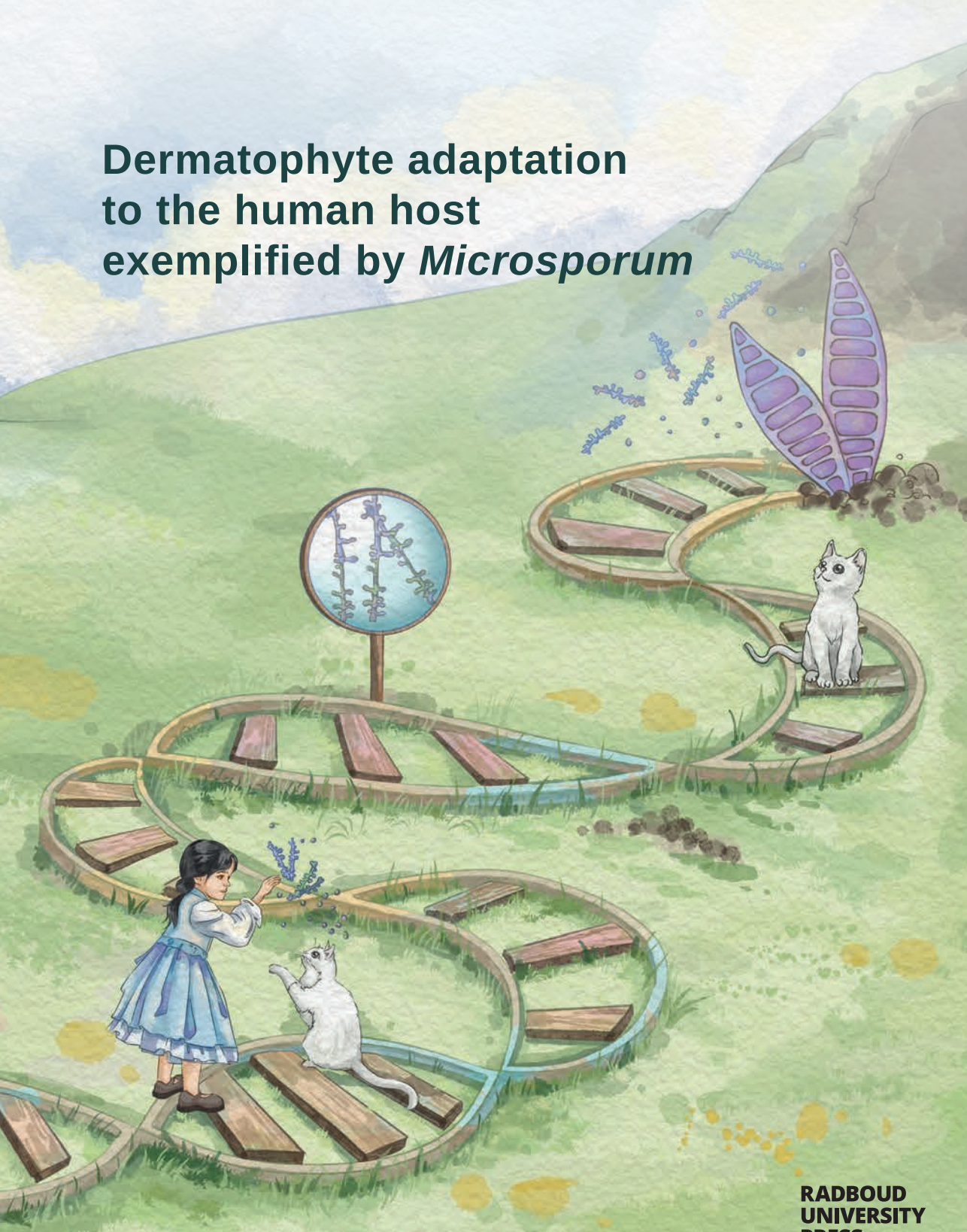
Dermatophyte adaptation to the human host exemplified by *Microsporum*



Xin Zhou

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CHAPTER 1

GENERAL INTRODUCTION, AIM, UNRESOLVED QUESTIONS, AND OUTLINE OF THE THESIS

Dermatophyte Infections

Dermatophytes, belonging to the *Ascomycota* phylum, *Eurotiomycetes* class, *Onygenales* order, and *Arthrodermataceae* family, are a group of filamentous fungi that infect the skin, nails, and hair. Currently, seven genera of dermatophytes are recognized: *Trichophyton, Epidermophyton, Nannizzia, Paraphyton, Lophophyton, Microsporum*, and *Arthroderma*. Among these, *Trichophyton spp., Epidermophyton spp.,* and *Microsporum spp.* are most frequently associated with human dermatophytosis [1].

Dermatophytes causes 30% of the most common fungal skin infections in pets and domestic animals, and as a zoonotic disease, dermatophyte infections affect approximately 20-25% of the global population [2]. In 2022, fungal skin infections were the most prevalent dermatological diseases in Europe, accounting for 8.9% of all skin conditions [3, 4]. Dermatophytes thrive in warm and humid environments, which contributes to the rising incidence of infections in tropical and developing countries. Furthermore, colonization, migration, and sports tourism have accelerated the global dissemination of dermatophyte pathogens [5]. The clinical manifestations of dermatophytoses vary widely depending on the causative dermatophyte species and the anatomical site affected. Factors such as overcrowding, shared living spaces, and urbanization have further contributed to the increasing prevalence of dermatophytosis. Additionally, age, sex, and geographical location play significant roles in the epidemiology of these infections [6, 7].

The global distribution of dermatophyte species has evolved over the past decades, influenced by changes in lifestyle, antifungal use, and pathogen adaptation. In the mid-20th century, species such as *Trichophyton interdigitale*, *Microsporum audouinii*, and *T. soudanense* were dominant in Europe, while *T. verrucosum*, *Epidermophyton floccosum*, *T. mentagrophytes*, and *M. canis* prevailed in parts of Asia [4]. However, over the past 50 years, *T. rubrum* has emerged as the globally dominant dermatophyte, likely due to its anthropophilic nature and persistence in urban environments. In India, the increasing prevalence of *T. indotineae* has been linked to widespread overuse of topical corticosteroid-antifungal combinations, poor treatment compliance, and close-contact community living [8]. In contrast, *M. canis* remains the leading zoonotic dermatophyte across Eurasia, particularly due to rising pet ownership and frequent human-animal interactions. Meanwhile, *T. tonsurans*, once limited to Southeast Asia and Australia, has become the main cause of tinea capitis in North America. These patterns reflect the impact of

socioeconomic development, shifts in urban–rural dynamics, and the remarkable adaptability of dermatophytes to both environmental and host-related pressures [4] .

Dermatophyte infections typically remain confined to the keratinized layers of the epidermis and hair shafts. However, in immunocompromised individuals with congenital or acquired immune deficiencies, dermatophytes can invade the dermis and deeper tissues, leading to systemic infections. Based on the affected site, dermatophytoses are classified into: Hair infections (tinea capitis, tinea barbae, tinea of vellus hair), Nail infections (onychomycosis), Glabrous skin infections (tinea corporis, tinea cruris, tinea pedis). The epidemiology and dominant causative species of dermatophyte infections vary according to the site of infection: tinea capitis and tinea barbae: Most commonly caused by *M. canis, T. mentagrophytes,* and *T. verrucosum*. Tinea corporis is frequently associated with *T. rubrum, T. mentagrophytes complex,* and *M. canis*. Tinea pedis primarily caused by *T. rubrum, T. mentagrophytes,* and *E. floccosum*. Tinea cruris is most caused by *T. rubrum* worldwide. However, in India, *T. mentagrophytes* is the leading pathogen, followed by *T. rubrum* and *T. interdigitale*. Onychomycosis predominantly caused by *T. rubrum* and *T. mentagrophytes*.

Hair Dermatophytosis

Hair originates from matrix cells in the lower part of the hair bulb and is classified into two types: terminal hair and vellus hair. Terminal hair is thick, pigmented, and contains a medulla, whereas vellus hair is fine, lightly pigmented, lacks a medulla, and is predominantly found on the trunk. Among dermatophyte infections affecting terminal hair, tinea capitis is the most common, representing one of the primary contagious fungal diseases in prepubescent children worldwide, often leading to outbreaks in schools and households. In certain regions, the prevalence of tinea capitis in children can reach up to 91.2%, with an average age of onset around six years and a higher incidence in males [9]. In adults, the prevalence ranges from 1.7% to 15%, with female predominance [10, 11].

Over the past 10 to 20 years, rapid economic development, increased human migration, and a rise in domestic pet ownership, alongside a decline in direct contact with stray animals and livestock, have contributed to significant shifts in the etiological spectrum of tinea capitis in certain regions. The emergence of *M. canis* as a dominant pathogen in Eurasia is a notable example [12] . Between 2019 and 2020, *M. canis* was identified as the most common causative agent of tinea capitis in China, responsible for 65.2% (n=354) of cases [13]. Between 1989

and 2018, *M. canis* was the most frequently isolated dermatophyte in South Korea, accounting for 73.16% of cases [14]. In Japan, anthropophilic dermatophytes had traditionally been the predominant causative agents. However, since the early 1990s, *M. canis* has been increasingly isolated among dermatophyte infections, rising from 2.6% between 1991 and 1995 to 10.2% between 2011 and 2015 [15] . Over the past 30 years, *M. canis* has remained the leading cause of dermatophyte infections in most Central and Southern European countries and Mediterranean regions, with prevalence rates ranging from 37.1% to 98.7% [9].

Another dermatophyte infection affecting human hair is tinea of vellus hair. Eby and Jett first reported a case in 1971, describing a three-year-old girl with a facial vellus hair infection caused by *Nannizzia incurvate* [16]. Tinea of vellus hair results from dermatophyte infection of vellus hair, with or without concurrent glabrous skin involvement. Direct mycological examination of affected hairs often reveals fungal hyphae and/or spores, both endothrix and ectothrix [17]. Despite its existence, few epidemiological studies and treatment reports on tinea of vellus hair have been published. Several factors may contribute to its underdiagnosis, including its rarity, frequent washing, scratching, or topical corticosteroid use—which may alter the typical clinical presentation—and inadequate sampling during direct mycological examination, such as failure to collect vellus hairs for testing [18, 19] . These factors may lead to misdiagnosis as tinea corporis or other superficial dermatophytoses. A report from Spain identified *Nannizzia gypsea* as the most common causative agent of tinea of vellus hair, followed by the *T. mentagrophytes* complex and *M. canis* [20] . In Eastern China, the *T. mentagrophytes* complex was the predominant causative agent, followed by *M. canis* and *N. gypsea* [21]. In Taiwan, the most frequently isolated pathogen was *T. rubrum*, accounting for 43.75% of cases, followed by *M. canis* at 37.50% [22].

Host Specificity of Dermatophyte Infections

Dermatophytes are traditionally classified into anthropophilic, zoophilic, and geophilic species based on their ecological niches and clinical parameters. However, these three categories exhibit overlapping characteristics and variable pleomorphism, which may reflect an evolutionary trend from geophilic species, through adaptation to animal hosts, ultimately leading to specialization in human hosts.

Geophilic dermatophytes are considered ancestral species that primarily inhabit soil, particularly in caves and areas rich in keratinous debris shed by animals. Some studies have reported their prevalence in rodent populations; however, they rarely

cause infections in humans or domesticated animals [23, 24] . The most common geophilic species associated with human and animal infections is *Nannizzia gypsea,* which causes tinea corporis and tinea capitis in humans. Notably, infections with geophilic dermatophytes tend to elicit the most intense inflammatory responses. Geophilic species maintain both mating types and can undergo sexual reproduction under moist soil conditions.

Zoophilic dermatophytes primarily colonize the fur of mammals and may be carried asymptomatically or cause overt infections [25] . Cats serve as the principal reservoir for *M. canis,* while *T. equinum* predominantly infects horses. *Trichophyton mentagrophytes* is commonly associated with rodents [26] , and *T. verrucosum* is frequently found in cattle and other livestock [27]. These animal-associated species pose a high risk of zoonotic transmission to humans through direct contact [28]. When zoonotic infections occur in humans, they often trigger moderate to severe inflammatory responses [1]. Similar to geophilic species, zoophilic dermatophytes maintain two mating types and can undergo sexual reproduction under specific conditions. However, many zoophilic species exhibit highly imbalanced mating-type ratios, with some strains lacking one of the mating types entirely. The loss of a compatible mating partner is believed to be a key evolutionary driver in these species, leading to the dominance of a single genotype. In these cases, asexual reproduction becomes the primary mode of propagation, allowing the retained genotype to outcompete others and persist in the environment [29].

Anthropophilic dermatophytes represent the most evolutionarily advanced group, having adapted to the human immune system. These species generally induce low inflammatory responses, resulting in mild clinical manifestations and chronic infections that can persist for months or even years. Transmission occurs through direct contact in households, schools, and communal settings. However, in individuals with underlying conditions, immune dysregulation, corticosteroid treatment, or genetic CARD9 deficiencies, infections can be severe and prolonged. *Trichophyton rubrum* and *T. interdigitale* are the primary causative agents of tinea corporis and tinea pedis [30], while *T. tonsurans, T. violaceum,* and *M. audouinii* are commonly implicated in tinea capitis in children [31] . Each anthropophilic dermatophyte species identified to date possesses only one mating type, suggesting a complete reliance on asexual reproduction for transmission and persistence in human populations.

