higher mRNA expression of OSMR-β in samples obtained from allergic animals.

OSMR-β is one of the subunits of heterodimeric receptor complex for IL-31. Upon binding of IL-31, the IL-31 receptor complex induces signaling via the JAK/STAT, PI3K/AKT, and several other pathways (Figure 13, Modified from figure 3 in Cornelissen et al.[97]). These pathways have implications in cytokine secretion, epidermal barrier function, cell migration and differentiation, and most notable, pruritus. Like IL-31, the IL-31 receptor complex is present on a wide range of cells, including keratinocytes, mast cells, eosinophil, and peripheral neurons. OSMR-β in particular is expressed in the skin, as demonstrated within the present study, but also has been found in other tissues such as the lungs and dorsal root ganglia.[96] Importantly, IL-31 is not the only ligand that can bind to OSMR-β. Research into the expression of its other ligands may reveal that they are potentially useful targets for therapeutic development. For example, OSM is involved in skin inflammation[186] and, when injected into mice, induced expression of several genes including those coding for Th2 cytokines IL-4, IL-5 and IL-13.[187]

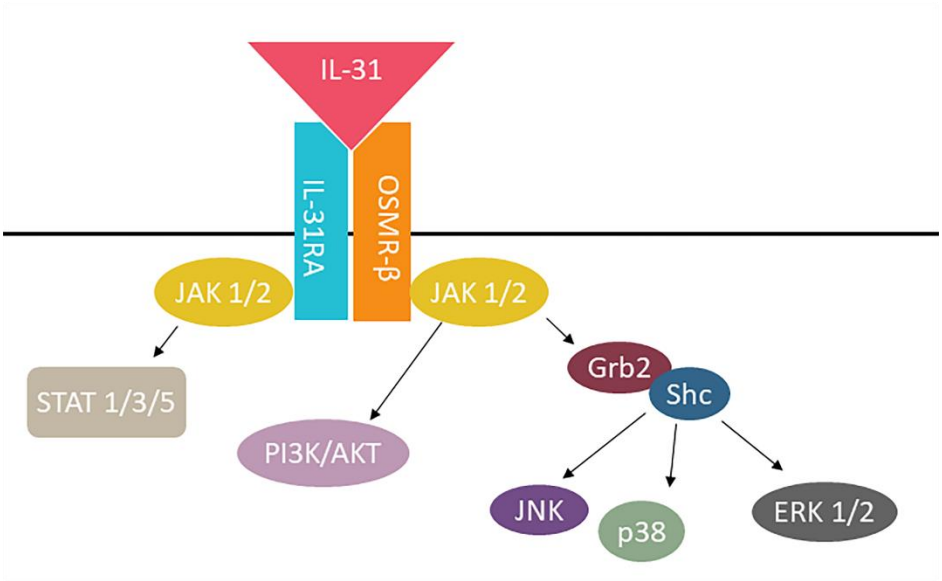

Figure 13: IL-31 signaling pathways.
Upon binding to the heterodimeric receptor composed of IL-31RA and OSMR-β, IL-31 results in activation of several pathways, including the JAK/STAT and PI3K/AKT pathways. Modified from figure 3 in Cornelissen et al., 2011.

Allergic dermatitis affecting cats has been known to be different from the disease affecting other species, particularly in terms of clinical patterns. Within this study, we provide evidence that supports the disease is distinct with respect to the immune system, suggesting potentially unknown pathomechanisms are involved in feline allergic dermatitis. While cats with allergic dermatitis do show an inflammatory infiltrate that is typical of allergic disease, our results do not suggest these cells are actively over-producing the characteristic Th2 cytokines observed in other species.

A strong Th2/Th22 response typically occurs in the acute phase of allergic dermatitis, which is then switched to a predominately Th1 response in the chronic phase.[9] In this study, FFPE samples were obtained from cats that did have current flares of disease, however these cases may have been in the chronic Th1 phase of the disease,

which was not evaluated in skin samples. Not all cats from which serum was collected from exhibited current flares of disease but did have a history of allergic disease, and therefore perhaps these could have had either a Th1 or Th2-skewed response, however neither was observed. A previous study in which qPCRs targeting 10 cytokines spanning both Th1 and Th2 response were performed in samples obtained from normal, non-lesional allergic and lesional allergic feline skin did not find evidence of a Th2-skewed cytokine profile. The results of the presented study support this previous study, which also did not identify significant differences in any of their targets.[13]

In the future, utilizing samples obtained from an experimental cohort would be ideal sample to sample differences, however the purpose of our study was to evaluate these cytokine profile in cats with naturally occurring disease, rather than experimentally induced. Besides a more controlled sampling population, a larger sample cohort would also be useful, particularly in the ELISA experiments where substantial variation could be observed. Additionally, although some animals from which samples were obtained from in this study may have been undergoing active flares of disease, challenging animals with allergens or using other methods to induce an allergic flare would allow us to see what immune response may occur upon activation.

The results of this study have greatly added to our knowledge the enigmatic allergic dermatitis affecting cats. Our results further support the hypothesis that cats do not exhibit the traditional allergy-associated Th2 response, and additionally show that a strong Th1 response is not apparent. Although the presented results do not demonstrate that IL-31 expression is heightened in allergic animals, targeting the molecule or others

involved in pruritus signaling may still be valuable, particularly due to the interesting findings with respect to OSMR-β. Further investigation is needed to evaluate how this signaling may be involved in feline allergic dermatitis. In particular, analysis of protein expression in both the skin and circulation would be useful. Additionally, expression quantification of this molecules and others evaluated in this study in others tissue involved in pruritus signaling, for example the dorsal root ganglia, may add to our understanding of the role of IL-31 signaling in feline allergic dermatitis.

CHAPTER V

CONCLUSIONS

Feline allergic disease is multifactorial; within this study we were able to evaluate both the microbial and immunological aspects of the disease. Collectively, our results indicate allergic dermatitis affecting cats is distinct from allergic dermatitides affecting other species. The pathomechanisms underlying this disease appear to be unique, involving incredibly diverse staphylococcal communities and unclear immune involvement.

We have demonstrated the feline skin microbiota is somewhat influenced by breed but not affected by whether cats reside indoors or outdoors. Although no significant findings were demonstrated, staphylococcal communities appear to be different depending on health status and warrant further research. Interestingly, *S. epidermidis, a* species that is known to convey benefits to the host, accounted for a high percentage of sequences in samples from healthy animals. Our study on the immune system in allergic and non-affected cats did not reveal a Th2-skewed system, either in the skin or systemically. While our results did not support our original hypothesis of IL-31 involvement in feline allergic dermatitis, another molecule important in IL-31 signaling, OSMR-β was found to have increased mRNA expression in affected individuals. These findings suggest further research into staphylococcal communities and OSMR-β involvement in this disease may yield findings that may have clinical applications.

The composition of staphylococcal communities on feline skin should be confirmed with other techniques, for example MALDI-TOF or sequencing of longer fragments. Upon confirmation of this work, further research to characterize functional capacity of the microbiome, particularly the different staphylococcal species identified, would be helping in understanding how these bacteria may contribute to or protect against the development of allergic dermatitis. Utilization of techniques that can discern strain-level identities would also be useful to better describe these communities.

Research into the immunologic aspect of the disease would benefit from further investigation into OSMR-β and other proteins that are involved in pruritus signaling. More specifically, evaluation of protein expression of OSMR-β in the skin would be a likely next step, in addition to evaluation of mRNA and protein expression of OSM. Expression should also be evaluated in other cells and tissues that are involved in this signaling, for example the dorsal root ganglia. Finally, techniques that are able to get a broader view of the immune system in these cats, such as RNA sequencing, would be helpful in identifying other targets for research and potential therapeutic development.

Although human and canine medicine have already began to develop more targeted therapeutics, which allow a larger number of patients to benefit from an improved quality of life, feline medicine is very much behind. Allergic feline patients and their owners are in need of safe and effective novel therapeutics, which will require research to identify specific targets. The results of this research have greatly added to our understanding of the immunological and microbiological factors of feline allergic

dermatitis, and more importantly, have identified multiple avenues for further research which may very well lead to therapeutic development.

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APPENDIX A

SUPPLEMENTARY TABLES

S1 Table. Signalment of sample cohort.

Cat #	Breed	Sex	Age	Indoor/outdoor (type)
1	Sphynx	CM	1y	100/0
2	Cornish Rex	M	7m	100/0
3	Siberian	SF	6y	100/0
4	Sphynx	F	5y	100/0
5	Sphynx	F	10m	100/0
6	Cornish Rex	SF	16y	100/0
7	Cornish Rex	M	4m	100/0
8	Devon Rex	M	9m	100/0
9	Cornish Rex	CM	3y	100/0
11	Bengal	SF	4y	100/0
12	Sphynx	F	2y	100/0
13	Cornish Rex	CM	10y	100/0
14	Siberian	CM	6y	99/1 (patio)
15	Siberian	SF	6y	99/1 (patio)
16	Cornish Rex	CM	15y	100/0
17	Bengal	CM	12y	100/0
18	Sphynx	SF	2.5y	100/0
19	Bengal	CM	9y	100/0
20	Bengal	SF	5y	100/0
21	Cornish Rex	CM	3y	100/0
22	Cornish Rex	CM	3y	100/0
23	Sphynx	F	3y	100/0
24	Sphynx	CM	4y	100/0
25	Sphynx	CM	4y	100/0
26	Bengal	SF	6y	30/70 (trees, grass, weeds)
27	Sphynx	CM	4y	100/0
28	Sphynx	M	2y	100/0
29	Sphynx	CM	3.5y	100/0
30	Bengal	M	3y	100/0
31	Bengal	F	3y	100/0
32	Devon Rex	SF	1y	100/0
33	Devon Rex	SF	10y	98/2 (grasses, patio)
34	Sphynx	SF	5m	100/0
36	Siberian	CM	3y	100/0
37	Sphynx	SF	5m	100/0
39	Devon Rex	SF	1.5y	100/0
40	Bengal	F	3m	100/0

S1 Table Continued

Cat #	Breed	Sex	Age	Indoor/outdoor (type)
41	Bengal	M	3m	100/0
43	Cornish Rex	CM	14y	100/0
45	Bengal	M	1y	100/0
51	Siberian	SF	4y	100/0
52	Siberian	SF	4y	100/0
53	Cornish Rex	SF	4y	100/0
54	Bengal	CM	5y	100/0
Indoor and outdoor cats				
Indoor 1	DLH	CM	6y	100/0
Indoor 2	DSH	F	4m	100/0
Indoor 3	DSH	M	1y 4m	100/0
Indoor 4	DSH	M	10y	100/0
Indoor 5	DSH	F	3y	100/0
Indoor 6	DMH	F	6y	95/5 (trees, grasses, weeds)
Indoor 7	DSH	CM	3y	100/0
Indoor 8	DSH	SF	9y	100/0
Indoor 9	DSH	SF	11y	98/2 (driveway)
Indoor 10	DSH	F	12y	100/0
Indoor 11	DSH	M	13y	100/0
Indoor 12	DSH	SF	3y	100/0
Indoor 13	DMH	CM	5y	100/0
Outdoor 1	DSH	SF	13y 3m	0/100
Outdoor 2	DSH	SF	13y 3m	0/100
Outdoor 4	DSH	SF	1y	0/100
Outdoor 5	DSH	F	1y	0/100
Outdoor 6	DSH	M	9.5m	0/100
Outdoor 7	DSH	M	10m	0/100
Outdoor 8	DSH	M	10m	0/100
Outdoor 9	DSH	F	2y	0/100
Outdoor 10	DSH	M	2.5y	0/100
Outdoor 11	DSH	F	2y	0/100
Outdoor 12	DSH	F	1.5y	0/100

S2 Table. P-values from pairwise Kruskal-Wallis tests on alpha diversity between cat breeds. P<0.05 are bolded.