Virulence and Pathogenicity of Dermatophytes

The virulence mechanisms of dermatophytes involve multiple factors, including adhesion molecules, cell wall components, keratin-degrading enzymes, pH adaptability, and heat shock proteins (HSPs). These factors work synergistically to enable dermatophytes to bypass host defence mechanisms and establish infections [32]. The first step in dermatophyte infection is adhesion to keratinized host tissues, such as skin, hair, and nails, as adhesion efficiency directly influences the success of infection [33]. Studies have shown that *T. rubrum* adheres to host keratinocytes through carbohydrate-specific adhesion molecules (CSA) on its microconidia, which bind to mannose and galactose residues on the host cell surface, enhancing attachment. *Trichophyton mentagrophytes*, in contrast, extends filamentous projections during adhesion, increasing its contact interface with host tissues [33]. Additionally, dipeptidyl peptidase IV (DppIV) plays an essential role in *T. rubrum* adhesion [34]. The mannan component of the dermatophyte cell wall also acts as a crucial adhesion factor, inhibiting keratinocyte proliferation, thereby facilitating infection persistence. *Trichophyton rubrum* produces significantly more mannan than *M. canis,* which may explain its greater ability to establish chronic infections [35].

The survival of dermatophytes depends on their ability to degrade keratin. These fungi use sulfite efflux pumps (Ssu1) and cysteine dioxygenases (Cdol) to release sulfites, which break disulfide bonds in keratin, disrupting its stable three-dimensional structure [36]. This process creates an entry point for subsequent enzymatic degradation by proteases. Dermatophytes secrete various enzymes to further degrade keratin, including: (i) Keratinases, which specifically degrade keratin into oligopeptides and amino acids [37, 38]; (ii) Serine proteases (Subfamily), such as Sub3 and Sub6, degrade specific peptide bonds in keratin and are highly expressed during early infection [39]; (iii) Metalloproteases (Mep family), such as Mep1-Mep5, which promote keratin degradation in later infection stages and may influence host immune responses [40, 41]; (iv) Leucine aminopeptidases (Lap1, Lap2) and dipeptidyl peptidases (DppIV, DppV), which participate in the final stages of keratin degradation by breaking down peptides into free amino acids for fungal absorption [42].

Dermatophytes can sense and adapt to host tissue pH fluctuations, allowing them to thrive in different environments. While normal skin and nail pH is slightly acidic, keratin degradation releases amino acids, causing a local pH shift toward alkalinity [43]. Studies have demonstrated that dermatophytes regulate pH adaptation through the PacC/Pal signaling pathway, which modulates keratinase and other

virulence factor expression [44, 45]. PacC is a key transcription factor that becomes activated in alkaline conditions, enhancing fungal secretion of more potent degradative enzymes, thereby accelerating infection progression [46]. Furthermore, dermatophytes can alkalinize their microenvironment, creating favorable conditions for their own survival within host tissues.

The dermatophyte cell wall plays a structural role in infection and modulates host immune responses. The cell wall is primarily composed of mannan, beta-glucans, and chitin, among which mannan is closely linked to fungal pathogenicity. Studies have shown that *T. rubrum* contains higher mannan levels than *M. canis*, contributing to its enhanced persistence in chronic infections [35, 47]. Mannan also interferes with keratinocyte proliferation, slowing down skin turnover and aiding fungal colonization while weakening host defenses. Additionally, dermatophytes can manipulate immune recognition by regulating the exposure of beta-glucans, helping them evade immune attacks.

During infection, dermatophytes must adapt to host temperature and other environmental stresses. Heat shock proteins (HSPs) function as molecular chaperones that stabilize fungal proteins under stress and regulate virulence gene expression [48]. Research indicates that Hsp30, Hsp60, Hsp70, and Hsp90 are significantly upregulated in dermatophytes during infection, enhancing fungal resistance to elevated temperatures, oxidative stress, and antifungal agents [49, 50]. Moreover, HSPs may influence fungal growth rates, providing dermatophytes with a competitive advantage in host tissues.

Microsporum canis

The genus *Microsporum* includes one zoophilic species, *M. canis*, and two anthropophilic species, *M. audouinii* and *M. ferrugineum*, which are the primary causative agents of tinea capitis in prepubescent children. Additionally, *M. canis* is frequently implicated in tinea corporis. *Microsporum canis* is carried asymptomatically or symptomatically on the fur of animals, primarily cats and dogs, whereas in humans, it often induces a highly inflammatory reaction on the scalp. In contrast, *M. audouinii* and *M. ferrugineum* are primarily transmitted from human to human, causing mild infections. However, *M. audouinii* has also been isolated from animals, soil, and environmental sources, and cases of intense immune reactions triggered by *M. audouinii* have been reported [51].

Since the 1950s, the epidemiological spectrum of tinea capitis has undergone significant changes, shifting from *T. schoenleinii* and *M. audouinii* to *M. canis* and

T. tonsurans. Today, *M. canis* is the dominant pathogen responsible for tinea capitis in most regions of Europe and Asia, often leading to small-scale outbreaks in schools with animal contact [52]. These outbreaks contribute to zoonotic transmission cycles between animals and humans, as well as human-to-human transmission. In the early 19th century, *M. audouinii* was highly prevalent in Europe and the Americas but later declined, becoming largely confined to Africa [53]. Similarly, *M. ferrugineum* has historically been restricted to China, Iran, and parts of North Africa [54]. However, recent reports indicate a resurgence of these two anthropophilic species in European countries, with new outbreaks being documented [31, 51, 55]. This suggests that the *M. canis* complex, consisting of *M. canis, M. audouinii*, and *M. ferrugineum*, spans multiple continents and remains a major causative agent of tinea capitis today [12].

Molecular Advances in *Microsporum canis*

Despite the distinct phenotypic and ecological differences among the three species within this complex, their close genetic relationship is supported by evidence of sexual reproduction. In 1975, fertile cleistothecia were first observed in mating experiments [56], leading to the classification of the teleomorphic (sexual) state of *M. canis* as *Arthroderma otae* (also referred to as *Nannizzia otae* or *M. canis MAT+*). Monoascospore culture studies further revealed that this fungus is heterothallic, whereas *M. audouinii* and *M. ferrugineum* are considered clonally reproducing species. Since then, an imbalance in Mat locus distribution has been observed in *M. canis*, with *MAT* (-) strains predominantly found in nature. Several researchers, including A. Kaszubiak [57] and Vanbreuseghem [58], have hypothesized that the early evolution of the *M. canis* complex may have occurred in Africa, with wild felines such as lions serving as hosts for the ancestral mating population. The anthropophilic species *M. audouinii* and *M. ferrugineum* likely originated from unknown sexual counterparts of *A. otae*, which may have since become extinct. *Microsporum ferrugineum* remains geographically restricted to certain regions in Asia and Africa, with no evidence of significant genetic variation over time. This suggests that clonal lineages emerged within a single species while sexual recombination events diminished in nature, leading to a substantial reduction or complete cessation of genetic recombination. In the 1970s and 1980s, Hironaga [59], Padhye [59], Weitzman [60], Kubo [61] *et al.* isolated fertile mating strains of *Arthroderma otae* from Japan, producing viable F1 progeny. The isolates VUT-77054 (=CBS495.96, *MAT1-2*) and VUT-77055 (=CBS496.96, *MAT1-1*) were among these fertile strains. VUT-77054 is currently classified as *M. audouinii*. Since these studies, there have been few documented reports of fertile *A. otae*, suggesting that mating

locus imbalances have persisted for a long time, likely restricting sexual reproduction within the *M. canis* complex.

The increasing prevalence of *M. canis* appears to be a sign of its adaptive evolution toward human hosts. Within the phylogenetic framework of the *M. canis* complex, multi-locus sequencing (ITS, *LSU*, *TUB*, and *60S L10*) has proven insufficient in clearly distinguishing the three species. Multi-locus sequence typing (MLST) provides higher resolution, but its correlation with symptoms and host sources remains inconsistent [62]. Zoophilic dermatophytes generally induce more severe inflammatory responses in human infections. However, Mariana *et al.* [37] compared the virulence and thermotolerance-associated enzyme expression profiles of *M. canis* strains isolated from dogs, cats, and humans and found no significant differences in pathogenic effects among these hosts. Additionally, no observable differences were found between symptomatic and asymptomatic feline isolates. Fernanda *et al.* [63] conducted a microsatellite analysis (McGT-13 and McGT-17 markers) to investigate the genetic variability of *M. canis* isolates from different animal species. Their study found no correlation between multi-locus genotypes and clinical or epidemiological factors, including host species, symptoms, clinical presentation, breed, age, sex, living conditions, and geographic origin. Shigeo *et al.* [64] demonstrated that *M. canis* strains from commercial breeding facilities and pet shops exhibit distinct MLMT genotypes compared to those isolated from outdoor environments, suggesting that MLMT typing may be useful for tracking *M. canis* transmission and dispersal patterns in Japan. These findings indicate that, despite the ancestral high virulence of dermatophytes, pathogenicity has gradually diminished over evolutionary time, allowing for enhanced coexistence with animal and human hosts [65].

Pathogenicity of *Microsporum*

Tinea capitis presents in three clinical forms: severe inflammatory - kerion, moderate inflammatory - black dot, and non-inflammatory - gray patch [66]. The zoophilic species *M. canis* is commonly associated with kerion and black dot tinea, while the anthropophilic species *M. audouinii* and *M. ferrugineum* typically cause gray patch tinea. Kerion is characterized by a severe inflammatory reaction, most often triggered by zoophilic *M. canis* species. Clinically, kerion represents a delayed-type hypersensitivity reaction to pathogenic dermatophytes. Children with kerion are more likely to develop regional lymphadenopathy and dermatophytid reactions. A study by Filiz *et al.* [67] found that 68% of patients with kerion celsi exhibited dermatophytid reactions. These dermatophytid lesions present with pruritic, diffuse papules predominantly on the trunk and, in some cases, may

extend to the limbs, neck, and face. Due to their atypical presentation, these lesions are often misdiagnosed as impetigo, contact dermatitis, or atopic dermatitis.

Like other dermatophytes, a range of virulence factors contribute to the ability of *M. canis* to infect human hosts. In *M. canis*, the most critical proteases involved in host invasion are keratinases, fungal proteases, and subtilisins, each exhibiting different levels of activity and secretion. These three enzyme classes have been the most extensively studied in *M. canis* and other dermatophytes, likely due to their high secretion levels and strong activity against skin, hair, and nail components [68]. Studies by Ramos *et al.* [37] and Viani *et al.* [69] reported that *M. canis* isolates from symptomatic cats exhibited significantly higher keratinase production and/or activity compared to those from asymptomatic cats. Subtilisins are among the most important keratinolytic enzymes in *M. canis* [70] . In this species, Sub1 and Sub2 subtilisins have been identified as virulence factors due to their ability to bind to host surfaces via conformational changes at the amino-terminal domain [71]. Sub3 plays a key role in adhesion and is essential for tissue invasion. It is expressed in both conidia and hyphae *in vitro*, as well as *in vivo* in infected cats and guinea pigs [68]. Additionally, these subtilisins appear to be associated with delayed-type hypersensitivity reactions, which may influence host susceptibility and pathogen persistence, thereby leading to chronic infections.

The relationship between hypersensitivity reactions and *Microsporum* infections, as well as the associated risk factors, remains poorly understood. In 1930, Wise and Sulzberger [72] first described the connection between dermatophyte infections and allergic diseases. Since 1973, the association between atopic dermatitis and dermatophytosis has become increasingly recognized, with studies showing that patients with atopic dermatitis are more susceptible to chronic dermatophyte infections. It is estimated that 40% of individuals with chronic dermatophytosis also have atopic conditions such as atopic dermatitis or asthma [73]. Acute dermatophyte infections may act as triggers for atopic dermatitis exacerbations, and antifungal treatment has been shown to improve atopic dermatitis symptoms in affected patients [74-76]. Furthermore, dermatophyte infections can exacerbate asthma symptoms, particularly through immediate hypersensitivity responses to dermatophyte antigens. Oral antifungal therapy has been shown to significantly improve allergic symptoms, indicating a strong link between dermatophytosis and allergic diseases such as asthma, rhinitis, and dermatitis [77, 78]. A study published in 2023 conducted in northern Odisha, India, involving 78,028 patients with dermatophytosis, identified atopic diathesis as a major comorbidity, with a

prevalence of 9.94% [79]. Additionally, 27% of the cases had a family history of dermatophytosis.

Unresolved Questions

Despite some research on the epidemiology, pathogenic mechanisms, and interactions between dermatophytes and the host immune system, several critical questions remain unanswered. One of the most pressing gaps concerns the dual role of dermatophytes as both pathogens and allergens. While studies have shown that dermatophyte infections can induce allergic responses such as atopic dermatitis, allergic rhinitis, and asthma, the specific fungal antigens responsible for these reactions remain poorly characterized. Additionally, the mechanisms by which these antigens modulate host immune responses are still unclear. Cross-reactivity between different dermatophyte species may lead to misdiagnosis, but the lack of standardized fungal allergen extracts complicates accurate diagnosis and clinical management of dermatophyte-related allergies.

Another unresolved issue is the association between pediatric tinea capitis and allergic diseases. Some studies suggest that dermatophyte infections may exacerbate host allergic responses, and children with atopic predisposition appear more susceptible to dermatophyte infections. However, long-term cohort studies are lacking, making it difficult to establish a causal relationship or identify the underlying molecular mechanisms. Additionally, the epidemiology of tinea capitis has shifted significantly over the past few decades, with variations in dominant causative species across different regions. The driving factors behind these changes, including the influence of host genetics, environmental shifts, and social behaviors, remain poorly understood.

Regarding the etiology of tinea of vellus hair, current epidemiological data are limited. Further studies are needed to clarify the causative dermatophyte species, clinical presentation, and misdiagnosis rates associated with this condition. Moreover, the differential keratin degradation capabilities of dermatophyte species on various keratin substrates have not been comprehensively investigated. This knowledge is crucial for understanding host adaptation and interspecies transmission dynamics.

The adaptive evolution of *Microsporum* species, particularly *Microsporum canis*, *M. audouinii*, and *M. ferrugineum*, remains a major unresolved topic. While phylogenetic studies have demonstrated the close genetic relationship between these three species, the molecular mechanisms underlying the shift from a

zoophilic to an anthropophilic lifestyle remain unclear. The adaptation of *Microsporum* species from animal to human hosts is influenced by multiple evolutionary pressures, including microenvironmental differences, immune selection, and changes in transmission dynamics. Human skin has a lower pH (4.5–5.5) compared to the fur of cats and dogs (6.0–7.5) and possesses a more complex lipid composition. The human immune system is also more adept at recognizing zoonotic pathogens, compelling dermatophytes to develop immune evasion strategies. Additionally, the shift from pet-to-human to human-to-human transmission requires adaptation to a lower transmissibility ecological niche. These selective pressures likely drive genome evolution in dermatophytes, yet the specific gene mutations or expression changes responsible for these adaptations remain unknown. It is also unclear whether key host-adaptive enzymes, such as keratinases, have undergone functional modifications. Furthermore, how dermatophytes regulate gene expression in different host environments to accommodate varying immune responses and ecological conditions remains an open question. In summary, how *M. canis* evolves to shift from animal to human hosts while maintaining its dual role as both a pathogen and an allergen requires further investigation.

The molecular mechanisms underlying dermatophyte adaptation to human hosts also remain a critical area of research. Comparative genomics has identified significant differences between zoophilic and anthropophilic dermatophytes in certain protein families, such as zinc finger transcription factors and transmembrane transporters. However, the role of these genes in dermatophyte virulence and host specificity is not yet fully understood. Additionally, the ability of dermatophytes to regulate their growth and invasion in different microenvironments, such as skin, scalp, and nail plates, requires further study.

Finally, the molecular regulation of spore germination remains a significant knowledge gap in dermatophyte pathogenesis. Spore germination is the initial step in host infection and determines colonization and invasion capacity. Previous studies suggest that certain proteases, such as Sub3 and PacC, along with specific signaling pathways and environmental factors, play a role in germination regulation. However, a comprehensive analysis of gene expression changes during different stages of germination is still lacking. It is also unclear how dermatophytes modulate spore dormancy, germination, and invasion in response to host immune defenses, skin barrier integrity, and environmental factors such as pH and temperature.

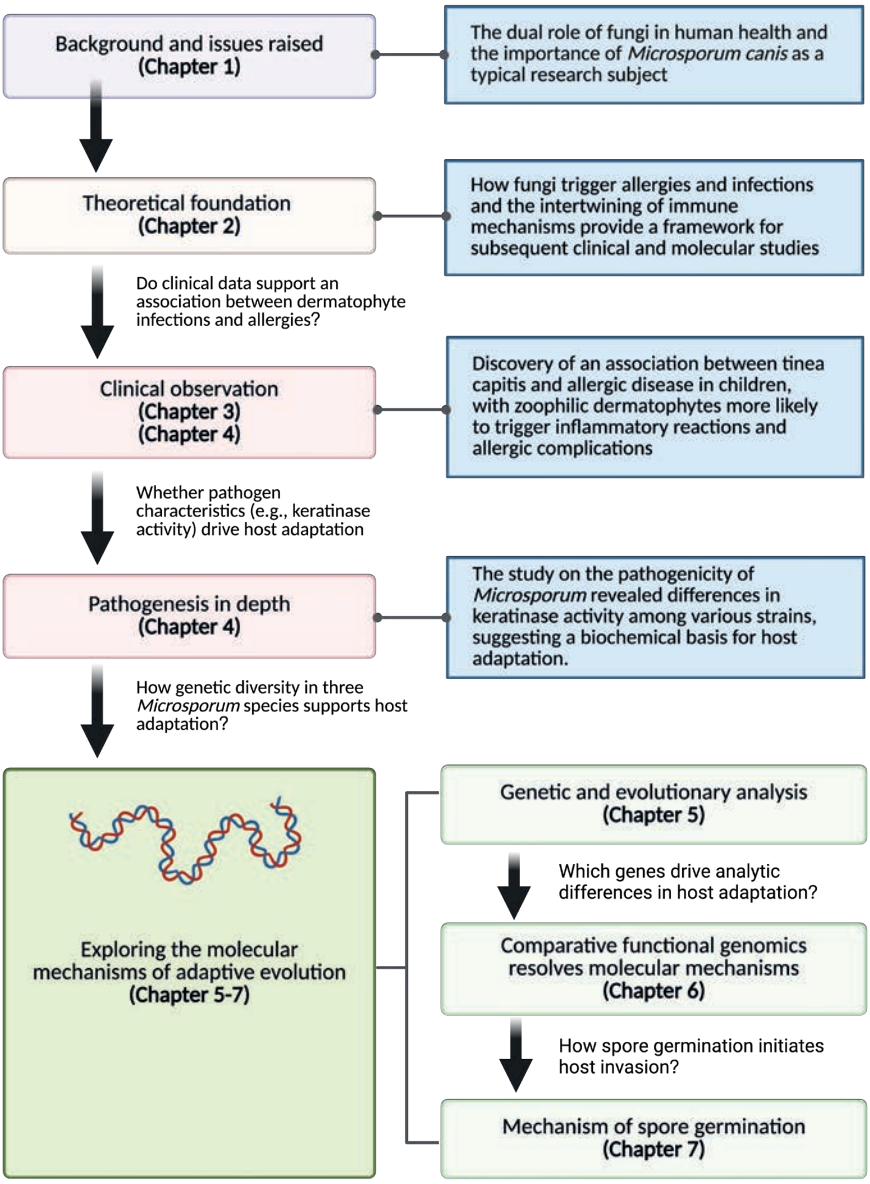
Aim of Thesis

The central research question of this study is how *M. canis* adapts from animal to human hosts through genetic, biochemical, and immunological mechanisms while balancing its pathogenicity and allergenicity.

Specific research objectives include:

a. Clinical correlation studies: Investigate the epidemiological association between *M. canis* infections, such as pediatric tinea capitis, and allergic diseases, as well as identifying risk factors.
b. Pathogen characterization: Analyze the relationship between keratinase activity, DNase activity, and host adaptation in different *M. canis* strains.
c. Evolutionary tracking: Use multi-gene phylogenetics and population genetics to trace the evolutionary shift of *M. canis* across hosts.
d. Molecular mechanism identification: Identify key genes driving host adaptation, including proteases, transport proteins, and transcription factors.
e. Intervention strategies: Develop novel diagnostic and therapeutic targets based on spore germination mechanisms and immune evasion pathways in *M. canis*.

Outline of the Chapters



This dissertation is structured into seven chapters, using *M. canis* as a model to investigate its evolutionary shift from animal hosts (such as cats and dogs) to human hosts and its dual role in infection and allergy. Each chapter explores a

distinct aspect of *M. canis* infection, including its function as both a pathogen and an allergen, its epidemiology, host adaptation, molecular mechanisms of virulence, and spore germination.

In Chapter 2, the discussion begins with how fungi act as both infectious agents and allergens. This chapter reviews immune responses triggered by fungal infections and hypersensitivity reactions induced by fungal exposure, emphasizing the molecular mechanisms underlying fungal antigenicity and cross-reactivity. The challenges in diagnosing fungal-induced allergic diseases are also discussed. Based on existing literature, the epidemiological associations between dermatophyte infections (such as tinea corporis, tinea pedis, and tinea capitis) and allergic diseases, including atopic dermatitis, allergic rhinitis, and asthma, are examined. These studies suggest that fungi may exacerbate allergic diseases through dual mechanisms. However, an unresolved question remains: do current clinical data support a definitive link between dermatophyte infections and allergic diseases? To address this, Chapter 3 presents an epidemiological study investigating the correlation between pediatric tinea capitis and allergic diseases. This study analyzes the clinical presentation, risk factors, and temporal trends of dermatophyte infections over 15 years. The findings indicate that zoophilic strains, such as *M. canis* and *T. mentagrophytes*, are more likely to induce inflammatory responses and allergic complications. Additionally, patients with kerion exhibit immune dysregulation, particularly Th2-skewed immune responses, which may contribute to their allergic phenotype.

A key question arising from these findings is whether specific characteristics of zoophilic pathogens, such as keratinase activity, drive host adaptation and immune polarization. Chapter 4 explores the epidemiology and pathogenesis of dermatophyte infections affecting vellus hair. This study analyzes three *Microsporum* species and their keratinase activity when exposed to various keratin substrates, including feline fur, human scalp hair, vellus hair, and nail keratin. The results reveal significant differences in keratin affinity between zoophilic and anthropophilic *Microsporum* species. But how does genetic diversity within *Microsporum* support human host adaptation? In Chapter 5, the evolutionary relationships between zoophilic *M. canis* and anthropophilic *M. audouinii* and *M. ferrugineum* are examined at the molecular level. Through phylogenetic analysis, population genetics, and mating experiments, this chapter explores the shift from zoophilic to anthropophilic lifestyles and the genetic changes associated with host specificity. These findings provide novel insights into dermatophyte adaptation and the potential for zoonotic transmission.

To further elucidate the key genes driving host adaptation and environmental fitness in *M. canis*, Chapter 6 focuses on the molecular basis of dermatophyte adaptation to human hosts, using *M. canis* as a model. This chapter presents a functional genomic analysis to identify and characterize genes associated with keratin degradation, immune evasion, and environmental adaptation. Comparative studies between zoophilic and anthropophilic species reveal potential mechanisms underlying host shift. Chapter 7 investigates the molecular regulation of spore germination in *M. canis*. By integrating transcriptomic data, this study identifies key signaling pathways controlling spore dormancy, activation, and germination. The impact of environmental factors such as pH, temperature, and nutrient availability on spore viability is also analyzed.

This dissertation integrates epidemiological, molecular, and genomic approaches to advance our understanding of *M. canis*, providing valuable insights into its pathogenic mechanisms, host adaptation, and clinical management.

CHAPTER 2

THE DUAL ROLES OF FUNGI: ALLERGENS AND PATHOGENS

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ABSTRACT

Fungal infections and fungal hypersensitivity represent the host immune system's distinct yet interconnected responses. While fungal infections primarily activate innate immune pathways to combat pathogens, fungal hypersensitivity arises from exaggerated immune responses to fungal allergens. This duality highlights the complex balance between immune defence against infection and the risk of hypersensitivity to fungal antigens. Fungal allergens can elicit allergic reactions within the respiratory, skin, and gastrointestinal tract. Inhalation of airborne spores from fungi such as *Aspergillus*, *Alternaria*, and *Penicillium* frequently leads to respiratory disorders like allergic rhinitis, allergic fungal rhinosinusitis, allergic asthma, allergic bronchopulmonary aspergillosis/mycosis. Cutaneous exposure to fungal allergens such as *Trichophyton can* provoke Id reactions, *Malassezia* and *Candida*, exacerbate atopic dermatitis or induce urticaria. Furthermore, fungal antigens may interact with the gut-associated immune system, potentially triggering or aggravating food allergies. Antibody- and T-cell-mediated hypersensitivity responses are central to these pathophysiological processes, with environmental influences and genetic predispositions modulating the intensity of allergic manifestations, especially in predisposed individuals. Despite the high prevalence of fungal-induced allergic diseases, precise diagnosis remains challenging due to the vast heterogeneity of fungal species and antigenic cross-reactivity. Conventional diagnostic tools, such as skin prick tests and serum total and specific IgE assays, are limited by the lack of standardized fungal extracts and variability in antigenic specificity. This dual role of fungal exposure in infection and hypersensitivity underscores the need to deepen our understanding of the immunopathological mechanisms underlying fungal sensitization, identify specific fungal allergens, and elucidate the molecular pathways involved. These areas are essential for enhancing diagnostic accuracy and developing standardized fungal allergen extracts, thereby facilitating the management and treatment of fungal-induced allergic conditions.

INTRODUCTION

Fungi represent a diverse group of eukaryotic organisms, encompassing micro- and macroorganisms which include yeasts, moulds and mushrooms. They are among the most widely distributed organisms on Earth. While an estimated 1.5 million species of fungi exist, only approximately 60,000 have been formally described. Most fungi are beneficial to humans and play integral roles in everyday life, being extensively utilized in fermentation and pharmaceutical industries despite their potential for pathogenicity. During the last decades, the incidence of fungal diseases has increased. Approximately 800 fungal species have been reported to cause diseases in humans and animals, resulting in a spectrum of clinical presentations that range from asymptomatic colonization and allergic reactions to superficial and life-threatening invasive infections.

The public health impact of fungal-related diseases needs to be adequately addressed. With changes in the living environment and human lifestyles, the World Allergy Organization estimates that allergic diseases impact 30–40% of the global population and have been categorized as experiencing a health concern, imposing a substantial burden on healthcare resources [80]. Fungi are one of the significant sources of sensitization involved in allergic diseases, with estimates of affecting approximately 3–10% of the global population, 20–30% of atopic individuals, and up to 80% of asthma patients [81, 82]. Meanwhile, emerging evidence suggests that fungal disease incidence and geographic distribution are expanding due to global warming and increased international travel and trade. It is estimated that nearly a billion people worldwide suffer from dermatomycoses (fungal infections of the skin, hair, and nails), and that over 300 million individuals of all ages experience serious fungal infections annually. Alarmingly, it is estimated that over 1.5 million of the latter patients succumb to their fungal diseases [83, 84]. In the late 2022s, the World Health Organization (WHO) published a first list of fungal priority pathogens, highlighted fungi of critical or high importance to human health, and called for improved surveillance, research and innovation and public-health interventions [85].