	Bacteria			Fungi		
Comparison	Chao1	Observed OTUs	Shannon	Chao1	Observed OTUs	Shannon
Bengal vs Cornish Rex	**0.001807**	**0.000952**	**0.005646**	0.064638	**0.006272**	**0.029991**
Bengal vs Devon Rex	**0.006034**	**0.002365**	**0.004035**	**0.009591**	**0.004491**	0.133539
Bengal vs Indoor	**0.001805**	**0.000804**	**0.024916**	0.326408	**0.045784**	0.113762
Bengal vs Siberian	**0.031257**	0.071091	0.092487	**0.000149**	**0.000305**	**0.027979**
Bengal vs Sphynx	0.570611	0.876334	0.943228	0.748411	0.977934	0.503447
Cornish Rex vs Devon Rex	0.578139	0.443209	0.136106	0.218611	0.477304	0.964838
Cornish Rex vs Indoor	0.835341	0.814794	0.411682	0.401696	0.332903	0.658185
Cornish Rex vs Siberian	0.392441	0.310163	0.419229	0.064769	0.446317	0.790466
Cornish Rex vs Sphynx	**0.008421**	**0.002674**	**0.006505**	**0.029194**	**0.004844**	**0.002707**
Devon Rex vs Indoor	0.609884	0.599932	0.055562	0.060383	0.149172	0.730382
Devon Rex vs Siberian	0.382405	0.232065	0.053137	0.845439	0.967255	0.770921
Devon Rex vs Sphynx	**0.027825**	**0.006384**	**0.005130**	**0.006701**	**0.003864**	**0.025404**
Indoor vs Siberian	0.292488	0.139056	0.848809	**0.004866**	**0.049773**	0.648264
Indoor vs Sphynx	**0.004992**	**0.000987**	**0.029604**	0.210413	**0.037494**	**0.012794**
Siberian vs Sphynx	0.210543	0.105923	0.093746	**0.000277**	**0.000498**	**0.002019**

S3 Table. Relative abundance of bacterial genera present at 1% in at least 10 samples. Average (min-max), P<0.05 are bolded.

Taxon					P-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
Thermi	Deinococci	Deinococcales	Deinococcaceae	Deinococcus	0.1001	0.6544	0.2 (0-2.7)	0.1 (0-1.5)	0.1 (0-0.6)	0.2 (0-1.8)	0.2 (0-2.1)	0.8 (0-11)	0.2 (0-3.5)
Acidobacteria	Acidobacteria-6	iii1-15			0.3141	0.2504	0.3 (0-3.9)	0.2 (0-2.9)	0.1 (0-0.6)	0.7 (0-4.3)	0.1 (0-0.8)	0.2 (0-2)	0.2 (0-2)
			RB40		0.1359	0.7162	0.2 (0-2.7)	0.3 (0-2)	0.1 (0-1.5)	0.1 (0-0.6)	0.4 (0-4.2)	0.4 (0-5)	1.1 (0-8.2)
Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces	**0.0164**	0.0655	3.7 (0.1-18.8)	3.8 (0.1-16.6)	3.4 (0.2-17.7)	3.8 (0.2-18.2)	3.7 (0.1-16.9)	2.9 (0.1-16.4)	4.7 (0.1-37.9)

S3 Table Continued

Taxon					P-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
			Corynebacteriaceae	Corynebacterium	0.1954	**0.0043**	0.1 (0-1.7)	0.1 (0-1.2)	0.1 (0-0.8)	0.1 (0-1)	0.1 (0-2.1)	0.2 (0-3.9)	0.1 (0-1.7)
			Microbacteriaceae	Cryocola	0.7561	0.7710	0.1 (0-0.8)	0.2 (0-2.1)	0 (0-0.3)	0.1 (0-1)	0 (0-0.3)	0.1 (0-2.5)	0 (0-0.6)
				Other	0.0088	0.1069	0.1 (0-1.6)	0 (0-0.7)	0.1 (0-1)	0 (0-0.4)	0.2 (0-2.9)	0.1 (0-1)	0.4 (0-4)
			Micrococcaceae	Micrococcus	0.1583	0.0659	7.8 (0.3-37.1)	9.2 (0.2-47.2)	8.2 (0.4-22)	11.9 (1.4-30.7)	8 (0.6-26.9)	12.5 (0.1-38.6)	8.7 (0.5-65.1)
				Rothia	0.3893	0.0561	0.7 (0-5.7)	0.3 (0-1.6)	0.3 (0-4.4)	0.1 (0-0.7)	0.6 (0-7.3)	0.2 (0-2.4)	0.8 (0-8.3)

Table S3 Continued

Taxon					P-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
			Nocardioidaceae		0.0526	0.4145	0.1 (0-3.4)	0 (0-1.1)	0 (0-0.1)	0.1 (0-0.4)	0.2 (0-2.2)	0.3 (0-7.4)	0.6 (0-4.4)
			Pseudonocardiaceae	Pseudonocardia	0.4262	0.4960	1.9 (0-30)	2.1 (0-30.2)	0.5 (0-2.8)	0.5 (0-3.6)	1.5 (0-41.9)	2 (0-27.2)	0.8 (0-5.7)
			Streptomycetaceae	Streptomyces	**0.0147**	0.3352	0 (0-0.3)	0 (0-0.3)	0 (0-0)	0 (0-0.1)	0 (0-0.3)	0.1 (0-1.4)	0.1 (0-1.8)

Table S3 Continued

Taxon					P-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
	Bifidobacteriales	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	**0.0002**	0.7460	0 (0-0.8)	0.1 (0-1.9)	0 (0-0)	0 (0-0.1)	0.1 (0-2.4)	0 (0-1)	0.2 (0-3.9)
	Coriobacteria	Coriobacteriales	Coriobacteriaceae	Collinsella	**0.0106**	0.3760	0 (0-0.2)	0 (0-1.5)	0 (0-0)	0 (0-0.3)	0 (0-0.6)	0.1 (0-5)	0.1 (0-1.5)
	[illegible]	Rubrobacterales	Rubrobacteraceae	Rubrobacter	**0.0029**	0.7473	8.2 (0.4-28.8)	7.1 (0.2-34.7)	6.3 (0.9-33.4)	5.5 (0.7-13.3)	7.9 (0.5-26.5)	6.4 (0.3-38)	8.6 (0.3-45.7)

Table S3 Continued

Taxon					P-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae		0.5554	0.7554	3.7 (0.1-18.8)	3.8 (0.1-16.6)	3.4 (0.2-17.7)	3.8 (0.2-18.2)	3.7 (0.1-16.9)	2.9 (0.1-16.4)	4.7 (0.1-37.9)
Bacteroidetes	Saprospirae	Saprospirales	Chitinophagaceae		0.3251	0.4033	0 (0-0.2)	0 (0-0.2)	0 (0-0.1)	0 (0-0.2)	0 (0-0.5)	0 (0-1.8)	0.1 (0-1.4)
				Flavisolibacter	0.1876	0.3733	0.1 (0-1.7)	0.1 (0-1.2)	0.1 (0-0.8)	0.1 (0-1)	0.1 (0-2.1)	0.2 (0-3.9)	0.1 (0-1.7)
	Bacteroidia	Bacteroidales	Paraprevotellaceae	Prevotella	0.5741	0.6041	0.1 (0-3.9)	0 (0-1.7)	0 (0-0.6)	0 (0-0.3)	0.1 (0-1.5)	0.1 (0-5.5)	0.4 (0-5.2)

Table S3 Continued

Taxon					P-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
			Bacteroidaceae	Bacteroides	0.2341	0.6015	3.9 (0.2-46.6)	6.9 (0.1-66)	2.6 (0.2-18.9)	9.5 (0.1-83.2)	6.7 (0.1-86)	7.9 (0.2-92.9)	5 (0.1-30.6)
			Porphyromonadaceae	Paludibacter	0.1415	0.7717	0.3 (0-1.4)	0.3 (0-2.2)	0.1 (0-0.2)	0.5 (0-4.7)	0.5 (0-5.1)	0.2 (0-2.6)	0.6 (0-3.7)
				Porphyromonas	**0.0003**	0.9016	1.9 (0.1-20.9)	1.1 (0-7.5)	0.9 (0-6.7)	1 (0-6.4)	2.3 (0-22.1)	1.3 (0-16.4)	1.5 (0-8.4)
			Prevotellaceae	Prevotella	**0.0003**	0.1303	0.6 (0-8.1)	1.2 (0-4.9)	0.5 (0-3)	1.7 (0-8.5)	0.9 (0-12.8)	0.5 (0-6.6)	0.4 (0-2.5)
	Cytophagia	Cytophagales	Cytophagaceae	Adhaeribacter	0.3376	0.7434	0.1 (0-0.8)	0.2 (0-2.1)	0 (0-0.3)	0.1 (0-1)	0 (0-0.3)	0.1 (0-2.5)	0 (0-0.6)

Table S3 Continued

Taxon					P-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
	Flavobacteriia	Flavobacteriales	Weeksellaceae		0.1113	0.7214	0 (0-0.1)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-1)	0 (0-0)	0.1 (0-2.1)
				Cloacibacterium	0.3915	0.8984	0.1 (0-1.6)	0 (0-0.7)	0 (0-0.2)	0 (0-0)	0.2 (0-2.9)	0 (0-0.8)	0.2 (0-4)
			Flavobacteriaceae	Capnocytophaga	0.0149	0.3680	0.2 (0-2.7)	0.1 (0-1.5)	0.1 (0-0.6)	0.2 (0-1.8)	0.2 (0-2.1)	0.6 (0-8.6)	0.2 (0-3.5)
	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Sphingobacterium	0.1053	0.9917	0.5 (0-9.3)	0 (0-0.6)	0.4 (0-1.4)	0.4 (0-5.1)	0.5 (0-11.6)	0.4 (0-7.4)	0.7 (0-6.1)
Chlorobi	OPB56				0.1878	0.3641	7.7 (0.3-37)	9 (0.2-44.2)	7.3 (0.4-22)	11.6 (1.4-30.7)	6.8 (0.3-26.8)	12.2 (0.1-36.2)	8.5 (0.5-65.1)

Table S3 Continued

Taxon					P-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
Firmicutes	Bacilli	Bacillales			0.1880	0.8464	0.1 (0-2.4)	0 (0-1.1)	0 (0-0.1)	0 (0-0.4)	0.2 (0-2.2)	0.3 (0-7.4)	0.5 (0-3.5)
			Bacillaceae		**0.0004**	0.7295	0.4 (0-5.4)	0.1 (0-0.5)	0 (0-0.5)	0.1 (0-0.5)	0.4 (0-12.3)	0.8 (0-27)	0.4 (0-4.5)
				Bacillus	0.0957	0.7483	1.9 (0-30)	2.1 (0-30.2)	0.5 (0-2.8)	0.5 (0-3.6)	1.5 (0-41.9)	2 (0-27.2)	0.8 (0-5.7)
				Geobacillus	0.3650	0.6103	0.3 (0-12.1)	4.6 (0-31.9)	0.9 (0-9.4)	0.5 (0-6)	0.1 (0-1.2)	0.1 (0-1.8)	0.1 (0-0.6)
			Planococcaceae		0.1055	0.4833	0 (0-0.8)	0.1 (0-0.9)	0 (0-0.1)	0 (0-0.2)	0.1 (0-1.6)	0.1 (0-4.1)	0 (0-0.7)
				Sporosarcina	<**0.0001**	0.3249	0 (0-0.8)	0.1 (0-1.9)	0 (0-0)	0 (0-0.1)	0.1 (0-2.4)	0 (0-1)	0.2 (0-3.9)
			Staphylococcaceae	Jeotgalicoccus	0.0647	0.7721	0 (0-0.1)	0 (0-1.5)	0 (0-0)	0 (0-0.3)	0 (0-0.6)	0.1 (0-5)	0.1 (0-1.5)
				Staphylococcus	0.3159	0.7741	0.1 (0-1.1)	0 (0-0.3)	0 (0-0.3)	0.2 (0-1.8)	0.2 (0-3.8)	0.1 (0-1.8)	0 (0-0.7)