Hence, it is necessary to comprehend the dual roles of fungi in developing allergic diseases and infections. This review aims to elucidate the current understanding of the etiology and pathogenesis of these conditions, with a specific focus on allergic sensitization, fungal allergens, allergenic mechanisms, and their clinical relevance to respiratory and cutaneous allergic diseases. Additionally, it addresses the allergic cross-reactivity between fungi and food, highlighting the urgent need to enhance diagnostic precision and develop standardized fungal allergen extracts.

Such advancements are crucial for the improved management and treatment of fungal-associated allergic conditions.

Hypersensitivity and infection

Fungi are ubiquitous, both *in vivo* and *in vitro*, and interact with humans through inhalation, ingestion, skin contact, and colonization daily. Exposure to high levels of fungi can pose a health threat to humans, leading to fungal hypersensitivity or infections, with certain fungi capable of causing both disorders simultaneously. Fungal infection is caused by the invasion and colonization of host tissues by fungi, where the fungi grow and reproduce within the host. Clearing the infection requires a direct immune response, and infections can become systemic, especially in immunocompromised individuals. Fungal allergy, not due to the invasion of host tissues but rather a response to fungal allergens, is an abnormal or exaggerated reaction to exogenous stimuli which involves various types of hypersensitivity reactions engaging antibodies, immune cell-mediated, tissue-driven, or metabolic mechanisms, and may result in the development of respiratory, skin, eye, gastrointestinal and other symptoms. Hypersensitivity is an undesirable, uncomfortable or damaging response arising from a tissue cell dysfunction or immune system overreaction [86]. According to the damage response framework theory [87], pathogenesis of microorganisms is considered the outcome of host-microorganism interactions. Disease arises when host damage disrupts homeostasis, and different immune states determine the occurrence and nature of the disease. The allergenic proteins of fungi can induce sensitization, giving rise to a broad spectrum of responses such as allergic respiratory disease, allergic rhinitis (AR), allergic fungal rhinosinusitis (AFRS), allergic asthma (AS), allergic bronchopulmonary mycoses (ABPM), hypersensitivity pneumonitis (HSP), cutaneous Id reaction, atopic dermatitis (AD), and urticaria. As fungi are complex eukaryotes, all forms of hypersensitivity reactions can lead to fungal allergies. A combination of different hypersensitivity reactions typically occurs, resulting in a mixed fungal allergy. A brief classification of the various types of hypersensitivity reactions in fungal sensitization and human allergic diseases is presented in Table 1.

Table 1. Types of hypersensitivity and relevant allergic diseases.

	Antibody-mediated reactions			**Cell-mediated reactions**			**Tissue-driven mechanism**
	Type I	**Type II**	**Type III**	**Type IVa**	**Type IVb**	**Type IVc**	**Type V**
Type of inflammatory response	Immediate response	Antibody-mediated cellular cytotoxicity reactions	Immune complex-mediated reactions	Th1 cell-mediated macrophage activation	Th2 cell-mediated eosinophilic inflammation	Cytotoxic T cell-mediated reactions	Barrier damage-mediated inflammation
Inflammation-related cells and factors	B-cell (IgE), ILC2, MCs, BAS, Th2	B-cell (IgM, IgG), NEU, MϕNK	B-cell (IgM, IgG), NEU, MϕBAS, MCs, Platelets	Mϕ, ILC1, NK,Th1, Tc1	EOS, ILC2, MCs, BAS, NK-T, B-cell (IgE), Th2, Tc2	NEU, ILC3, Th17, Tc17	Mϕ, EOS, ILC, Th1, Th2, Th17
	IL-4, IL-5, IL-9, IL-13	C-dependent cytotoxicity, ADCC	Immune complexes, complement	IFN-γ, TNF-α, granzyme B, perforines	IL-4, IL-5, IL-9, IL-13, IL-31, TSLP,	IL-17, IL-22, IL-23	TSLP, IL-25, IL-33
Fungal related allergic diseases	AFRS, AS, ABPM, Urticaria, FFAS, AR	Dermatophytid, ABPA	ABPM, HSP	HSP, Dermatophytid	AFRS, KC, Dermatophytid	AD, Dermatophytid	AFRS, AS, AD

AR, Allergic rhinitis; AFRS, Allergic fungal rhinosinusitis; AS, Allergic asthma; HSP, Hypersensitivity pneumonitis; ABPM, Allergic bronchopulmonary mycosis; ABPA, Allergic bronchopulmonary aspergillosis; FFAS, Fungal food allergy syndrome; AD, Atopic dermatitis; ADCC, antibody-dependent cellular cytotoxicity; B, B-lymphocyte; BAS, basophil; EOS, eosinophil; IFN-γ, interferon-gamma; Ig (E, G, M), immunoglobulin (type E, G, M); IL, interleukin; ILC1/2/3, innate lymphoid cells type 1/2/3; Mϕ, macrophage; MCs, mast cells; NEU, neutrophil; NK, natural killer cell; NK-T, natural killer T-cell; Tc1/2/17, T-cytotoxic lymphocyte type 1/2/17; Th, T-helper lymphocytes; TNF-α, tumour necrosis factor-alpha; KC, kerion celsi.

There is considerable uncertainty regarding the factors determining the relationship between fungal exposure and the development of hypersensitivity reactions or infection. In broad terms, fungi can be classified as either thermotolerant or non-thermotolerant. The latter, such as many species of *Alternaria* and *Cladosporium*, have an optimal growth temperature of 18–22°C and cannot grow at body temperature, thus rarely causing infection but often triggering allergic responses. Thermotolerant fungi, such as *Aspergillus*, *Candida*, and *Mucorales*, exhibit optimal growth within a temperature range of 20–50°C; this adaptability to both environmental and body temperatures supports their potential to colonize the respiratory system, increasing the risk of sensitization and infection [88].

To date, over 30 fungal genera have been identified as producers of allergens [82], primarily belonging to *Ascomycota* (e.g., *Alternaria*, *Aspergillus*, *Candida*, *Penicillium*, and *Trichophyton*), *Basidiomycota* (e.g., *Malassezia*, *Trichosporon*, *Ustilago*, and *Rhodotorula*), and Mucorales (e.g., *Mucor* and *Rhizopus*). Moreover, members of approximately 60 genera belonging to the above three phyla are implicated in developing fungal infections. Dermatophytes (including *Trichophyton*, *Microsporum*, and *Epidermophyton*) and *Malassezia* species primarily cause superficial infections. Notably, species such as *Candida albicans*, *Can. auris*, *Can. glabrata*, *Can. krusei*, *Can. tropicalis*, and *Can. parapsilosis*, *Aspergillus fumigatus*, *Cryptococcus neoformans*, *C. gattii*, and *Pneumocystis jirovecii* are responsible for more than 90% of reported deaths due to invasive fungal diseases, representing half of the fungi in the WHO fungal priority pathogens list [89, 90]. A typical example of patterns of fungus-host interaction can be observed in the thermotolerant fungus *Asp. fumigatus* (Figure 1). This fungus can cause allergic diseases of the upper and lower airways, chronic pulmonary aspergillosis, and *Aspergillus* bronchitis in non-immunocompromised individuals, as well as invasive infections in immunocompromised individuals [91]. However, another medically relevant species, *Can. albicans*, which is a thermotolerant commensal and a common cause of IgE sensitization, does not appear to induce the same disease pattern as *Asp. fumigatus* [92]. *Malassezia*, another skin-resident colonizer, is not thermotolerant but equally capable of causing superficial fungal disease and skin sensitization liberating allergens; the reasons for this discrepancy are unclear. *Trichosporon asahii* is a basidiomycetous yeast-like fungus that causes severe disseminated disease in patients with neoplastic disease. It is also the most common allergen-causing summer-type HSP in Japan (Walsh *et al.*, 1986; Matsunaga *et al.*, 2003).

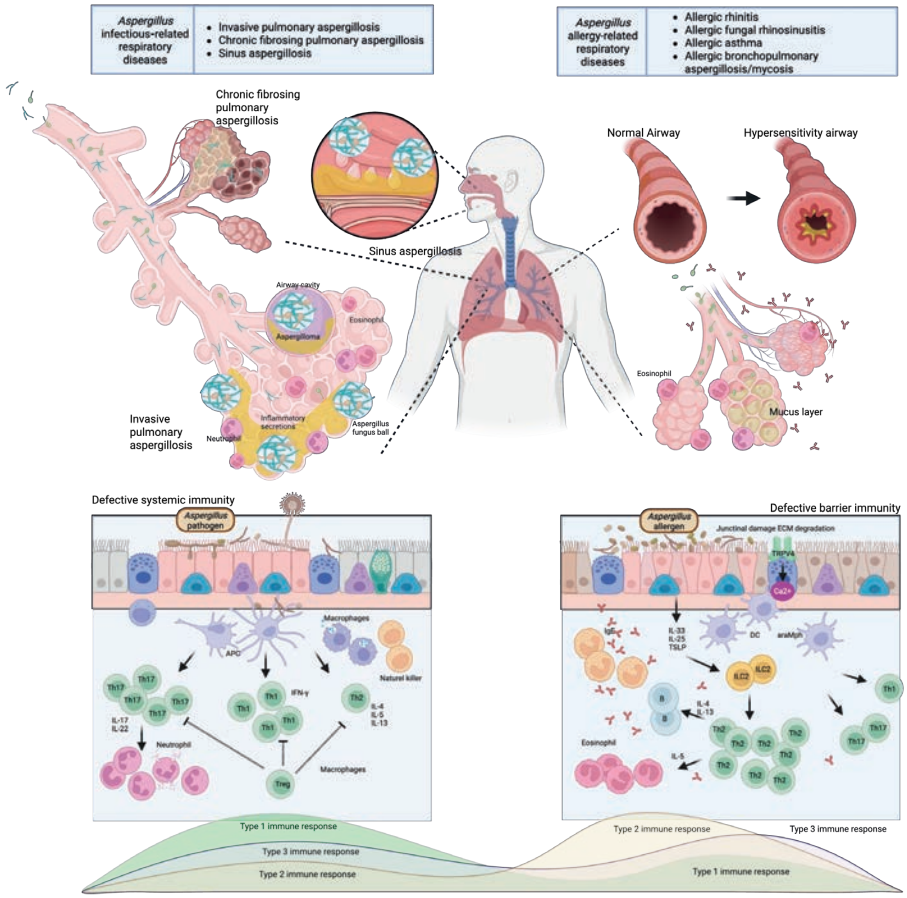

Figure 1. Example of fungus-host interactions involving inhaled *Aspergillus* in humans' hypersensitivity reactions and infections. *Aspergillus*-related respiratory diseases and immune responses. The left panel illustrates infection-related respiratory diseases (e.g., invasive pulmonary aspergillosis, chronic fibrosing pulmonary aspergillosis, and sinus aspergillosis) due to *Aspergillus* invasion. The right panel shows allergy-related respiratory diseases (e.g., allergic rhinitis, allergic fungal rhinosinusitis, allergic asthma, and allergic bronchopulmonary aspergillosis) triggered by *Aspergillus* allergens. The gradient at the bottom reflects the dominance of Type 1, Type 2, and Type 3 immune responses in various *Aspergillus*-related respiratory conditions.

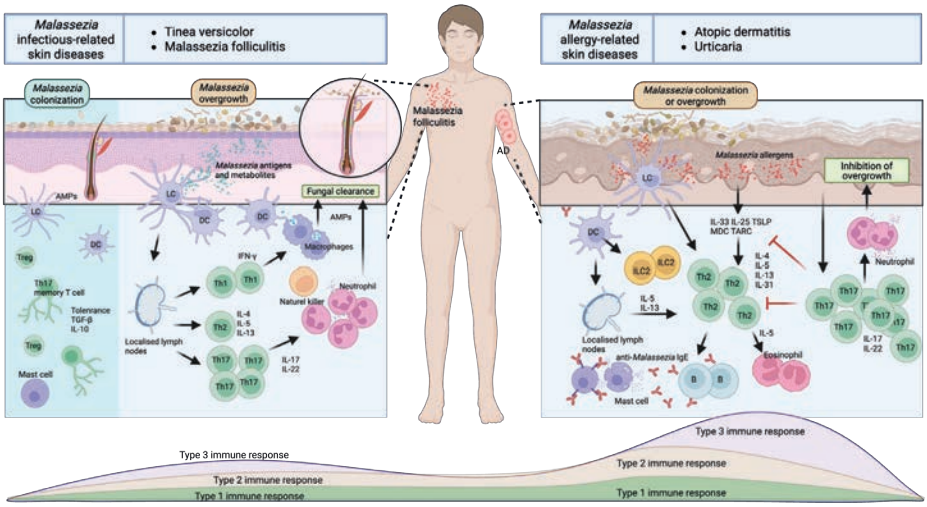

Figure 2. Example of fungus-host interactions with commensal cutaneous *Malassezia* involved in hypersensitivity reactions and infections in humans. *Malassezia*-related skin diseases and immune responses. The left panel represents infection-related skin diseases (e.g., pityriasis versicolor and *Malassezia* folliculitis) caused by *Malassezia* colonization or overgrowth. The right panel represents allergy-related skin diseases (e.g., atopic dermatitis and urticaria) characterized by *Malassezia* allergen-induced immune activation. At the bottom, the gradient indicates the relative activation of Type 1, Type 2, and Type 3 immune responses across different *Malassezia*-related skin disease scenarios.