Table S3 Continued

Taxon					P-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
		Lactobacillales	Aerococcaceae		**0.0100**	0.7491	0 (0-0.2)	0.1 (0-0.5)	0 (0-0.5)	0.1 (0-1.5)	0.1 (0-1.1)	0.1 (0-0.9)	0.1 (0-1.1)
			Enterococcaceae	Enterococcus	0.0729	0.7520	0.1 (0-2.2)	0 (0-1.7)	0 (0-0.6)	0 (0-0.3)	0.1 (0-1.1)	0 (0-0.3)	0.3 (0-4)
			Lactobacillaceae	Lactobacillus	**<0.0001**	0.7196	0.3 (0-8.2)	0.4 (0-4.5)	0.1 (0-0.7)	0.2 (0-1.5)	0.2 (0-5.3)	0.4 (0-7.2)	0.1 (0-1.1)

Table S3 Continued

Taxon					P-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
			Streptococcaceae	Streptococcus	**0.0074**	0.7986	0.4 (0-3.3)	0.2 (0-1.5)	0.8 (0-11.3)	0.2 (0-1.1)	0.3 (0-2.1)	0.7 (0-7)	0.5 (0-3.9)
			Other	Other	0.0973	0.3742	0.2 (0-1.7)	3 (0-22.5)	0.3 (0-1.9)	0.2 (0-0.8)	0.3 (0-1.9)	0.3 (0-6)	0.6 (0-7.1)
	Clostridia	Clostridiales			0.1394	0.9228	1.3 (0-15.2)	0.2 (0-1.7)	0.2 (0-1)	0.5 (0-2.5)	0.8 (0-6.8)	1.5 (0-12.2)	1.2 (0-12.4)
			Acidaminobacteraceae	Fusibacter	0.0722	0.5523	0 (0-0.1)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0.1)	0 (0-0)	0.1 (0-1.3)

Table S3 Continued

Taxon					P-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
			Tissierellaceae	Parvimonas	0.3897	0.2270	0.8 (0-9.7)	0.2 (0-2.5)	0.5 (0-1.4)	0.6 (0-5.6)	0.7 (0-11.6)	0.5 (0-7.4)	1.7 (0-18.5)
			Clostridiaceae		**<0.0001**	0.0517	9 (0.3-37.2)	9.7 (0.3-47.4)	8.6 (0.5-22.5)	12.4 (1.9-32.1)	9 (0.7-27.5)	13.2 (0.2-38.6)	10.6 (0.6-65.2)
				Clostridium	**0.0003**	0.1172	17 (0.8-49.8)	26 (0.4-62.1)	10.9 (2.4-24.7)	16.1 (2.1-44.5)	14.7 (0.9-51.8)	16.2 (0.5-58.2)	14 (0.6-40)
			Lachnospiraceae		0.1578	0.7787	0.3 (0-3.9)	0.2 (0-2.9)	0.1 (0-0.6)	0.7 (0-4.3)	0.1 (0-0.8)	0.2 (0-2)	0.2 (0-2)
				Ruminococcus	0.0010	0.5449	0.2 (0-2.7)	0.3 (0-2)	0.1 (0-1.5)	0.1 (0-0.6)	0.4 (0-4.2)	0.4 (0-5)	1.1 (0-8.2)
				Blautia	**<0.0001**	0.1946	23.2 (3.3-75.2)	15.7 (0.6-46.5)	15.4 (1.8-50.3)	17.1 (2.6-48.2)	26 (2.4-85.4)	20.4 (0.9-97.5)	21.9 (2-71.8)
				Other	0.1888	0.4973	0.2 (0-4.9)	0.1 (0-1.7)	0 (0-0.6)	0 (0-0.4)	0.2 (0-1.7)	0.2 (0-5.5)	0.5 (0-6.5)
			Peptococcaceae	Peptococcus	0.3438	0.3400	43.7 (8.5-76.8)	41.7 (7.1-98.5)	59.9 (24.6-90.9)	44.9 (12.4-88.5)	42.3 (7.3-89.4)	43.3 (1.4-97)	43.2 (13-92.2)

Table S3 Continued

Taxon					P-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
			Peptostreptococcaceae		**0.0477**	0.9783	0.6 (0-8.1)	1.2 (0-4.9)	0.5 (0-3)	1.7 (0-8.5)	0.9 (0-12.9)	0.5 (0-6.6)	0.5 (0-2.5)
				Filifactor	**0.0190**	0.7937	0.6 (0-9.9)	0.1 (0-0.6)	0.1 (0-0.8)	0.2 (0-2.1)	0.4 (0-6)	0.7 (0-12.9)	0.4 (0-3.3)
			Ruminococcaceae		0.0529	0.3857	0.1 (0-0.8)	0.2 (0-2.1)	0 (0-0.3)	0.1 (0-1)	0 (0-0.3)	0.1 (0-2.5)	0 (0-0.6)
			Veillonellaceae	Megamonas	**0.0079**	0.4475	0.2 (0-2.6)	0.2 (0-2.1)	0 (0-0.5)	0.1 (0-1.2)	0.1 (0-3.2)	0 (0-0.7)	0.5 (0-11.1)
				Megasphaera	**<0.0001**	0.1501	0.5 (0-9.3)	0 (0-0.6)	0.4 (0-1.4)	0.4 (0-5.1)	0.5 (0-11.6)	0.4 (0-7.4)	0.7 (0-6.1)
				Veillonella	0.3405	0.7380	0.7 (0-5.7)	0.3 (0-1.6)	0.3 (0-4.4)	0.1 (0-0.7)	0.6 (0-7.3)	0.2 (0-2.4)	0.8 (0-8.3)

Table S3 Continued

Taxon					P-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	**0.0237**	0.0991	0.3 (0-7.9)	0.2 (0-2.4)	0.1 (0-0.6)	0.3 (0-5.4)	0.1 (0-1)	0.1 (0-1.8)	0 (0-0.3)
			Leptotrichiaceae		0.1170	0.1979	0 (0-0.9)	0 (0-0)	0 (0-0.1)	0.1 (0-1)	0 (0-0.9)	0.1 (0-2.5)	0.3 (0-3.2)
				Other	0.1114	0.7550	0.5 (0-7.7)	0.1 (0-0.5)	0.3 (0-1.6)	0.3 (0-2.3)	1 (0-23.5)	0.9 (0-29)	0.7 (0-15.1)
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae		0.4821	0.9959	1.9 (0-30)	2.1 (0-30.2)	0.5 (0-2.8)	0.5 (0-3.6)	1.5 (0-41.9)	2 (0-27.2)	0.8 (0-5.7)

Table S3 Continued

Taxon					P-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
		Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	0.7002	0.7279	0 (0-0.3)	0 (0-0.6)	0 (0-0)	0 (0-0.6)	0.1 (0-2.2)	0.1 (0-1.1)	0.3 (0-3)
			Methylobacteriaceae	Methylobacterium	0.0734	0.6048	14.9 (0.5-72.7)	8.5 (0.4-32.6)	9 (0.9-22.9)	11.6 (0.4-45.5)	18 (0.8-83.2)	13.9 (0.3-97)	13.2 (0.9-66)
			Phyllobacteriaceae		**0.0103**	0.6097	8.2 (0.4-28.8)	7.1 (0.2-34.7)	6.3 (0.9-33.4)	5.5 (0.7-13.3)	7.9 (0.5-26.5)	6.4 (0.3-38)	8.6 (0.3-45.7)
				Phyllobacterium	0.6666	0.8235	0 (0-0.2)	0.1 (0-0.5)	0 (0-0.5)	0.1 (0-1.5)	0.1 (0-1.1)	0.1 (0-0.9)	0.1 (0-1.1)
		Rhodobacter	Rhodobact		0.1756	0.7603	0 (0-0.2)	0 (0-0.2)	0 (0-0.1)	0 (0-0.2)	0 (0-0.5)	0 (0-1.8)	0.1 (0-1.4)

Table S3 Continued

Taxon					P-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
				Paracoccus	0.2732	0.7788	0.1 (0-1.7)	0.1 (0-1.2)	0.1 (0-0.8)	0.1 (0-1)	0.1 (0-2.1)	0.2 (0-3.9)	0.1 (0-1.7)
		Rhodospirillales	Acetobacteraceae		**0.0100**	0.3738	3.9 (0.2-46.6)	6.9 (0.1-66)	2.6 (0.2-18.9)	9.5 (0.1-83.2)	6.7 (0.1-86)	7.9 (0.2-92.9)	5 (0.1-30.6)
		Sphingomonadaceae	Sphingomonadaceae		**0.0240**	0.7521	1.9 (0.1-20.9)	1.1 (0-7.5)	0.9 (0-6.7)	1 (0-6.4)	2.3 (0-22.1)	1.3 (0-16.4)	1.5 (0-8.4)
				Sphingomonas	0.0504	0.9843	0.6 (0-9.9)	0.1 (0-0.6)	0.1 (0-0.8)	0.2 (0-2.1)	0.4 (0-6)	0.7 (0-12.9)	0.4 (0-3.3)
	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Sutterella	<**0.0001**	0.9573	0.2 (0-2.7)	0.1 (0-1.5)	0.1 (0-0.6)	0.2 (0-1.8)	0.2 (0-2.1)	0.6 (0-8.6)	0.2 (0-3.5)

Table S3 Continued

Taxon					P-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
			Burkholderiaceae	Lautropia	0.5172	0.5193	0.5 (0-9.3)	0 (0-0.6)	0.4 (0-1.4)	0.4 (0-5.1)	0.5 (0-11.6)	0.4 (0-7.4)	0.7 (0-6.1)
			Comamonadaceae		0.6615	0.7546	7.7 (0.3-37)	9 (0.2-44.2)	7.3 (0.4-22)	11.6 (1.4-30.7)	6.8 (0.3-26.8)	12.2 (0.1-36.2)	8.5 (0.5-65.1)
				Acidovorax	0.3180	0.7445	0.2 (0-3.9)	0.2 (0-3)	0.9 (0-15.8)	0.2 (0-2.5)	1.2 (0-12.1)	0.3 (0-3.6)	0.2 (0-1.7)
				Lampropedia	0.1730	0.9038	0.7 (0-5.7)	0.3 (0-1.6)	0.3 (0-4.4)	0.1 (0-0.7)	0.6 (0-7.3)	0.2 (0-2.4)	0.8 (0-8.3)
				Other	0.4444	0.9955	0 (0-0.9)	0 (0-0)	0 (0-0.1)	0.1 (0-1)	0 (0-0.9)	0.1 (0-2.5)	0.3 (0-3.2)
			Oxalobacteraceae		**0.0015**	0.4690	0.1 (0-2.4)	0 (0-1.1)	0 (0-0.1)	0 (0-0.4)	0.2 (0-2.2)	0.3 (0-7.4)	0.5 (0-3.5)
		Neisseriales	Neisseriace		**<0.0001**	0.7428	1.9 (0-30)	2.1 (0-30.2)	0.5 (0-2.8)	0.5 (0-3.6)	1.5 (0-41.9)	2 (0-27.2)	0.8 (0-5.7)
				Conchiformibius	**0.0004**	0.1355	0.3 (0-12.1)	4.6 (0-31.9)	0.9 (0-9.4)	0.5 (0-6)	0.1 (0-1.2)	0.1 (0-1.8)	0.1 (0-0.6)