Fungal allergens

Most allergens are proteins or glycoproteins with molecular weight ranging from 5,000 to 100,000 Da, although polysaccharides and low-molecular-weight substances may also exhibit allergenic properties. Fungi are considered complex sources of allergens, with their allergenic molecules located in spores and mycelia. The WHO and the International Union of Immunological Societies (WHO/IUIS) maintain the primary allergen database in the field of allergies, which currently contains 120 component allergens from 31 different fungal species (https://allergen.org/). Among these, 95 belong to ascomycetous species, 23 to basidiomycetes, and two to *Mucorales* (Table 2). The order *Eurotiales* includes 55 allergens from *Aspergillus* (n = 38) and *Penicillium* (n = 17), and the order *Pleosporales* comprises 18 allergens from *Alternaria* (n = 12), *Epicoccum* (n = 1), *Ulocladium* (n = 1), and *Curvularia (*n = 4) species. The basidiomycetous yeast *Malassezia* accounts for 13 allergens, and the ascomycetous yeast *Candida* contains three allergens. Compared with the increased number of allergens from plant and animal sources, the number of fungal allergens has remained relatively static, except in 2009, when 23 new fungal allergens were added to the database [93].

Fungal allergens have historically received little attention, mainly due to their undetermined regional and seasonal distribution, which complicates establishing a clear association with disease exposure. Furthermore, the allergenic potential of sensitizing fungi is complex. Fungi inherently exhibit variability, and different strains, culture media, growth temperatures, and culture durations, and various extraction methods can influence the allergenicity of extracts. Furthermore, different antigenic determinant clusters may exist within the same fungal species, and cross-reactions of allergens can directly impact the accurate identification of the actual allergen. Moreover, fungi can induce diverse allergic reactions simultaneously.

Allergic cross-reactivity is widely observed among fungal species, stemming from structural similarities in exposed surface areas that form allergen epitopes. Generally, the closer the taxonomic affinities between species, the larger the degree of structural and immunological similarity between the allergens – although some allergens are specific to certain species, genera, or families. For example, the primary fungal allergen Asp f 2 of *Aspergillus fumigatus*, a cell wall-associated extracellular protein, is considered a genus-specific allergen, with homologous, cross-reactive molecules found in other species of the genus *Aspergillus*. Alt a 1 is a family-specific major allergen for the common outdoor mould *Alternaria alternata*, and more than 80% of *Alternaria*-allergic patients are sensitive to this allergen. Allergic cross-reactivity has been observed in closely related species, such as *Stemphylium botryosum* and *Ulocladium chartarum*, which belong to the same family *Pleosporaceae*. Allergens cross-reactive with Alt a 1 are absent from the more distantly related allergen sources, such as members of genera *Cladosporium* and *Aspergillus*. Interestingly, allergic cross-reactivity between *Alt. alternata* allergens and food allergens, such as with edible mushrooms and spinach, has been reported as *Alternaria*-spinach syndrome [94]. Selected fungal allergens and allergic cross-reactivity data are shown in Table 2.

Table 2. Fungal allergens and cross-reactivity.

Species	Number allergens	Selected example of allergen	Protein type	Potential cross-reactive allergens	Relevant allergic diseases	Relevant infectious diseases	Major infectious virulence factors
Alt. alternata, *Alt. chrysanthemi*, *Alt. brassicae*, *Alt. carotiincultae*, *Alt. oregonensis*, *Alt. sonchi*, *Alt. tenuissima*	12	Alt a 1	Glutathione-S-transferase	Alt b 1 *Alt. brassicicola* a.o.Ulo c 1 *Ul. chartarum*	AR, AS	Paranasal sinusitis (Didehdar et al., 2021),Ocular infections (Colosi et al., 2023), (Sub)cutaneous infections (Ando & Takatori, 1987)	Tripeptidyl-peptidase 1,TSPc domain-containing protein, thick cell wall, toxins, proteases, enzymes, ROS (Strader et al., 2024)
		Alt a 3	HSP 70	Pen c 19 *P. citrinum*Mala s 10 *M. sympodialis*			
		Alt a 8	Mannitol dehydrogenase	Cla h 8 *Cl. herbarum*			
		Alt a 15	Vacuolar serine protease	Cur l 4 *Cu. lunata*Cla h 9 *Cl. Herbarum* a.o.Pen o 18 *P. oxalicum* a.o.Tri r 2 *T. rubrum* (Abel-Fernandez et al., 2023)			

Table 2. (continued)

Species	Number allergens	Selected example of allergen	Protein type	Potential cross-reactive allergens	Relevant allergic diseases	Relevant infectious diseases	Major infectious virulence factors
Asp. fumigatus, Asp. flavus, Asp. niger, Asp. Oryzae	38	Asp f 3	Peroxysomal protein	Mala f 3 *M. furfur*Pen c 3 *P. citrinum*	AR, ABPM, AS, HSP	Pulmonary aspergillosis (Seif et al., 2022)Sinusitis (Singh et al., 2013)	Afmp1, Hydrophobic protein Rod A, Fumagillin, Gliotoxin,Helvolic acid, Fumigaclavin C, Asp-hemolysin (Gu et al., 2021)
		Asp f 6	Mn superoxide dismutase	Mala s 11 *M. sympodialis*Psi c 2 *P. cubensis*			
		Asp f 8	Ribosomal protein P2	Cla h 5 *Cl. herbarum* Alt a 5 *Alt. alternata*			
		Asp f 22	Enolase	Pen c 22 *P. citrinum*Alt a 6 *Alt. alternata*Cla h 6 *Cl. herbarum*(Abel-Fernandez et al, 2023; Strader et al., 2024)			
Can. albicans, Can. boidinii	3	Cand a 3	Peroxysomal protein	Asp f 3 *Asp. fumigatus*Pen c 3 *P.citrinum* (Fukutomi & Taniguchi, 2015)	AD, AS	Candidiasis (Villar & Dongari-Bagtzoglou, 2021; Lu et al., 2023)	Hsp 90 (Robbins & Cowen, 2023)Adhesin and invasin in Als and Hwp1 families (Lopes & Lionakis, 2022)

Table 2. (continued)

Species	Number allergens	Selected example of allergen	Protein type	Potential cross-reactive allergens	Relevant allergic diseases	Relevant infectious diseases	Major infectious virulence factors
Cl. herbarum, Cl. cladosporoides	10	Cla h 5	Acid ribosomal protein P2	Alt a 5 *Alt. alternata*Asp f 8 *Asp. fumigatus*Fus c 1 *F. culmorum*	AD, AS, Urticaria, HSP	Keratitis(Pritchard & Muir, 1987)Cutaneous infection	/
		Cla h 6	Enolase	Alt a 6 *Alt. alternata*Asp f 22 *Asp. fumigatus*Cur l 3 *Curvularia lunata*			
		Cla h 12	Acid ribosomal protein P1	Alt a 12 *Alt. alternata*(Abel-Fernandez et al., 2023; Strader et al., 2024)			
Cu. lunata	4	Cur l 2	Enolase	Alt a 6 *Alt. alternata*	AR, AS	Respiratory tract,cutaneous, corneal (Carter & Boudreaux, 2004)	/
		Cur l 3	Cytochrome C	Asp f 22 *Asp. fumigatus*Pen c 22 *P. citrinum*(Abel-Fernandez et al., 2023; Strader et al., 2024)			
E. purpurascens	1	Epi p 1	Serine protease	Cur l 1 *Cu. lunata* (Simon-Nobbe et al., 2008)	HSP, AS	Cerebral (Charron et al., 2022)Sepsis after transplant(Suchitra et al., 2012)	/

Table 2. (continued)

Species	Number allergens	Selected example of allergen	Protein type	Potential cross-reactive allergens	Relevant allergic diseases	Relevant infectious diseases	Major infectious virulence factors
F. culmorum, F. solani	4	Fus c 2	Thioredoxin-like protein	Cop c 2 *Coprinus comatus*(Mari et al., 2009)	AS, FFAS	/	RodA protein,Homolog of ceruloplasmin,PacC/Rim1 (Abbondante et al., 2023)
P. citrinum, P. chrysogenum, P. oxalicum	17	Pen c 13 Pen c 19	Alkaline serine protease HSP 70	Pen c 2 *P. citrinum*Asp f 13 *Asp. fumigatus* Alt a 3 *Alt. alternata*Mala s 10 *M. sympodialis*(Abel-Fernandez et al., 2023; Strader et al., 2024)	AR, ABPA, AS	Urinary tract (Perry, 1964)Pulmonary (Zhao et al., 2020)Endophthalmitis (Garg et al., 2016)	/
T. rubrum, T. mentagrophytes	4	Tri r 2 Tri t 4	Secreted alkaline protease Alp1 Serine protease	Alt a 15 *Alt. alternata*Cur l 4 *Cu. Lunata* Asp f 13 *Asp. fumigatus*Cla c 9 *Cl. cladosporioides*(Mari et al., 2009; Abel-Fernandez et al., 2023; Strader et al., 2024)	AR, AS	Tinea corporisOnychomycosis (Deng et al., 2023)	Keratinolytic enzyme (Nenoff et al., 2014)

Table 2. (continued)

Species	Number allergens	Selected example of allergen	Protein type	Potential cross-reactive allergens	Relevant allergic diseases	Relevant infectious diseases	Major infectious virulence factors
M. furfur, M. sympodialis	13	Mala f 3	Peroxysomal membrane protein	Asp f 3 *Asp. fumigatus*Pen c 3 *P. citrinum*	AD, Urticaria	FolliculitisPityriasis (Pedrosa et al., 2014)	Aspartyl protease (Goh et al., 2022) SMG1 (Guo et al., 2015)
		Mala s 13	Thioredoxin	Asp f 28 *Asp. fumigatus*Cop c 2 *Coprinus comatus*(Mari et al., 2009; Strader et al., 2024)			
Rhizopus oryzae	2	Rhi o 2	Cyclophilin	Mala s 6 *M. sympodialis*Asp f 27 *Asp. fumigatus* (Mari et al., 2009)	ABPM	Mucormycosis (Ervens et al., 2014)	CotH2(Ibrahim et al., 2012)CotH3 (Gebremariam et al., 2014)

a.o., and other members of the same genus; *Alt., Alternaria; Asp., Aspergillus; Can., Candida; Cl., Cladosporium; Cu., Curvularia; E., Epicoccum; F., Fusarium; M. Malassezia; P., Penicillium; T., Trichophyton.*

Fungal allergy and host immune mechanism

Fungal sensitization has been associated with hypersensitivity reactions, with variable levels of evidence available to link types of fungi with clinical conditions. The fungal cell wall is essential for growth and survival and is essential for cell viability, morphogenesis, and pathogenesis. The cell wall must be robust but flexible, protective and shielded yet porous to nutrients and membrane vesicles and receptive to exogenous signals. Its composition must be highly regulated in response to environmental conditions and imposed stresses. The cell wall is mainly composed of polysaccharides (https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/polysaccharide), such as glucans (β- and α-linked), chitin (https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/chitin), chitosan, mannan, and galactosaminogalactan [95, 96], which induce innate and adaptive immune responses. The impairment of host epithelial barrier function due to microbial dysbiosis, resulting in biodiversity loss, a reduction of symbiotic organisms, and the colonization by opportunistic pathogens, is a prerequisite for initiating epithelial immune responses [97]. The nature of these immune responses varies depending on the location (e.g., skin, airways, intestines). For example, airway biopsies from patients with asthma reveal patchy disruptions in tight junctions and decreased expression of epithelial markers such as zonula occludens-1 (ZO-1), E-cadherin, and α-catenin [98]. AR patients exhibit reduced transepithelial resistance, increased permeability to FITC-dextran 4 kDa, and decreased expression of claudin and ZO-1, both *in vitro* and *in vivo*. In AD patients, genetic and/or acquired epidermal barrier dysfunction plays a critical role in the pathogenesis. In the skin of patients with chronic urticaria, genes associated with terminal epidermal differentiation and barrier function are overexpressed compared to healthy control skin [99], where increased filaggrin expression reduces skin barrier formation through the activation of histamine receptor 1 [100]. These disruptions in skin barriers facilitate the penetration of microbial allergens into the skin and promote allergic sensitization. Fungal allergens, including proteases, protease activity receptors, glucans, and membrane receptors, chitosanases, glycosidases, ribosomes, mycotoxins, and volatile organic substances, can cause mainly type I, III, and IV allergic reactions [101-103]. Innate and adaptive immune responses can be categorized into three types based on the involvement of T-cells and innate lymphoid cells (ILCs). Type 2 immunity or allergic inflammation comprises innate and adaptive immune arms [104]. Adaptive type 2 immunity (Type IVb) is characterized by the induction of T-helper 2 (Th2) cells and B-cell class switch recombination towards IgE antibodies that bind to FcεR1 on mast cells (MCs) and basophils (BAS), resulting in anaphylactic reactions upon secondary exposure to allergen.