Table S3 Continued

Taxon					P-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
				Other	0.0741	0.5908	0 (0-0.8)	0.1 (0-0.9)	0 (0-0.1)	0 (0-0.2)	0.1 (0-1.6)	0.1 (0-4.1)	0 (0-0.7)
		Other	Other	Other	**0.0003**	0.9368	9.4 (0.2-69.2)	3.7 (0.2-18.3)	4.4 (0.2-14.3)	7.7 (0.3-44)	10.6 (0.3-80.8)	10.1 (0.2-96.2)	8.9 (0.3-65.4)
	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Arcobacter	**<0.001**	0.4275	3.7 (0.1-18.8)	3.8 (0.1-16.6)	3.4 (0.2-17.7)	3.8 (0.2-18.2)	3.7 (0.1-16.9)	2.9 (0.1-16.4)	4.7 (0.1-37.9)
				Campylobacter	0.0539	0.7412	0.1 (0-1.7)	0.1 (0-1.2)	0.1 (0-0.8)	0.1 (0-1)	0.1 (0-2.1)	0.2 (0-3.9)	0.1 (0-1.7)
			Helicobacteraceae	Flexispira	0.1717	0.7036	0.1 (0-2.2)	0 (0-1.7)	0 (0-0.6)	0 (0-0.3)	0.1 (0-1.1)	0 (0-0.3)	0.3 (0-4)
				Helicobacter	**0.0041**	0.4905	0.3 (0-8.2)	0.4 (0-4.5)	0.1 (0-0.7)	0.2 (0-1.5)	0.2 (0-5.3)	0.4 (0-7.2)	0.1 (0-1.1)

Table S3 Continued

Taxon					P-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
	Gammaproteobacteria	Cardiobacteriales	Cardiobacteriaceae		0.3025	0.9993	1.3 (0-15.2)	0.2 (0-1.7)	0.2 (0-1)	0.5 (0-2.5)	0.8 (0-6.8)	1.5 (0-12.2)	1.2 (0-12.4)
		Enterobacteriales	Enterobacteriaceae		**0.0028**	0.6890	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
		Pasteurellales	Pasteurellaceae		**0.0277**	0.7337	23.2 (3.3-75.2)	15.7 (0.6-46.5)	15.4 (1.8-50.3)	17.1 (2.6-48.2)	26 (2.4-85.4)	20.4 (0.9-97.5)	21.9 (2-71.8)
				Aggregatibacter	0.0723	0.3748	0 (0-0.7)	0 (0-0.2)	0 (0-0.1)	0 (0-0.4)	0.1 (0-1.3)	0.1 (0-1.8)	0.2 (0-1.9)
				Haemophilus	0.1293	0.6177	0.1 (0-1.7)	0.1 (0-1.2)	0.1 (0-0.8)	0.1 (0-1)	0.1 (0-2.1)	0.2 (0-3.9)	0.1 (0-1.7)
				Pasteurella	0.4416	0.1904	0.2 (0-4.9)	0.1 (0-1.7)	0 (0-0.6)	0 (0-0.4)	0.2 (0-1.7)	0.2 (0-5.5)	0.5 (0-6.5)
		Pseudomona-	Moraxellac-		**<0.0001**	0.3694	43.7 (8.5-76.8)	41.7 (7.1-98.5)	59.9 (24.6-90.9)	44.9 (12.4-88.5)	42.3 (7.3-89.4)	43.3 (1.4-97)	43.2 (13-92.2)
				Acinetobacter	**<0.0001**	0.7615	0.6 (0-8.1)	1.2 (0-4.9)	0.5 (0-3)	1.7 (0-8.5)	0.9 (0-12.9)	0.5 (0-6.6)	0.5 (0-2.5)

Table S3 Continued

Taxon					P-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
				Enhydrobacter	**0.0078**	0.8193	0.6 (0-9.9)	0.1 (0-0.6)	0.1 (0-0.8)	0.2 (0-2.1)	0.4 (0-6)	0.7 (0-12.9)	0.4 (0-3.3)
				Moraxella	0.6662	0.1947	0.1 (0-0.8)	0.2 (0-2.1)	0 (0-0.3)	0.1 (0-1)	0 (0-0.3)	0.1 (0-2.5)	0 (0-0.6)
			Pseudomonadaceae		0.1348	0.7508	0.1 (0-1.6)	0 (0-0.7)	0.1 (0-1)	0 (0-0.4)	0.2 (0-2.9)	0.1 (0-1)	0.4 (0-4)
				Pseudomonas	0.3212	0.7616	0.2 (0-2.7)	0.1 (0-1.5)	0.1 (0-0.6)	0.2 (0-1.8)	0.2 (0-2.1)	0.8 (0-11)	0.2 (0-3.5)
		Xanthomonadales	Xanthomonadaceae		**0.0295**	0.4996	0.2 (0-2.6)	0.2 (0-2.1)	0 (0-0.5)	0.1 (0-1.2)	0.1 (0-3.2)	0 (0-0.7)	0.5 (0-11.1)
				Luteimonas	0.0726	0.6181	0.5 (0-9.3)	0 (0-0.6)	0.4 (0-1.4)	0.4 (0-5.1)	0.5 (0-11.6)	0.4 (0-7.4)	0.7 (0-6.1)

Table S3 Continued

Taxon					P-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema	0.0572	0.6046	0.7 (0-5.7)	0.3 (0-1.6)	0.3 (0-4.4)	0.1 (0-0.7)	0.6 (0-7.3)	0.2 (0-2.4)	0.8 (0-8.3)
SR1					0.0878	0.7876	0.3 (0-7.9)	0.2 (0-2.4)	0.1 (0-0.6)	0.3 (0-5.4)	0.1 (0-1)	0.1 (0-1.8)	0 (0-0.3)
Tenericutes	Mollicutes	Acholeplasmatales	Acholplasmataceae	Acholeplasma	0.1893	0.3537	0.1 (0-3.4)	0 (0-1.1)	0 (0-0.1)	0.1 (0-0.4)	0.2 (0-2.2)	0.3 (0-7.4)	0.6 (0-4.4)

S4 Table. Relative abundance of fungal genera present at 1% in at least 10 samples. Average (min-max). P<0.05 are bolded.

Taxon					p-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
Ascomycota	Ascomycota class incertae sedis	Ascomycota order incertae sedis	Ascomycota family incertae sedis	Thermomyces	**0.0051**	1.0331	0.7 (0-34.8)	0 (0-0.4)	0 (0-0.1)	0 (0-0.2)	2 (0-32.8)	0.5 (0-28.9)	0.1 (0-1.8)
	Dothideomycetes	Capnodiales	Capnodiales family incertae sedis	Cladosporium	**0.0009**	0.9761	5.4 (0.2-30.7)	12.8 (0.1-84.3)	3.9 (0.1-27.8)	6.1 (0.1-50.9)	8.6 (0.1-67.8)	23.4 (0.1-96.9)	25.9 (0.1-97.3)
			unclassified Capnodiales family|	unclassified Capnodiales genus	0.6370	0.9063	0.5 (0-18.3)	0.1 (0-2.1)	0.8 (0-7.5)	5.8 (0-93.2)	0.1 (0-2.5)	0 (0-0.4)	0.2 (0-4.7)

S4 Table Continued

Taxon					p-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
			Dothioraceae	unclassified Dothioraceae genus	0.1859	1.0604	0.3 (0-5.7)	2.6 (0-81.9)	0.3 (0-4.1)	0.7 (0-10.3)	1.1 (0-17.7)	1.8 (0-51.8)	0.4 (0-12.6)
		Pleosporales	Pleosporaceae	Alternaria	**0.0064**	1.0030	1.6 (0-26.9)	0.1 (0-3.2)	0.7 (0-13.3)	0.1 (0-1.2)	3.9 (0-94.2)	0.4 (0-10.8)	0.6 (0-5.8)
				Other	**0.0086**	0.9593	2.6 (0-31.5)	1.9 (0-42.6)	2.9 (0-30.2)	3.1 (0-39.9)	3.5 (0-33.9)	5 (0-73.9)	0.5 (0-4.2)
				unclassified Pleosporaceae genus	<**0.0001**	0.8535	1.9 (0-26.8)	2.5 (0-37.1)	0.2 (0-0.9)	5 (0-69.5)	11.3 (0-77.4)	2 (0-44)	5.9 (0-95.9)
			Pleosporales family incertae	Leptosphaerulina	0.4270	0.9483	12.1 (0-90.1)	0.3 (0-5.9)	3.2 (0-55.7)	0.5 (0-9.2)	8.9 (0-74.2)	1.7 (0-81.5)	3.6 (0-49.5)

S4 Table Continued

Taxon					p-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
			unclassified Pleosporales	unclassified Pleosporales genus	0.1099	0.8909	2.6 (0-44.2)	3.8 (0-55.2)	0.5 (0-5.4)	5.7 (0-92)	2.9 (0-48.1)	4.3 (0-96.3)	8.6 (0-82.6)
	Eurotiomycetes	Eurotiales	Trichocomaceae	Aspergillus	**0.0026**	0.9543	7 (0-77.1)	1.1 (0-24.6)	6.4 (0-97.7)	0.8 (0-11.7)	3.4 (0-94.2)	7.7 (0-98.1)	1.4 (0-15.4)
				Other	**<0.0001**	0.9087	3.2 (0-83.2)	2.7 (0-64.9)	0 (0-0.2)	0.2 (0-3.6)	2.4 (0-59.9)	0.8 (0-42)	0.2 (0-3.3)
				Penicillium	0.0712	1.2943	1.1 (0-13.3)	5.1 (0-95.1)	2.4 (0-37.7)	5 (0-93.7)	5.3 (0-98.3)	3 (0-90.4)	3 (0-78.4)
	Other	Other	Other	Other	0.1201	0.9434	0.3 (0-3.8)	0 (0-0.7)	0 (0-0.2)	0 (0-0.1)	0.1 (0-0.7)	1.9 (0-75.3)	0.1 (0-1.6)
	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	Saccharomyces	0.1055	0.9235	0.6 (0-12.7)	0.4 (0-5.6)	0.2 (0-3.2)	0.1 (0-0.6)	0.8 (0-45.6)	0.4 (0-10.4)	0.3 (0-9.4)

S4 Table Continued

Taxon					p-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
			Saccharomycetales family incertae sedis	Candida	**0.0136**	0.9470	0.2 (0-2.6)	0.2 (0-3.6)	0.1 (0-0.9)	0.1 (0-0.9)	1.1 (0-28.9)	0.1 (0-2.6)	0 (0-0.5)
				Debaryomyces	0.5947	0.8895	2.8 (0-67.8)	15.6 (0-91.3)	0.1 (0-0.3)	1.6 (0-45.9)	0.1 (0-1.1)	0.3 (0-10)	2.3 (0-88.7)
			unclassified Saccharomycetales	unclassified Saccharomycetales genus	0.0955	0.9248	0.5 (0-15.6)	0.4 (0-13.6)	5.2 (0-97.7)	0.1 (0-1.1)	0.2 (0-2.2)	0 (0-0.3)	0 (0-0.3)
	Sordariomycetes	Hypocreales	Hypocreales family incertae sedis	Fusarium	0.0557	1.2009	4.2 (0-42.3)	0.6 (0-10.1)	4.5 (0-71.7)	3.4 (0-87.3)	3 (0-24.4)	6.5 (0-40.6)	7.6 (0-91.4)
				Myrothecium	0.0905	1.6716	0.3 (0-6.8)	1.8 (0-47.7)	0 (0-0.1)	0.2 (0-4.8)	0.3 (0-9)	0.3 (0-9.8)	0.3 (0-10)
				Sarocladium	**0.0290**	1.0319	0.2 (0-2.2)	2.6 (0-98)	0 (0-0.1)	1.7 (0-49.4)	0.9 (0-21.5)	0.3 (0-3.6)	0.1 (0-2.2)

S4 Table Continued

Taxon					p-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
			Other	Other	0.1156	0.8520	0.2 (0-4)	0.1 (0-1.5)	0 (0-0.1)	0.7 (0-20.8)	0.3 (0-5.5)	0.4 (0-17.8)	0.4 (0-14.4)
			unclassified Hypocreales	unclassified Hypocreales genus	**0.0051**	1.0046	0.2 (0-7.1)	0 (0-0.6)	0 (0-0.2)	0 (0-0.3)	1.1 (0-38.7)	0.2 (0-2.9)	0.1 (0-1.6)
		Trichosphaeriales	Trichosphaeriales family incertae	Nigrospora	**0.0075**	0.9847	1.8 (0-36.1)	0.2 (0-3.3)	0.1 (0-1.1)	5.4 (0-88.3)	1.9 (0-46.6)	7.5 (0-97.4)	3.3 (0-41.9)
	unclassified Ascomycota class	unclassified Ascomycota order	unclassified Ascomycota family	unclassified Ascomycota genus	**0.0189**	0.8147	0.4 (0-9.1)	0.2 (0-6.4)	2 (0-11)	0.1 (0-0.9)	1.7 (0-66.5)	2.5 (0-97.7)	0.4 (0-7.3)

S4 Table Continued

Taxon					p-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
Basidiomycota	Agaricomycetes	Agaricomycetes order incertae sedis	Corticiaceae	Phanerochaete	0.8788	1.0515	0.2 (0-5.4)	0.3 (0-9.3)	0.1 (0-1.5)	0 (0-0.3)	0 (0-0.2)	1.8 (0-93.5)	0.1 (0-2.9)
			Peniophoraceae	Peniophora	0.4179	0.9887	0.5 (0-14.1)	1 (0-44.6)	0.5 (0-8.7)	1.9 (0-50.6)	0.5 (0-25.4)	0.9 (0-25.8)	0.1 (0-1.3)
			Stereaceae	Stereum	**0.0034**	1.0299	1.1 (0-26.5)	2.9 (0-83.1)	4 (0-74.7)	0.4 (0-7.3)	0.1 (0-2.1)	0.1 (0-1.6)	0 (0-0.3)
		Polyporales	Polyporales family incertae sedis	Fomes	**0.0349**	0.9736	0.3 (0-4.3)	0.1 (0-0.5)	0.9 (0-16.3)	0.2 (0-2.3)	0.1 (0-1)	2 (0-96.5)	0.9 (0-32.6)
				Gelatoporia	0.3585	1.4040	0.9 (0-34.5)	0.1 (0-1.3)	1.5 (0-20.3)	0 (0-0.1)	0.4 (0-20.4)	0 (0-0.6)	0.1 (0-1.6)

S4 Table Continued

Taxon					p-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
				Trametes	0.5798	1.0264	0.5 (0-11.9)	1.5 (0-30.3)	2.4 (0-41.8)	0 (0-0.1)	0.5 (0-27.1)	0 (0-0.9)	0.2 (0-6.4)
	Agaricostilbomycetes	Agaricostilbomycetes order incertae sedis	Agaricostilbomycetes family incertae sedis	Sterigmatomyces	0.0926	0.5152	0.2 (0-7.9)	0.3 (0-12.9)	3.5 (0-45.7)	0 (0-0.1)	0 (0-2.1)	0 (0-0.2)	0.6 (0-10.1)
	Basidiomycota class incertae sedis	Basidiomycota class incertae sedis	Basidiomycota family incertae sedis	Wallemia	0.3670	1.2529	0.1 (0-2.2)	0.9 (0-38.3)	0.1 (0-2.4)	0.1 (0-1.9)	0.7 (0-24.8)	0.1 (0-2.5)	0.2 (0-4.6)
		Malasseziales	Malasseziales family incertae	Malassezia	**0.0026**	0.9414	1.3 (0-12.5)	5.6 (0-98.4)	17.2 (0.1-98.2)	11.2 (0-94.4)	2.6 (0-59.7)	0.8 (0-9.5)	5.2 (0-96.9)

S4 Table Continued

Taxon					p-value		Sample type						
Phylum	Class	Order	Family	Genus	Breed	Environ-ment	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
		Sporidiobolales	unclassified Sporidiobolales	unclassified Sporidiobolales genus	**0.0006**	0.8599	0.3 (0-6.8)	0.4 (0-17.2)	0.1 (0-2.3)	0.1 (0-2.1)	1.2 (0-19.3)	0 (0-0.4)	0.3 (0-10.7)
	Tremellomycetes	Tremellales	Tremellales family incertae sedis	Cryptococcus	0.6497	1.0284	0.2 (0-5.4)	0.8 (0-9.9)	0.1 (0-1.9)	0.6 (0-12.8)	0.7 (0-29.6)	0.1 (0-1.4)	1.1 (0-46)
Fungi phylum	Fungi class incertae sedis	Mucorales	Choanephoraceae	unclassified Choanephoraceae genus	0.1253	0.9444	0.9 (0-38.2)	0 (0-0.3)	2.6 (0-34.5)	0 (0-0.1)	0.2 (0-7.7)	0.2 (0-5.6)	0.3 (0-9.1)
Other	Other	Other	Other	Other	0.0057	1.1681	32.2 (0.5-95.2)	16.6 (0.5-98.5)	21.3 (0.7-80.2)	32.3 (0.5-98.5)	17.3 (0.5-93.7)	12.5 (0.4-99.1)	15.5 (0.5-98.9)

S5 Table. Relative abundance of Malassezia species. Average, median (min-max).

Malassezia species	Bengal	Cornish Rex	Devon Rex	Siberian	Sphynx	Indoor	Outdoor
M. dermatis	0.4, 0 (0-5.2)	0.2, 0 (0-7.1)	0.1, 1.0 (0-1.3)	0.1, 0 (0-1.8)	2.9, 0 (0-73.1)	0.3, 0 (0-10.0)	0.6, 0 (0-14.3)
M. furfur	6.9, 0 (0-40.6)	4.3, 0 (0-25.0)	0.2, 0 (0-3.7)	4.0, 0 (0-34.0)	13.9, 3.0 (0-84.8)	5.3, 0 (0-40.0)	6.9, 0 (0-60.0)
M. globosa	22.5, 17.7 (0-94.3)	22.7, 17.0 (0-80.0)	34.2, 23.9 (0-100.0)	26.0, 16.9 (0-96.2)	18.1, 12.1 (0-96.5)	26.3, 20.0 (0-99.0)	25.9, 23.1 (0-82.2)
M. japonica	0, 0 (0-0.3)	0, 0 (0-0)	0, 0 (0-0)	0, 0 (0-0.6)	0, 0 (0-0.4)	0, 0 (0-0)	0.1, 0 (0-6.7)
M. nana	5.3, 0 (0-90.9)	0.5, 0 (0-7.8)	4.6, 0 (0-59.5)	0.4, 0 (0-3.2)	10.9, 0.5 (0-88.6)	2.8, 0 (0-100.0)	1.1, 0 (0-16.7)
M. obtusa	0, 0 (0-0)	0, 0 (0-0)	0, 0 (0-0)	0, 0 (0-0)	0, 0 (0-0.1)	0, 0 (0-0)	0, 0 (0-0)
M. pachydermatis	0.7,0 (0-30.2)	0.7, 0 (0-21.9)	1.4, 0 (0-14.1)	0.2, 0 (0-4.3)	1.3, 0 (0-63.3)	2.4, 0 (0-46.2)	1.2, 0 (0-33.3)
M. restricta	32.5, 23.8 (2.2-93.9)	31.9, 25.1 (0-98.0)	42.3, 36.8 (0-96.8)	52.3, 52.9 (0.9-99.8)	31.1, 13.9 (0-100.0)	40.7, 34.7 (0-100.0)	38.8, 25.0 (0-99.4)
M. slooffiae	8.1. 0.1 (0-58.8)	18.5, 0 (0-100.0)	8.0, 0 (0-64.0)	5.0, 0 (0-63.6)	6.5, 0 (0-89.0)	4.9, 0 (0-66.7)	9.8, 0 (0-89.4)
M. sympodialis	0.5,0 (0-25.5)	0.3, 0 (0-8.3)	0.1, 0 (0-1.3)	0, 0 (0-0)	0, 0 (0-0.9)	0, 0 (0-0.2)	0.1, 0 (0-3.2)
Unclassified *Malassezia*	23.0, 7.6 (0-92.1)	21.1, 8.3 (0-88.5)	9.0, 4.0 (0-46.7)	12.0, 4.7 (0-96.8)	15.1, 6.1 (0-96.8)	17.2, 9.5 (0-100.0)	15.5, 4.9 (0-66.7)

S6 Table. Taxa determined to be differentially abundant on the skin across age groups and sex with LEfSe (LDA>2.5, p<0.01).

	Bacteria			Fungi		
	Taxa	**Group**	**LDA score**	**Taxa**	**Group**	**LDA score**
Sex	Geobacillus	M	2.606929			
	RB40	M	2.723305			
	Unclassified RB40 genus	M	2.73251			
	Thermus	M	2.724025			
Age group	Nocardioidaceae	Senior	2.544902	Marasmius	Adult	2.82278
	Oxalobacteraceae	Senior	2.991608	Agaricales_family_incertae_sedis	Adult	2.83465
	Ralstonia	Senior	2.988875	Auriculariaceae	Adult	3.40098
	N09	Senior	2.481695	Auricularia	Adult	3.406538
	Planctomycetes	Senior	2.635051	Auriculariales	Adult	3.476242
				Clavicipitaceae	Senior	3.262227
				unclassified_Clavicipitaceae_genus	Senior	3.270884
				Eurotiales	Senior	4.265596
				Trichocomaceae	Senior	4.265596

Table S7: Signalment of sample cohort.

Cat	Breed	Sex	Age	Indoor/Outdoor	Status
Indoor 3	DSH	M	1y 4m	100/0	Healthy
Indoor 4	DSH	M	10y	100/0	Healthy
Indoor 5	DSH	F	3y	100/0	Healthy
Indoor 6	DMH	F	6y	95/5	Healthy
Indoor 7	DSH	CM	3y	100/0	Healthy
Indoor 8	DSH	SF	9y	100/0	Healthy
Indoor 9	DSH	SF	11y	98/2	Healthy
Indoor 10	DSH	F	12y	100/0	Healthy
Indoor 11	DSH	M	13y	100/0	Healthy
Indoor 12	DSH	SF	3y	100/0	Healthy
Indoor 13	DMH	CM	5y	100/0	Healthy
F12	DSH	CM	9y	100/0	Allergic
F13	Siamese	CM	8y	100/0	Allergic
F14	DSH	CM	11y	95/5	Allergic
F15	Siamese	SF	9y	100/0	Allergic
F16	DSH	SF	5y	60/40	Allergic
F17	DSH	SF	9y	100/0	Allergic
F18	Persian	CM	4y	100/0	Allergic
F19	DSH	SF	11y	95/5	Allergic
F20	DSH	SF	7y	100/0	Allergic
F21	DSH	SF	8y	95/5	Allergic

Table S8: Oligos used in quantitative PCRs.