Table 3 . The difference of inflammatory mechanism in different type 2 inflammatory diseases and the main targeted therapeutic drugs

Location of Involvement	Diseases	Key inflammation-related cells and factors	Major targeted biologics
Upper respiratory tract	AR	B cell (IgE), MCs, BAS, IL-4, IL-5, IL-9, IL-13	Dupilumab, Omalizumab (Wise *et al.*, 2023; Zhang *et al.*, 2022)
	AFRS	B cell (IgE), ILC2, MCs, BAS, Th2, IL-4, IL-5, IL-13	Benralizumab, Dupilumab,Omalizumab (Chua *et al.*, 2023; Luong *et al.* 2022)
Lower respiratory tract	AS	B cell (IgE), EOS, ILC2, EOS, Th2, IL-4, IL-5, IL-9, IL-13, TSLP, IL-25, IL-33,	Benralizumab, Etokimab, Omalizumab, Reslizumab, Tezepelumab, Tralokinumab (Breiteneder *et al.*, 2024; Caminati *et al.*, 2024)
	ABPA / ABPM	B cell (IgM, IgG), MC, EOS, Mϕ, NEU, BAS, NK, Th2, IL-4, IL-5, IL-9, IL-13, IL-31, TSLP	Benralizumab, Dupilumab, Mepolizumab, Omalizumab, Tezepelumab (Agarwal *et al.*, 2024)
	HSP	B cell (IgM, IgG, CD20+), NEU, MϕBAS, MCs, Platelets, ILC1, NK,Th1, Tc1, IFN-γ, TNF-α	Rituximab (Morisset *et al.*, 2017; Ferreira *et al.*, 2020)
Skin	AD	Mϕ, EOS, ILC2, Th1, Th2, Th17, IL-4, IL-5, IL-13, IL-33, IL-17, TSLP, IL-25, IL-33	Abrocitinib, Baricitinib, Dupilumab, Etokimab, Ligelizumab, Nemolizumab, Tralokinumab, Upadacitinib(Müller *et al.*, 2024)
	Urticaria	B cell (IgE), MCs, Mϕ, BAS, Th2, IL-4, IL-17	Barzolvolimab, Dupilumab, Fenebrutinib, Ligelizumab, Omalizumab, Remibrutinib, Tezepelumab (Casale *et al.* 2022; Kolkhir *et al.*, 2022; Paller *et al.*, 2024)
Digestive tract	FFAS	Th2, IgE, IL-4, IL-13, IL-33	Acalabrutinib, Dupilumab, Etokimab, Omalizumab(Sindher *et al.* 2022)

Type 2 immunity is intricately linked to fungal sensitization and predominantly involves two signaling pathways [105]: (i) Following fungal exposure, antigen-presenting cells migrate to regional lymph nodes, process and present antigens to naive $CD4^+$ T-cells, thereby initiating their activation and differentiation into Th2 cells, which subsequently secrete type 2 cytokines, including interleukin (IL)-4, IL-5, and IL-13; (ii) Fungal allergens stimulate epithelial cells to release IL-33, IL-25, and

thymic stromal lymphopoietin (TSLP), which activate ILC2, promoting Th2 cell differentiation and the secretion of type 2 cytokines. IL-4, IL-5, and IL-13 affect B-cells, EOS, BASs, MCs, and macrophages (Mϕs), resulting in eosinophilic inflammation, goblet cell hyperplasia, and airway remodeling, among other pathological alterations (Figure 1-2). Table 3 lists the difference of inflammatory mechanism in different type 2 inflammatory diseases and the main targeted therapeutic drugs.

Innate immune responses to fungal allergens

The innate immune system is critical for proper host defense against opportunistic pathogens. Innate cells, e.g., Mϕs, neutrophils (NEUs), dendritic cells (DCs), and eosinophils (EOS) are equipped with an intricate cell machinery to detect fungi and facilitate killing, to recruit additional immune cells, and to direct the adaptive immune responses [106, 107]. Additionally, the innate immune system is well equipped to recognize invaded fungi through specialized cells expressing a broad range of pattern recognition receptors (PRRs). Several families of PRRs are grouped according to phylogeny, structure, and function. Toll-like receptors (TLRs), C-type lectins (CLRs), nod-like receptors (NLRs) and RIG-I-like receptors (RLRs) are the critical families involved in antifungal immunity. Innate immune cells recognize the different components of the fungal cell wall and release pro-inflammatory factors that vigorously promote Th2 cell-mediated reactions, thereby exacerbating hypersensitivity symptoms.

β-Glucan is an essential molecule for the recognition of fungi by the immune system. Evidence indicates that persistent exposure to β-(1,3)-glucan can lead to respiratory allergic symptoms and airway inflammation. Signaling through Dectin-1, the receptor for β-glucan, triggers a pathway that exists in a Syk-independent manner, and Raf-1 kinase activation leads to the activation of an alternative pathway of NF-κB through the NF-κB subunit RelB, which regulates cytokine production and Th differentiation characteristics (Gringhuis *et al.*, 2009). NF-κB has been shown to attach to two enhancer sites in the IL-4 motif and to contact nuclear factor-activated T cells (NFAT) to stimulate their induction, in addition to being involved in the expression of GATA3, a significant regulator of Th2 differentiation. Zhang *et al.* [108] reported that fungal exposure enhanced allergen-driven Th2 cell-mediated response, promoting severe AS, which was independent of fungal sensitization and could be reconstituted with β-glucan and abrogated by neutralization of IL-17A, especially in severe steroid-resistant asthma. β-(1,3)-glucan comprises a group of polysaccharides with a natural origin in fungi but also in plants, bacteria, algae, mites, *etc.* Recently, Kanno *et al.* [109] reported that plants

containing β-(1,3)-D-glucan in Japanese cedar pollen could act as adjuvants for allergen-specific IgE production via Dectin-1. β-(1,3)-glucan is frequently detected in house dust mite (HDM) feces. Hadebe *et al.* [110] reported that highly purified β-glucan exacerbated HDM-induced eosinophilic, Th2 cell-mediated airway responses by acting as an adjuvant, promoting activation, proliferation, and polarization of HDM-specific T-cells (1-Derβ T-cells). Oluwole *et al.* [111] examined the association between indoor endotoxin and β-(1,3)-D-glucan exposure and asthma severity in children. The authors reported that higher mattress endotoxin concentrations were associated with increased odds of moderate/severe asthma, whereas higher β-(1,3)-D-glucan loads in play areas were inversely associated with moderate/severe asthma. However, a higher β-(1,3)-D-glucan concentration was not associated with a lower forced vital capacity (FVC) or forced expiratory volume in 1 second (FEV1). The effects of different types of β-glucans, exposure routes and exposure times on allergies remain unclear and need further study.

Chitin is an essential cell wall component of all pathogenic fungi and affects protozoa, arthropods, and nematodes. Fungal chitin is a linear polymer composed of N-acetylglucosamine residues linked by β-(1,4)-glycosidic bonds. Chitin is known to act as a potential allergy-promoting pathogen-associated molecular pattern (PAMP) that elicits strong innate immune responses, as well as an immune adjuvant that enhances the adaptive immune response [112-114]. Previous studies have shown that the size of the chitin particles is important for the type of response elicited by the immune system. Particle sizes above 70 μm do not appear to be immunogenic, whereas large particles (>40−70 μm) have been reported to induce a classical Th2 'allergic' response. Chitin particles were shown to induce innate eosinophilic infiltration into the lungs of mice mediated by the release of epithelial cell-derived cytokines, such as TSLP, IL-25, and IL-33, with subsequent activation of innate ILC2s. Small particles (≤40 μm, especially 1−10 μm) induced protective Th1 and anti-inflammatory responses. Research has shown that intravenous administration of fractionated chitin particles (1−10 μm) into the lung activates alveolar Mϕs to express cytokines such as IL-12, tumour necrosis factor (TNF)-α, and IL-18, leading to INF-γ production mainly by natural killer cell (NK) [115, 116]. Humans with AS sensitized to fungi often have more severe symptoms than those who are not sensitized. Cell wall chitin has been implicated as a pathogenic factor in AS. Ellis *et al.* [117] demonstrated that chronic exposure to fungal chitin alone cannot modulate lung function, even in the presence of differential lung chitinase activity. Chitin-positive *Aspergillus fumigatus* conidia are detectable in the allergic lung during chronic exposure. Lung function in mice deficient in AMCase (*Chia*$^{-/-}$) and overexpressing AMCase (SPAM) is directly correlated with the level of chitinase activity during chronic fungal exposure [117]; however, chronic exposure of normal

mice to purified *Asp. fumigatus* chitin resulted in only moderate inflammatory changes in the lungs that were not sufficient to induce airway hyperreactivity (AHR). Moreover, despite dramatic differences in chitinase activity, chronic exposure of *Chia*$^{-/-}$and SPAM mice to purified *Asp. fumigatus* chitin likewise did not modulate AHR.

Proteolytic enzymes play roles in fungal physiology and development. Proteases such as extracellular serine, aspartic, and metalloproteases are considered virulence factors of many pathogenic species. Research has shown that proteases act as pathogenic agents in fungal infection and in allergic disorders. Proteases from fungi induce inflammatory responses by altering the permeability of the epithelial barrier and by inducing proinflammatory cytokines through protease-activated receptors (PARs), which are components of the innate immune response that are modulated by proteolytic activity and are involved in potentiating Th2 responses. A recent study revealed that the fungal allergen Asp f 13 damaged airway epithelial tight junctions and E-cadherin [118]. In bronchiolar club cells, a distinct subset of epithelial cells, this junction injury triggered a calcium influx sensed by the mechano-sensor TRPV4, leading to allergic inflammation [119]. Fungal protease allergens can selectively promote the fibrinogen cleavage product-TLR4 axis [120]. Activated and/or damaged epithelium produces intracellular ROS, leading to the release of an extensive collection of proinflammatory cytokines (IL-6, GM-CSF, TSLP, and IL-25), Damage-associated molecular patterns (IL-1α, IL-33, Uric acid, and ATP) and chemokines (CCL2) which activate ILC2 and cDC2 cells. IL-4 produced by activated ILC2s initiates Th2 differentiation. The production of IL-4, IL-5, and IL-13 by Th2 cells and ILC2s induces the production of allergen-specific IgE, the associated sensitization/degranulation of BAS/MCs, and EOS recruitment. The contact of protease allergens with immune cells, facilitated by the epithelial barrier degradation, allows the cleavage of surface receptors on these target cells, optimizing the Th2-biased inflammatory response. Matrix metalloproteinases (MMP) are other protein hydrolases that play important roles in various physiological and pathological processes, regulating host barrier function, inflammatory cytokine and chemokine activity, and the production of chemokine gradients. Excessive activity of MMP-2 and MMP-9 may contribute to the bronchial wall in the late stages of allergic airway inflammation and abnormal tissue remodeling with adverse effects. MMP-2-deficient mice had significantly increased numbers of inflammatory cells in the lungs and higher levels of Th2 cytokine expression compared to allergic wild-type mice. Greenlee *et al.* found that MMP-2 cleaves and produces fragments of Th2 chemokines, including CCL7, CCL11, CCL17, MCP-3, eosinophil chemokine-1, and thymic and activation-regulated chemokines (TARC), which play critical roles in

inducing allergic inflammation under asthmatic conditions. In addition, matrix metalloproteinases affect macrophages' M1/M2 polarization.

In the list of allergens from the WHO/IUIS allergen database, more than 60 allergens are classified as proteases or peptidases based on their protein sequence/structural similarities with archetypal serine, cysteine, aspartic or metalloproteases. A limited number of allergenic sources contain protease allergens: HDMs, cockroaches, fungi, pollen, fruits, and bee venoms. Protease allergens, primary sensitizers with IgE binding frequencies higher than 50%, are usually stable and have abundant proteins in their allergenic sources. Allergenic fungal proteases can act as adjuvants, potentiating responses to other allergens. Proteolytic enzymes from fungi contribute to inflammation through interactions with the kinin system and the coagulation and fibrinolytic cascades [118, 121].

Adaptive immune responses to fungal allergens

At the beginning of the 21st century, the European Academy of Allergology and Clinical Immunology (EAACI), in an effort to standardize the nomenclature of allergies, provided a new classification of allergic and nonallergic hypersensitivities, mediated by adaptive immune responses (allergen-specific antibodies or lymphocytes) and by other (innate) mechanisms, respectively [122]. The concepts have been continuously updated and are currently extended into nine types comprising antibody- (I-III), cell-mediated (IVa-c), tissue-driven mechanisms (V-VI) and direct response to chemicals (VII) [86]. In addition to innate immune responses, the adaptive immune mechanisms of fungal allergies include mainly type I (IgE-dependent reactions), type III (IgG/IgM-mediated hypersensitivity), type IV (T-cell-mediated), and type V (epithelial barrier defect) immune responses (Table 1), but a combination of different hypersensitivity reactions as a mixed type may appear in the clinical setting. Mould spores are among the classical allergens that initiate type I hypersensitivity, which may be responsible for allergic reactions in patients with AR, AS, AD, acute urticaria/angioedema, and food allergy. The IgE-dependent allergies are Th2-driven. The Th2 branch of the adaptive immune system favors $CD4^+$ Th2 cells, EOS, BAS, MCs, and type 2 innate lymphoid cells, as well as the production of cytokines such as IL-4, IL-5, IL-9, and IL-13 and humoral antibody responses of the IgE isotype [123]. Allergic reactions to ABPM and AFRS include types I, III and IV. The hallmark feature of both diseases is eosinophil infiltration into the airway mucosa caused by localized type 2 inflammation and concomitant viscid secretions in the airways. Patients may show a cutaneous hypersensitivity to specific allergens, specific IgE and IgG antibodies, and elevated total IgE levels.