Oligo	**Sequence (5'→3')**	**Final concentration**
RPS7		
RPS7_460F	GTC CCA GAA GCC GCA CTTT	400 nM
RPS7_549R	CAC GGA TTC TCT TGC CCA CA	400 nM
TBP1		
TBP1_746F	GAA TAA GAG AGC CCC GAA CC	400 nM
TBP1_824R	TGC TCT TCA CTC TTG GCT CC	400 nM
IL-31		
IL31_498F	CAACCAAGTGACGTCCGAAA	900 nM
IL31_561R	GACTACCTGAAGAAGGAGATTG	900 nM
IL31_533Probe	ACGGCCCATGTCTAAGGGACTTTTGCA	400 nM
IL-31RA		
IL31RA_619F	TTGGGTGTCAAACGAATGGT	900 nM
IL31RA_737R	ACTTCCATCCAGTAGGCACT	900 nM
IL31RA_654P	ACGGCCTGTGTTGGCTCCTGTT	400 nM

APPENDIX B

SUPPLEMENTARY FIGURES

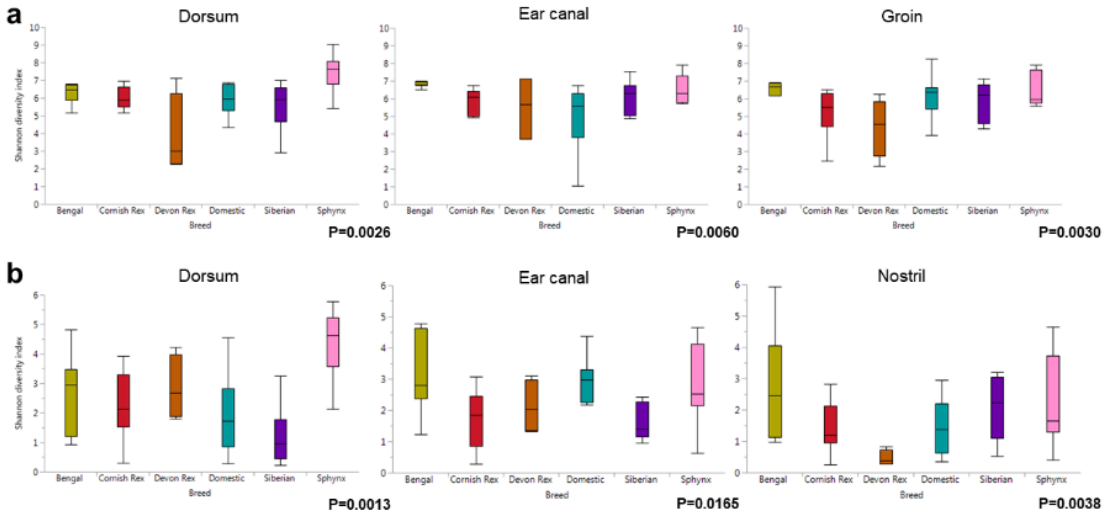


Figure S1. Significant differences in Shannon diversity index between cat breeds by site. Differences were found in the Shannon diversity index when comparing the (a) bacterial sequences in the dorsum, ear canal, and groin and when comparing the (b) fungal sequences in the dorsum, ear canal, and nostril.

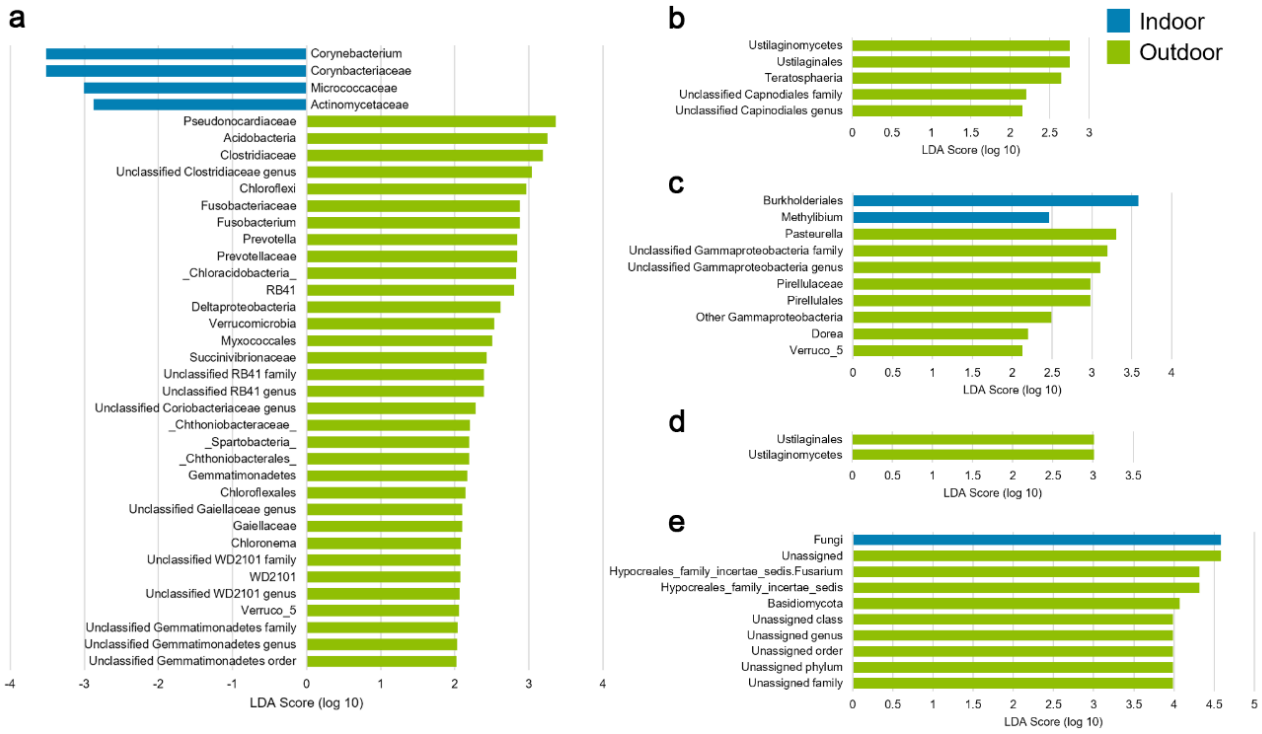


Figure S2. Taxa found to be differentially abundant between indoor and outdoor cats as determined by LEfSe. When comparing all body sites but the oral cavity, many (a) bacteria and (b) fungi were identified as differentially abundant between indoor and outdoor cats. Additionally, differentially abundant taxa were found when looking at just the bacterial sequences in the (c) nostril samples and the fungal communities in the (d) dorsum and (e) the nostril.

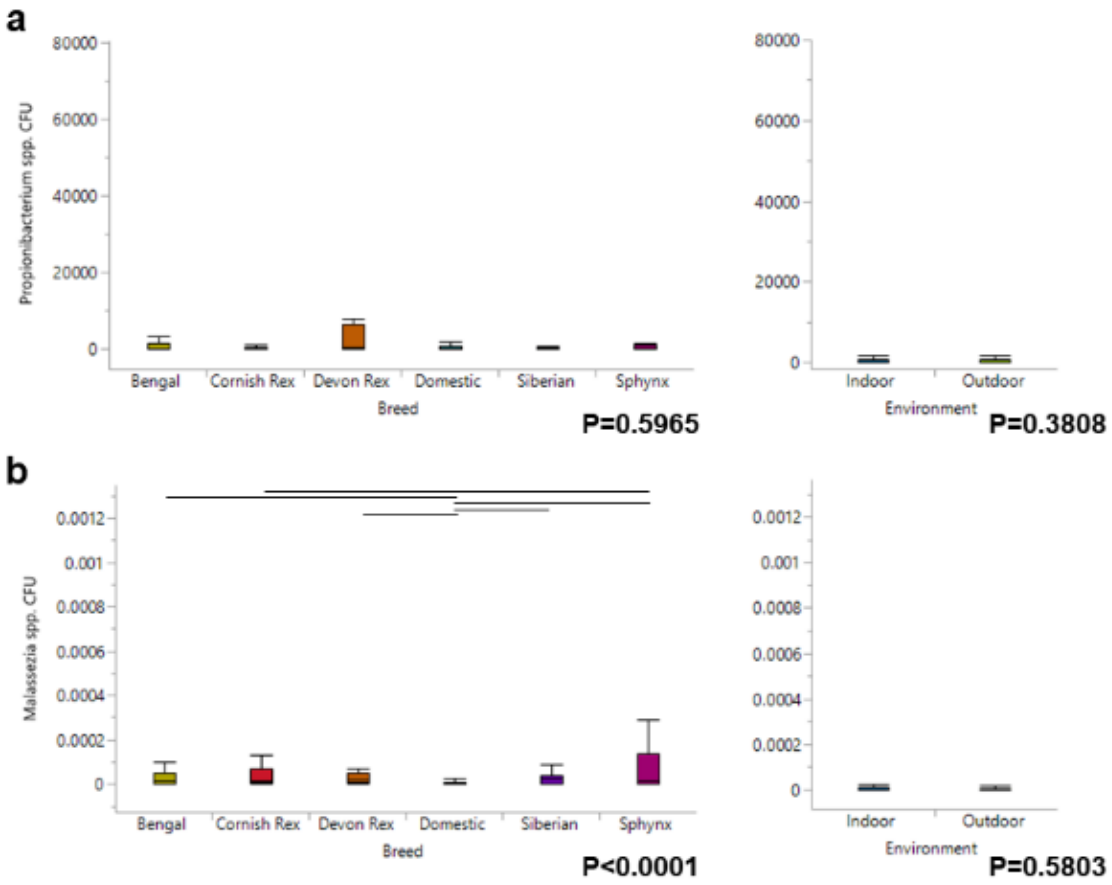


Figure S3. Results of *Propionibacterium* spp. and *Malassezia* spp. qPCRs. (a) With the *Propionibacterium* spp. qPCR, no significant differences were found between cat breeds (P=0.5965) or between indoor and outdoor cats (p=0.3808). (b) Significant differences in *Malassezia* spp. as quantified by qPCR were found between the different cat breeds (p<0.0001) but not between indoor and outdoor cats (p=0.5803). Plots do not show points for extreme outliers, however statistical analyses and box plots were made when including the outliers. Lines show significant pairwise tests where p<0.01.

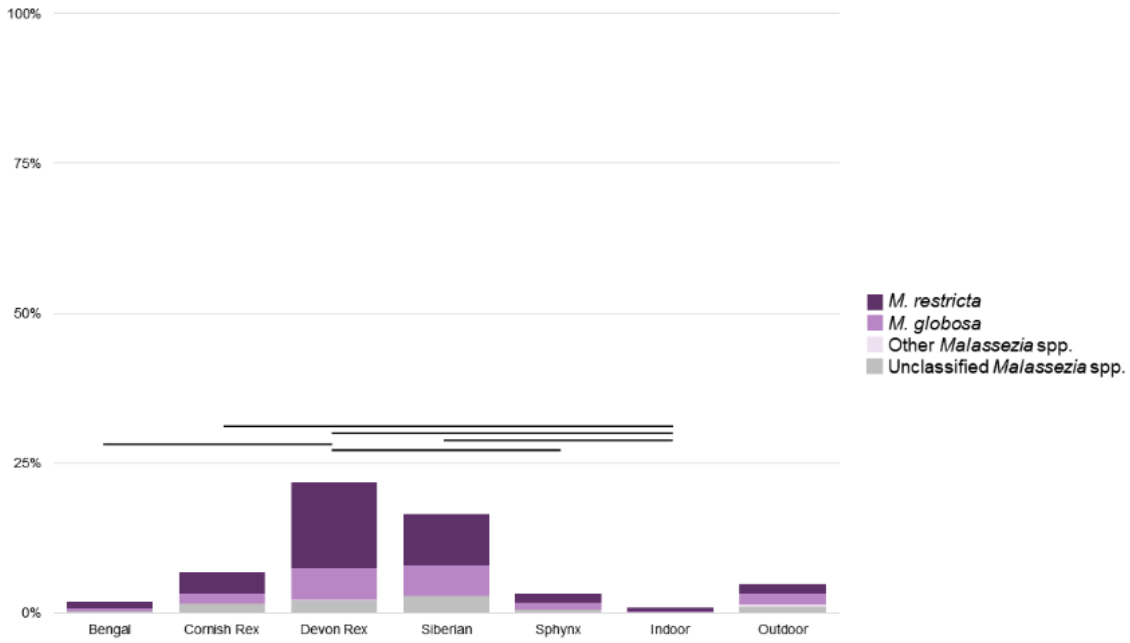


Figure S4. Average relative abundance of *Malassezia* spp. on feline skin. The height of the bar shows the average relative abundance of *Malassezia* spp. in each sample type, while the specific species are shown in terms of median relative abundance. *M. restricta* and *M. globosa* were the most abundant. Lines show significant pairwise tests of *Malassezia* spp. abundance where $p<0.05$.

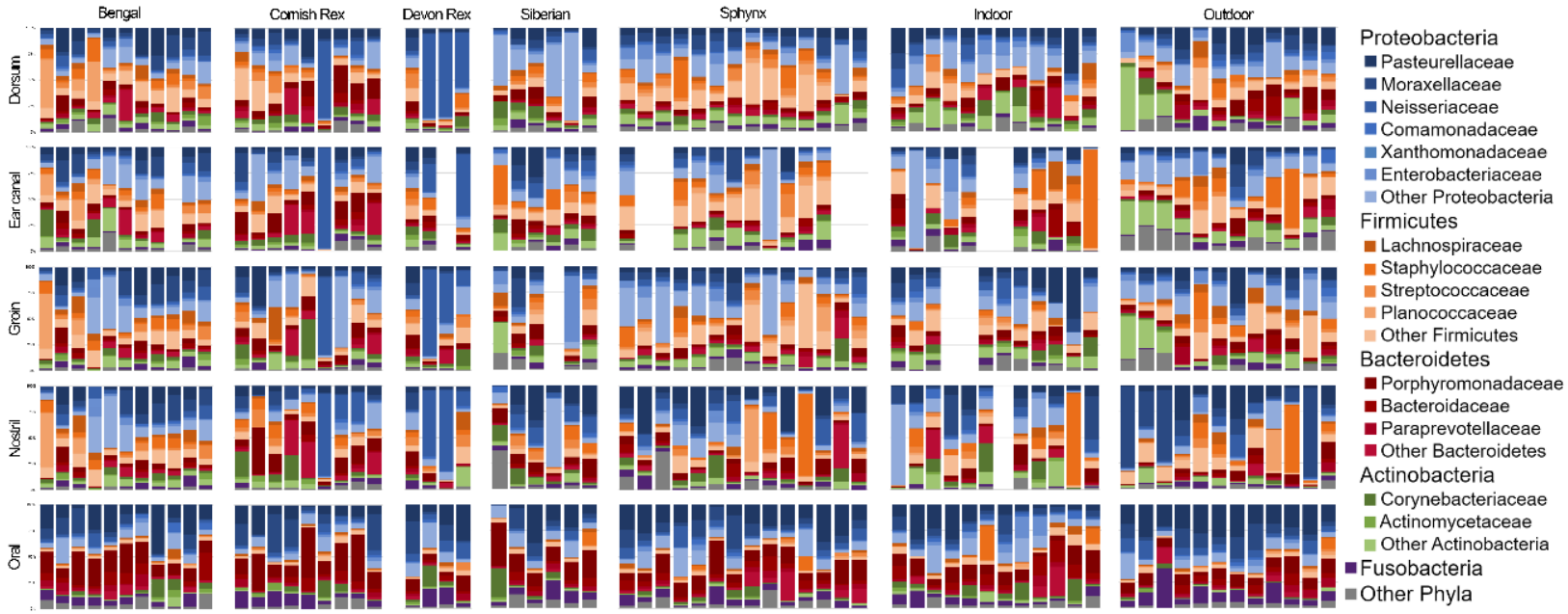


Figure S5. Relative abundance of bacterial taxa in each sample.

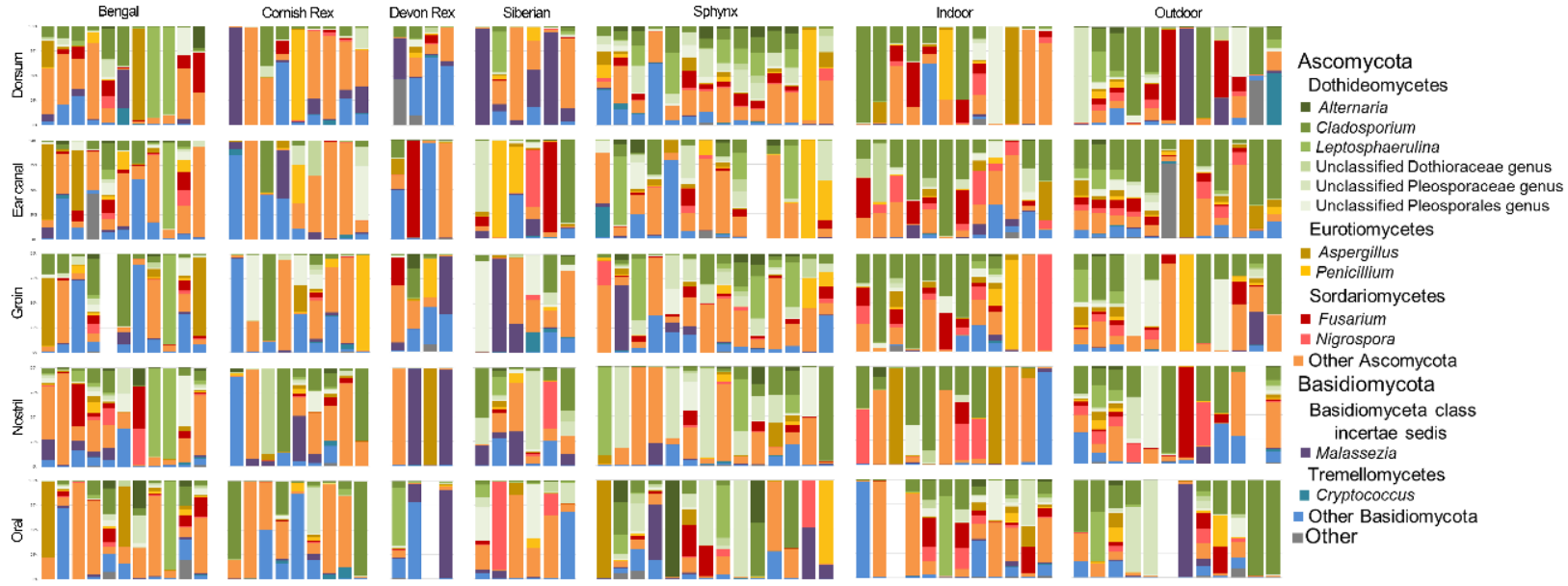


Figure S6. Relative abundance of fungal taxa in each sample.

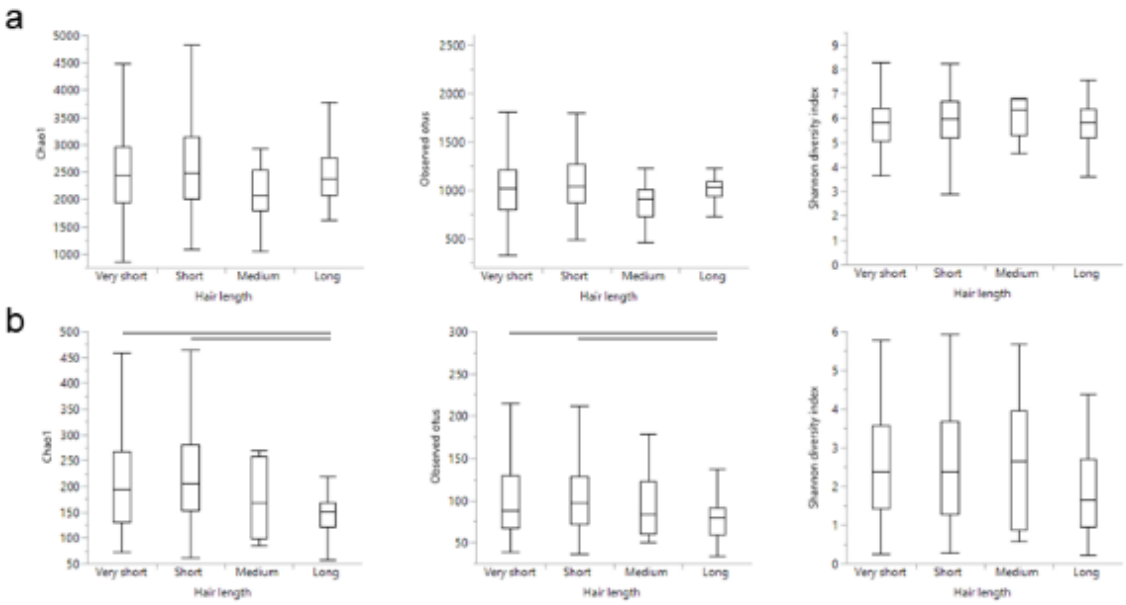


Figure S7. Evaluating the influence of hair length on alpha diversity. Evaluating the influence of hair length on (a) bacterial alpha diversity did not reveal any differences, but significant differences were observed in two metrics of (b) fungal alpha diversity. Cats with short (DSH and Bengal cats) and very short (Cornish Rex, Devon Rex, and Sphynx cats) hair have significantly more diverse communities than long haired cats (DLH and Siberian cats) with the Chao1 and observed OTUs alpha diversity metrics. Bars indicated significant pairwise comparisons where the $p<0.05$.