Hypersensitivity pneumonitis is based on type III/IV allergic reactions to repeated inhalation of allergens and may lead to chronic disease with irreversible lung damage [124]. A type III hypersensitivity reaction involving immune complex deposition and subsequent inflammation, is suspected in the acute inflammatory phase. This is supported by increased serum IgG titers and lung NEUs on diagnostic testing. Chronic fibrosis, on the other hand, is thought to be due to a type IVc-mediated immune response. Studies have indicated that the involvement of $CD4^+$ cells, characterized by an increase in the $CD4^+/CD8^+$ ratio, plays a significant role in chronic fibrosis. Specifically, Th17 cells are implicated in this response, as the numbers seem to increase after antigen exposure. Genetic deletion and antibody depletion of IL-17 protects the lungs from fibrosis. Type IV (delayed-type) reactions involve interactions between memory T-lymphocytes, ILC, NK-T cells, EOS, and Mϕs. The classic clinical manifestation of the chronic phase of allergic pneumonitis in type IVa patients is mediated by memory Th1 and T-cytotoxic lymphocyte type cells (Tc1), leading to a type 1 (T1) response. Type IVb (type2, T2) reactions are mediated by Th2 cells, with the most typical presentation observable in AFRS, asthma, and AD, involving key players such as Th2, ILC2, Tc2, IgE, and effector cells. When IgE synthesis is triggered, type IVb and type I hypersensitivity reactions overlap in the final stages. Furthermore, type IVb and type V hypersensitivity reactions overlap in terms of epithelial cell activation, the disruption of the epithelial barrier, and the drainage of inflammation into the bronchial lumen. Type IVc (type 3, T3) reactions target extracellular fungi, characterized by Th17 cells producing cytokines from the IL-17 family, recruiting NEUs, and increasing the production of type 2 cytokines. In type IVc responses, Th17, Tc17, ILC3 and other IL-17A- and IL-17F-producing cells have been implicated in neutrophilic inflammation and the pathogenesis of AD, chronic rhinosinusitis with nasal polyposis and neutrophilic asthma. Approximately 50% of patients with AD have a dysregulated humoral response to *Malassezia*, characterized by elevated *Malassezia*-specific IgE. Sparber *et al.* [125] demonstrated that specific $CD4^+$ T-cells against *Malassezia* in healthy human hosts belong predominantly to the Th17 subpopulation and that, in a mouse model of AD, *Malassezia* selective Th17 polarization of $CD4^+$ T-cells specific for *Malassezia* controls cutaneous *Malassezia* over-proliferation and promotes an inflammatory response in barrier-damaged skin via the IL-23/IL-17 axis. In addition to IL-17- and IL-22-producing T cell subsets, an increase in type 2 responses was also observed. Type V hypersensitivity involves immune dysregulation due to epithelial barrier defects and microbial dysbiosis, including extensive activation of T1, T2, and T17 responses and the loss of regulatory T-cells (Tregs), regulatory B-cells, and innate lymphoid cells.

In the context of fungal infections, successful host clearance of fungi and restoration of tissue homeostasis involves both Th1/Th17 type responses, whereas lymphokines produced by Th2 cells are generally characterized by an inability to clear fungal pathogens, accompanied by deleterious allergic inflammation. The two preferential activation pathways, Th1 or Th2, are considered antagonistic. In fact, it has been suggested that the Th2 subset tends to be activated in hosts with allergic reactions, leading to strong B cell stimulation and inefficient Mϕs activation. Fungi are evident inducers of type 2 inflammation, but it is unclear why this immune response pattern occurs specifically against such microbes. The most extensively studied fungal proteases, mannose-binding lectin and chitin, are associated with the induction of type 2 inflammation.

The role of helminth infections in the development of allergic diseases is another controversial topic. Helminths are potent modulators of the host type 2 immune response, which increases the risk of allergy. Producing a strong type 2 response has a genetic-evolutionary advantage in preventing helminth infections, and conversely, chronic helminth infections may reduce the prevalence of allergic diseases [126, 127]. A meta-analysis of 99,967 participants showed that helminth infections may increase the risk of bronchial hypersensitivity in children and allergies in adults [126]. The use of biologic therapies targeting type 2 inflammation, like anti-IL-5R antibody increases the risk of parasitic infections, especially worms [128]. The dramatic increase in the prevalence of allergies over a short period may be explained by environmental factors in genetically susceptible patients or epigenetic events, which indicate the importance of environmental factors in abnormal immune responses. The environments in which parasitic infections may occur, and unhygienic living conditions may also expose populations to a wide range of microorganisms such as fungi, which are abundant in the environment and may contribute to the regulation of inflammatory responses [129, 130]. This regulatory action may have an inhibitory effect on fungal-induced allergic diseases. For example, intestinal helminth infections may reduce the severity and incidence of fungal allergy by reducing the response to specific fungal allergens through the action of regulatory T-cells.

Fungal allergy-related respiratory diseases

Allergic rhinitis

Allergic rhinitis (AR) is a chronic inflammation of the nasal mucosa caused by IgE-mediated reactions to aeroallergens. It is characterized by sneezing, a running nose, nasal congestion, and itching. The prevalence of AR has been steadily

increasing for decades, and AR currently affects approximately 500 million individuals worldwide, significantly impairing the quality of life across all age groups and imposing a substantial socioeconomic burden [131]. The etiology of AR is not fully understood and is believed to be linked to genetic factors, lifestyle, climate change, and environmental exposures, with mould exposure being identified as one of the primary triggers [132]. Sensitization to fungal allergens is commonly observed in individuals with AR, particularly those with concurrent asthma, and is especially prevalent in perennial AR patients, with seasonal exacerbations peaking between April and November. This seasonal variation may play a crucial role in fungal sensitization to diseases, as fungi typically thrive in hot and humid environments during the summer and fall.

Multiple meta-analyses have indicated a significant association between fungal allergen exposure and the development and exacerbation of AR. Caillaud *et al.* [133] combined data from 11 meta-analyses, longitudinal studies, and birth cohorts and revealed a substantial relationship between mould exposure and AR, with an odds ratio generally exceeding 1.35. Ma *et al.* [134] conducted a cross-sectional study in northern China and identified mould allergy as an independent risk factor for adult AR and AR combined with asthma. Additionally, a comprehensive review categorized exposure to indoor mould as a class II environmental risk factor for AR, with highly suggestive evidence that was upgraded to convincing evidence in the children subgroup. Sio *et al.* found a significant association between sensitization to *Curvularia lunata* allergen and the risk of AR in a cohort of Singapore / Malaysia Chinese adults [135]. Sensitization to *Aspergillus* species was the most common fungal sensitization, with increasing specific-IgE (sIgE) titers against this allergen correlating with an elevated risk of AR and related symptoms. Studies in Türkiye and elsewhere have also reported associations between mould sensitization and the severity of AR, although other studies reported contradictory conclusions [136, 137]. Further research involving larger participant cohorts and comprehensive factors is necessary to conclusively establish the role of fungal allergens in AR and rhinitis severity.

The epithelial barrier disruption, which allows allergens to penetrate and initiate a type 2 inflammatory response, is a significant pathogenic factor in AR [131]. *Alternaria*, likely the most prevalent fungal allergen in AR, can induce or exacerbate AR by impacting the barrier function of nasal epithelial cells through the interaction of serine proteases or by increasing the production of reactive oxygen species [138]. Daines *et al.* [139] demonstrated that the activation of the epidermal growth factor receptor (EGFR) induced by *Alternaria* was responsible for epithelial inflammation. Furthermore, a systematic review revealed that several cellular

mechanisms, including PAR2, EGFR, ATP and IL-33 signaling, as well as oxidative stress, drive the allergic inflammatory response to fungal allergens in the respiratory epithelium [140].

Allergic fungal rhinosinusitis

Fungal rhinosinusitis encompasses a spectrum of diseases rather than a single distinct entity related to host immune status and is clinically categorized into non-invasive or invasive types [141]. Allergic fungal rhinosinusitis (AFRS) is diagnosed when the criteria defined by Bent and Khun are met [142, 143]. This disorder is considered a subset of non-invasive fungal rhinosinusitis and chronic rhinosinusitis with nasal polyps (CRSwNP) characterized by antifungal IgE sensitivity, eosinophil-rich mucus, and characteristic computed tomographic and magnetic resonance imaging findings of paranasal sinuses. It is estimated that 5–15% of chronic rhinosinusitis cases are related to fungal allergens, and AFRS accounts for 6–9% of fungal rhinosinusitis cases [144].

Predisposing factors for AFRS include host genetic diversity, structural abnormalities, geographic residence, and lower socioeconomic status [145]. HLA-DQB1*03 and HLA-DQB1*0302 have been identified as risk factors for AFRS and hypertrophic sinus disease [146]. Notably, bony erosion is a characteristic finding in AFRS, with a prevalence ranging from 20–56%, likely attributed to pressure from nasal polyps and eosinophilic mucin [147]. AFRS is frequently observed among individuals residing in arid and tropical regions with warm temperatures and high humidity, with a relatively high prevalence in the Americas, southern Asia, the Middle East, and Sudan. The causative fungi responsible for AFRS display substantial variation across different geographic regions. For example, *Aspergillus* species account for up to 96% of all AFRS cases in India, while dematiaceous fungi such as *Alternaria*, *Bipolaris*, *Cladosporium*, and *Curvularia* are more prevalent in the U.S.A. [148]. In Egypt, *Aspergillus* was detected in 42 out of 68 patients (61.7%) with AFRS, whereas *Alternaria* was isolated from only a single sample [149]. Interestingly, reports of AFRS in Japan have indicated that the basidiomycetous fungus *Schizophyllum commune* is one of the most common species (Inoue *et al.*, 2019). Furthermore, a study in the U.S.A. revealed a significant difference in the prevalence of asthma between CRSwNP and AFRS, with 48.3% of CRSwNP patients and 23.6% of AFRS patients having AS symptoms confirmed by pulmonary function testing [150].

Although the exact pathogenic mechanisms of AFRS are not fully understood, the role of type I and type II hypersensitivity reactions to fungal antigens is

highlighted, being induced by multiple factors such as barrier dysfunction of nasal epithelial cells, congenital immune deficiency, fungal co-colonization, and bacterial superantigens. Fungi within the sinus cavities can upregulate type 2 immune responses, leading to the characteristic type I hypersensitivity, eosinophilic inflammation, and type 2 cytokine profiles associated with AFRS. Sun *et al.* [151] demonstrated that AFRS patients exhibit a defect in STAT3 activation, specifically limited to IL-6-dependent STAT3 phosphorylation, which is crucial for Th17/Th22 differentiation. This defect results in a local insufficiency of IL-17/IL-22 cytokines and deficient AMP expression within the diseased sinus mucosa of AFRS patients [151]. Furthermore, patients with AFRS display type I IgE hypersensitivity to fungi as well as other aeroallergens. Recent studies have reported that *Alternaria alternata* is a widespread fungal species in airway discharge, and the pathogenic hypersensitivity and inflammatory reactions correspond to the development of chronic rhinosinusitis, bronchial asthma, and AR [152]. The surfactant protein D is a natural defense peptide in the human nasal mucosa, which may have an essential role in fungal allergen-induced inflammation [153]. *Alternaria* allergens increase the secretion of surfactant protein by respiratory epithelial cells in patients with CRSwNP. Additionally, Dutre *et al.* [154] reported that *Staphylococcus aureus* co-existed with *Aspergillus* species within the sinuses of patients with AFRS, and nearly all subjects had serum IgE to *Staphylococcus aureus* enterotoxin superantigens, with levels correlating with total serum IgE levels. They hypothesized that *Staphylococcus aureus* might play a crucial role in AFRS by synergizing with or using of *Aspergillus* species to create a Th2 tissue signature.

Allergic asthma

Asthma is a prevalent, chronic inflammatory airway disease characterized by bronchial hyperresponsiveness, inflammation, and reversible airflow obstruction. Symptoms include recurrent wheezing, dyspnea, chest tightness, and cough. The condition is influenced by genetics, family history, socioeconomic status, infections, and various environmental factors [155]. Allergic fungal airway disease (AFAD) is a broad term encompassing allergic airway diseases caused or worsened by fungi [156]. Fungal AS can range from mild to severe, with extensive lung damage characterized by bronchiectasis. This includes various allergic fungal syndromes complicating asthma, such as asthma associated with fungal sensitization (AAFS), *Aspergillus fumigatus*-associated asthma (AFAA), severe asthma with fungal sensitization (SAFS), thunderstorm asthma, ABPA, and ABPM. Fungal sensitization is now recognized as an asthma attack trigger, which occurs in 3–10% of the healthy population and 7–20% of asthmatics, with prevalence rates as high as 35–75% in patients with severe asthma [157, 158]. Important allergenic fungi associated with

asthma include *Alternaria alternata*, *Cladosporium herbarum*, *Aspergillus fumigatus*, and *Penicillium* and *Trichophyton* species [159].

Fungal sensitization rates may vary due to different exposure levels related to the local biome and climate. For example, an outdoor airborne fungal survey in Japan revealed that *Cladosporium* and *Alternaria* were the predominant genera, followed by *Epicoccum*, *Aureobasidium*, *Curvularia*, and *Ulocladium*. Indoor airborne fungal isolates in Japan were predominantly *Cladosporium*, *Penicillium*, *Aspergillus* section *Restricti*, and *A. versicolor* [160]. A multicenter study in seven European countries revealed that sensitization rates to *Alt. alternata* and *Cladosporium herbarum* varied between 3% and 20% in children and adults (n = 877) with asthma and / or rhinitis, with approximately 9.5% being skin prick positive to at least one or both fungal species [81]. A study from the U.S.A. found that 22% of patients were positive for at least one fungal allergen, with 13.7% positive for more than two fungal allergens [161]. Specific positivity rates were highest for *Candida albicans*, *Alt. alternata*, *Stemphylium herbarum*, and *Asp. fumigatus*. In another study, Agarwal *et al.* [157] estimated the prevalence of *Aspergillus* sensitization in adults with bronchial asthma from systematically reviewed literature. They reported that the prevalence of *Aspergillus* sensitization in asthma patients ranged from 1.6% to 73%, with a pooled prevalence of 25.1%. Studies from Sweden and Japan have shown varying rates of fungal sensitization in patients with severe asthma, with *Aspergillus* being a common sensitizing species [162, 163]. Additionally, atmospheric mould may also contribute to asthma exacerbations. Exposure of sufficient intensity and duration to moulds has been linked to the development of asthma and the worsening of pre-existing asthma [164, 165]. Additionally, *Trichophyton*, known as the causal pathogen of dermatophytosis, has been associated with more severe asthma in some studies, although its significance in asthma remains controversial [166, 167].