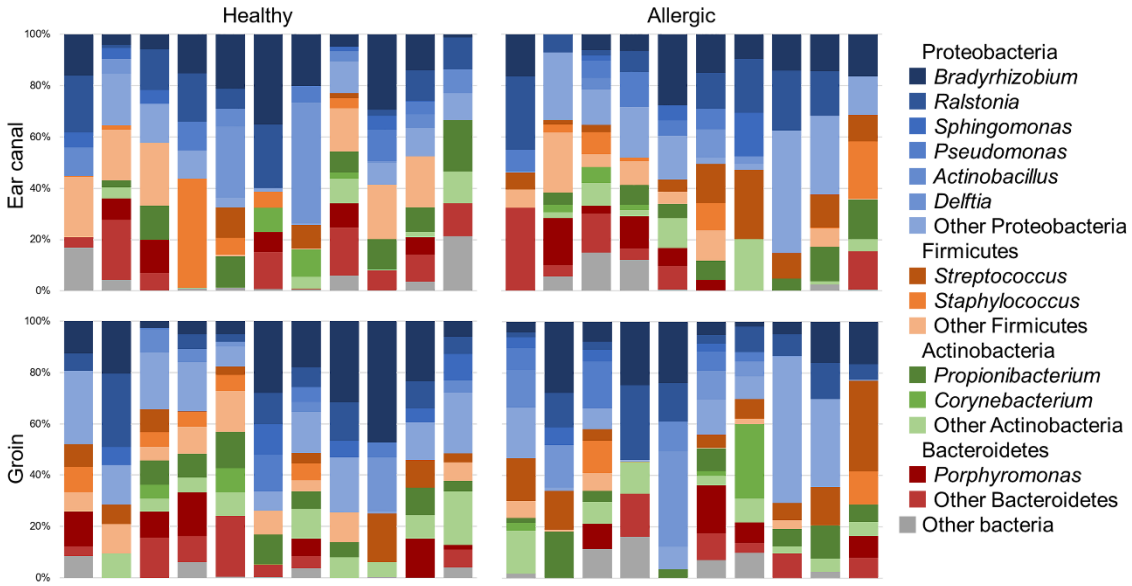
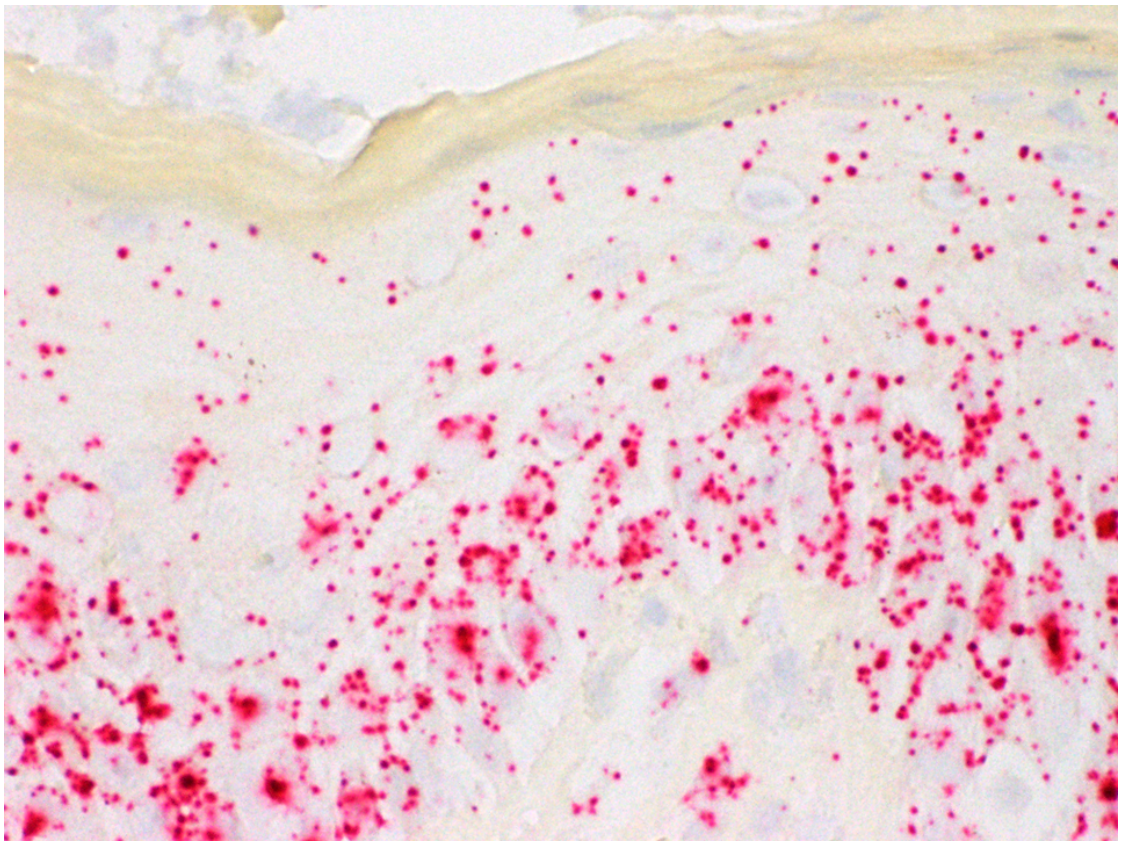
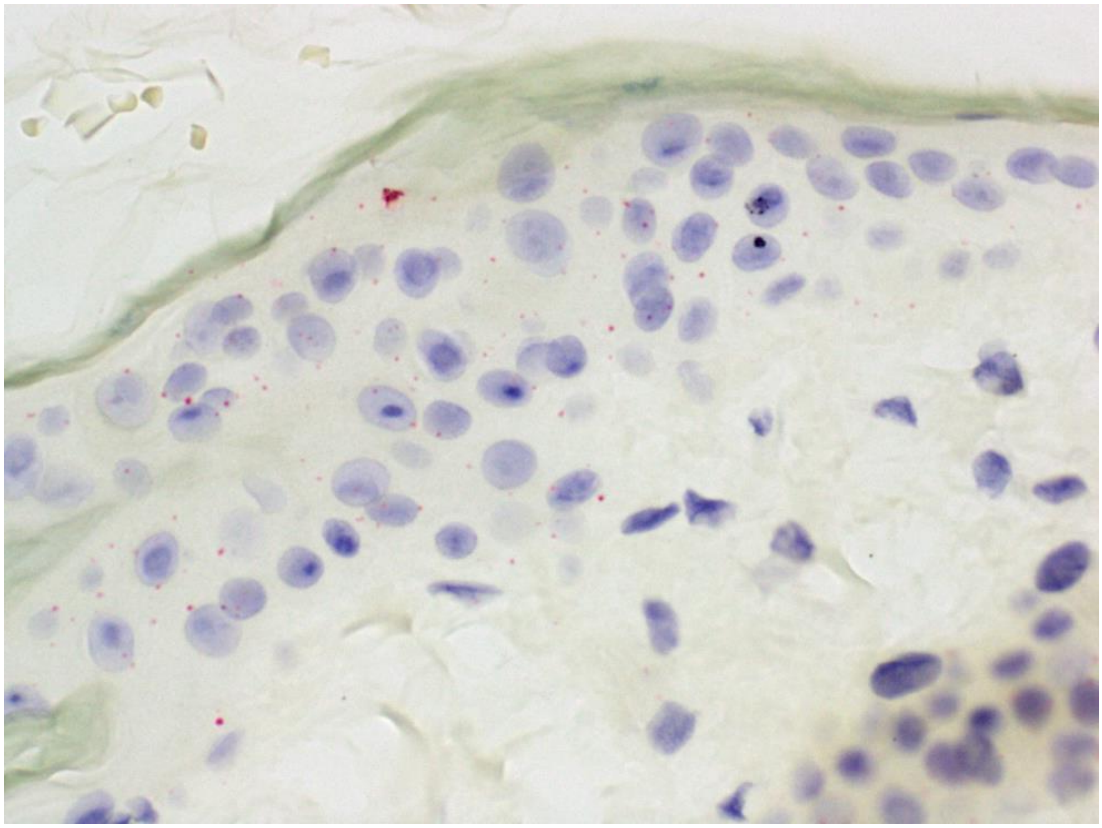


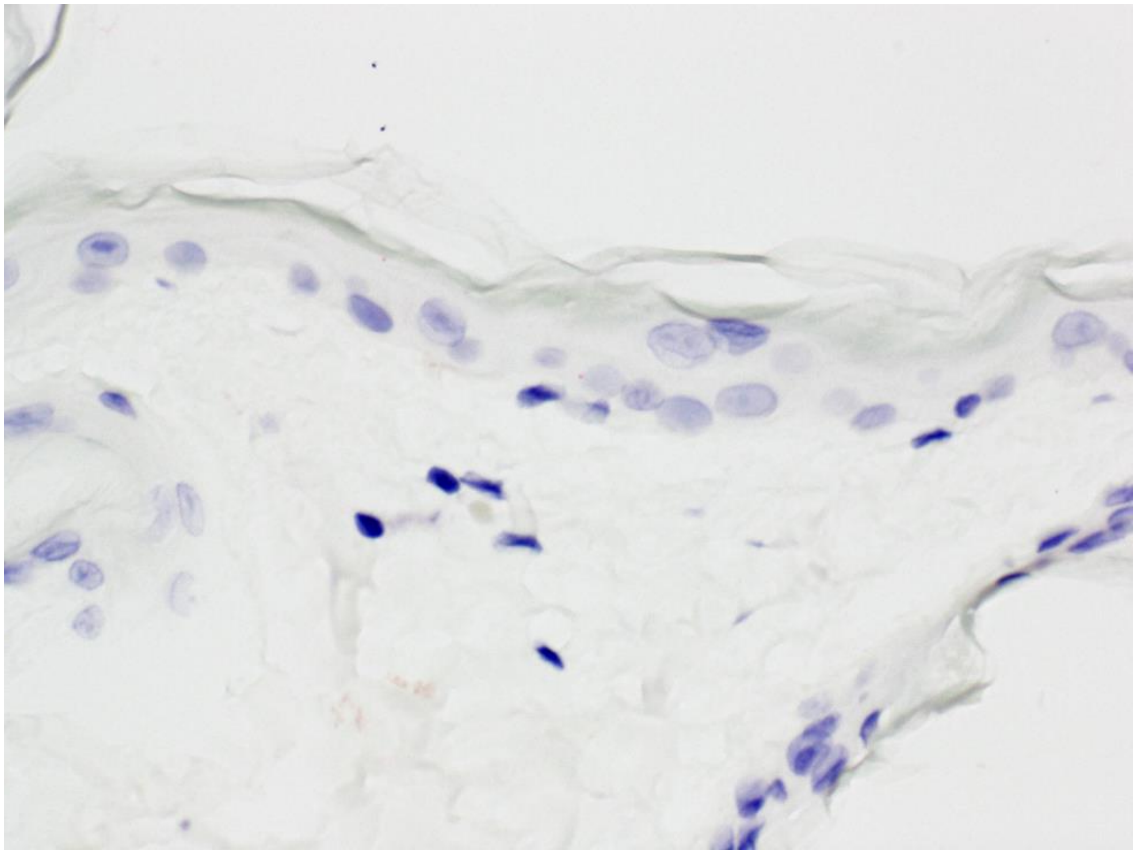
Figure S8: Relative abundance of bacterial taxa across individual samples.



S9 Fig. OSMR-β mRNA expression in feline skin. Evaluating OSMR-β mRNA expression with RNAScope demonstrated high expression levels in samples from both allergic and control cats. Staining was predominately observed in the epidermis (shown above) as well as in follicular epithelial cells in the hair bulb and outer root sheath, and occasionally in sebaceous and apocrine glands, muscle, and infiltrating inflammatory cells.



S10 Fig. IL-31RA mRNA expression in feline skin. RNAScope targeting IL-31RA revealed low numbers of mRNA in the skin, which was primarily in the epidermis and around hair follicles.



S11 Fig. IL-31 mRNA expression in feline skin. IL-31 mRNA expression, as visualized with RNAScope, was very low. Rare transcripts could be observed primarily in the epidermis, although also sometimes within dermal cells.