Thunderstorm asthma is defined as a sudden surge in acute asthma cases affecting a large population following a thunderstorm, attributed to elevated levels of airborne allergens [168]. Numerous studies have documented increased morbidity and emergency hospital visits for asthma following exposure to fungal spores during such events. Targonski *et al.* [169] demonstrated that asthma-related mortality in Chicago more than doubled on days with spore counts reaching 1,000 per cubic meter. A systematic review revealed that several studies reported a rise in the concentration of fungal aeroallergens during thunderstorm asthma outbreaks, suggesting that fungal spores are potential triggers [170]. Sensitivity to *Alternaria* has been identified as a risk factor for severe asthma attacks and epidemic asthma [169]. A study in England indicated that sensitivity to *Alt. alternata* was associated

with and could serve as a predictor of thunderstorm asthma [171]. Additionally, a recent study in Iran revealed that the genus *Curvularia* was significantly correlated with thunderstorm asthma outbreaks [172].

Fungal exposure has been strongly linked to the initiation, persistence, and exacerbation of asthma, although the underlying mechanisms have not been fully elucidated. Broadly speaking, three different mechanisms contribute to the worsening of asthma caused by mould exposure. (i) A mechanism involving non-thermotolerant fungi, such as *Alt. alternata* and *Cladosporium* species, whose spores or hyphal products act as allergens in atopic patients. Research by Liu *et al.* [173] demonstrated that treatment with rAlt a 1 alleviated asthma progression and regulated Th and Breg cells in an *Alternaria*-induced asthmatic mouse model. (ii) A second mechanism is associated with thermotolerant fungi such as *Asp. fumigatus* and *Penicillium* species, which can trigger a persistent IgE-driven eosinophilic inflammatory response leading to severe asthma. Fungal proteinases involved in SAFS can lead to the predominant activation of type 2 cells and subsequent synthesis of IL-4, IL-13, and IL-5. A phase 3 study (Liberty Asthma Quest) established the efficacy of dupilumab monotherapy in treating uncontrolled, moderate-to-severe asthma with fungal sensitization [174]. (iii) A third mechanism is associated with skin fungal infections, such as dermatophytosis caused by *Trichophyton* species, which can worsen asthma control through allergic cross-reactive T-cells. Limited evidence exists for this mechanism, although allergenic proteins from the genus *Trichophyton* have been identified to elicit immediate and delayed-type hypersensitivity skin reactions in different individuals [160].

Allergic bronchopulmonary aspergillosis / mycosis

Allergic bronchopulmonary mycoses (ABPM) are complex pulmonary disorders caused primarily by type I, III, and IV hypersensitivity reactions to fungi, which colonize the airways of patients with chronic lung diseases, particularly asthma or cystic fibrosis [175]. The term allergic bronchopulmonary aspergillosis (ABPA) is used when *Aspergillus* is the causative agent. In addition to *Aspergillus*, the most common fungi responsible for ABPM include *Schizophyllum commune*, and *Bipolaris* and *Curvularia* species [176, 177], with occasional cases involving *Candida albicans* [178] and *Alternaria alternata* [179].

Recently, the International Society for Human and Animal Mycology (ISHAM) published the revised diagnostic criteria for ABPA/ABPM [177], which require consideration in patients with predisposing conditions or a compatible clinical-radiological presentation. Essential components include documenting sensitization

to the implicated fungus (using fungus sIgE) and demonstrating immunological activity (elevated serum total IgE). Additional features such as fungal-specific IgG, peripheral blood eosinophilia, and consistent imaging confirm the diagnosis. The Japan ABPM Research Program also proposed a new 10-component diagnostic criterion for ABPA/ABPM [175], demonstrating improved sensitivity and specificity compared to existing criteria. Sensitivity rates for pathological ABPM using different criteria were 25.3%, 77.2%, and 96.2% for Rosenberg-Patterson, ISHAM, and the new criteria, respectively. For physician-diagnosed ABPA/ABPM, the sensitivity rates were 49.2%, 82.7%, and 94.4%, and for culture-positive non-*Aspergillus* fungi ABPM cases, the rates were 13.0%, 47.8%, and 91.3%, respectively.

The etiology of ABPA/ABPM is multifaceted and remains speculative. The initial step in developing of ABPA/ABPM involves the germination of inhaled fungal conidia in the lower airway lumen. For conidia to germinate in the bronchi, the fungi must be thermotolerant, and their conidia must be small enough to reach the lower airways. Proteases and pathogen-associated molecular patterns of fungi subsequently induce various immune responses. Specific genetic factors related to innate and adaptive immunity, including surfactant protein A2, TLR9, TLR10, human leukocyte antigen HLA-DQ, HLA-DR, IL-10, and IL-4 receptor alpha, are associated with ABPA [180]. Recent studies have indicated that EOS and Th2 cells may play a crucial role in the pathogenesis. Murine models have demonstrated that Th2 cells and IL-4 are essential for eosinophilic infiltration, whereas flow cytometry has shown skewing towards Th2 cells in ABPA patients [181]. Cystic fibrosis (CF) is the most common autosomal recessive disease in Caucasians and is characterized by frequent respiratory infections and progressive lung disease. Hong *et al.* (2018) found that 1541 (9.6%) subjects developed persistent *Aspergillus* colonization in 16,095 CF patients (median age 16.4 years) followed up for 7 years, and this rate may be higher in older patients. These CF patients present with specific IgE or IgG sensitization to *Aspergillus fumigatus*, total IgE and eosinophilia, 8% (median 6–10%) of patients eventually develop ABPA. The innate defenses of mucosal cilia clearance are impaired in CF patients. *Aspergillus* spores penetrate and adhere to collagen and fibronectin fibers in the basement membrane, thus contributing to their persistence in the airway. The fungal metabolite gliotoxin has also been shown to down-regulate vitamin D receptor expression in macrophages and airway epithelial cells in CF patients and to increase IL-5 and IL-13 levels (Wiesner *et al.*, 2017). A phase one open-label study demonstrates that daily supplementation with 4,000 IU of vitamin D reduces IL-13-mediated Th2 responses and *Aspergillus*-specific IgE levels in 12-year-old CF children with ABPA.

Hypersensitivity pneumonitis

Hypersensitivity pneumonitis (HSP, also known as extrinsic allergic alveolitis) is based on type III/IV allergic reactions to repeated inhalation of allergens and may lead to a chronic disease with irreversible lung damage. This condition can occur in association with specific environmental exposures, such as mouldy hay (farmer's lung), bird proteins (bird fancier's lung), mouldy wood (woodworker's lung), or mould-contaminated air systems [182]. In salami/sardine workers, air conditioning exposures, or contaminated water sources, *Aspergillus*, *Clostridium* and *Penicillium* are the primary causative fungi; on mouldy wood, *Bacillus subtilis* and species of *Alternaria*, *Mucor*, and *Rhizopus* are the main allergenic organisms. Diagnosing HSP requires a combined evaluation of exposure, high-resolution computed tomography and, if necessary, analysis of histopathological material. Serum IgG-specific antibodies can be used for an additional antigen test for diagnosing HSP, with a sensitivity of 93 % and specificity of 100% when compared with controls without a history of exposure [183].

HSP pathophysiology results from complex antigen exposure pathways, aberrant immune mechanisms, and genetic susceptibility. Common and rare genetic variants have been found to occur more frequently in HSP, such as HLA-DRB1, HLA-A, -B and -C, MUC5B loci, and TNF-gene promotor, which increase the susceptibility of patients to HSP [184, 185]. Fungal spores or mycelial fragments (<3 µm) are repeatedly inhaled into distal bronchioles, and alveolar Mϕs and DCs are stimulated to initiate innate immune responses by TLR2, 6, 9 and Dectin-1. Inhaled fungal antigens also bind to IgG antibodies, which initiate the complement cascade and produce by-products, including C5, further stimulating Mϕs. Mϕs fuse with multinucleated giant cells and epithelioid cells to form granulomas mediated by Th1 cytokine production. Granuloma production of chemokines enhanced Th2 to Th1 responses, diminished regulatory T-cell responses, $CD8^+$ T-cell production and Th17 differentiation (induced in part by $CD103^+$ on DCs) promote fibroblast proliferation [186].

Fungal allergy-related skin diseases

Cutaneous Id reaction

Cutaneous Id reaction is an autosensitization dermatitis characterized by pruritic, erythematous, maculopapular, and papulovesicular lesions appearing at sites distant from the primary inflammatory infection and has been linked to various

fungal, bacterial, viral, and parasitic skin infections. Ilkit *et al.* [187] published a comprehensive review of the clinical manifestations, epidemiology, etiology, and management of cutaneous Id reactions. A literature review revealed that Id reactions secondary to cutaneous fungal infections are most associated with superficial infections, particularly dermatophytosis. Apart from these, other deep fungal infections, such as histoplasmosis [188], coccidioidomycosis, sporotrichosis [187], and candidiasis [189], can also induce Id reactions, often presenting as erythema nodosum and erythema multiforme [190].

The Id reaction associated with dermatophytosis, known as the dermatophytid reaction, is most linked to zoophilic and geophilic dermatophytes. The estimated incidence of dermatophytid reactions ranges from 0.7% to 17% [187]. The occurrence of dermatophytid reactions is influenced by factors such as the degree of local infection inflammation, the causative agent, and the host's immune response, resulting in variable manifestations. Based on clinical symptoms and histopathological changes, the disease can be categorized into four clinical types: (i) epidermal type, (ii) dermal type, (iii) subcutaneous type, and (iv) vascular type. Tinea pedis and kerion celsi (KC) are common dermatophyte infections that induce dermatophytid reactions, with the former often associated with eczematous-type dermatophytids and the latter frequently observed in children, presenting as acute disseminated and nodular erythema multiforme-like dermatophytid. Veien *et al.* [191] reported that 37 out of 213 patients (17%) diagnosed with tinea pedis developed dermatophytids on their hands, and the authors described Id reactions in 27 (34.6%), seven (5.5%), and one (16.7%) case of *T. mentagrophytes* (n = 78), *T. rubrum* (n = 128), and *Epidermophyton floccosum* (n = 6) foot lesions, respectively. In a recent prospective study of dermatophytid reactions in KC, 68% of 19 pediatric patients experienced dermatophytid reactions prior to treatment initiation [67]. Among these patients, 36.8% presented with eczematous patches or plaques, 15.8% presented with papules, 10.5% exhibited eczematous lesions, papules, and pustules, and 5.3% showed signs of an angioedema-like reaction. In a recent retrospective study involving 127 children with tinea capitis, *M. canis* and *T. mentagrophytes* were the most common pathogens, with 26.8% of cases complicated by allergic diseases [192]. Among these cases, 26.5% of children presented with dermatophytid reactions, all occurring in the context of KC. The intense immune responses triggered by zoophilic dermatophytes are thought to play a significant role in the pathogenesis of KC [193]. Notably, an increasing number of cases of KC caused by *M. canis* with dermatophytid reactions have been reported [194-196]. The epidemiological and clinical patterns of fungal infections due to *M. canis* have undergone significant changes in recent years. It is important

to raise awareness of this issue to establish an accurate diagnosis and implement successful treatment strategies.

The mechanism of Id reaction remains unclear. The epidermal type with eczematous eruptions is triggered by a type IVa hypersensitivity reaction involving the secretion of large amounts of IFN-γ and TNF-α, which activates Mϕs. The subcutaneous type with erythema nodosum lesions is a hypersensitivity reaction triggered by multiple factors involving type I, II, and IV reactions [187]. Subtilisins are important virulence factors for dermatophytes, particularly when invading the host's epidermal barrier. Shi *et al.* [197] reported that Sub6 triggers both IgE-dependent reactions and delayed-type hypersensitivity reactions. Vermout *et al.* [198] used a crude exoantigen from *M. canis* to induce a strong delayed-type hypersensitivity response in a guinea pig model characterized by significant erythema and skin swelling.

Atopic dermatitis

Atopic dermatitis (AD) is a chronic, inflammatory skin disease affecting approximately 15–20% of children and 1–3% of adults. It is considered a systemic disease due to its association with an increased risk of comorbidities, including food allergies, asthma, AR, and mental health disorders. The complex pathophysiology involves genetic predisposition, skin barrier dysfunction, T-cell driven inflammation, allergen sensitization, and microbial interactions [199]. Increasing evidence suggests the presence of fungal infections in the eczematous lesions of AD patients, with reports indicating that *Malassezia*, *Candida*, and dermatophytes can impact the symptoms of AD and that even aeroallergens can act as triggers for cutaneous reactions in AD [161, 200].

Although lipophilic *Malassezia* yeasts constitute the primary commensal fungal microbiota of healthy human skin, they have been implicated in the development and persistence of AD. A significant proportion of AD patients exhibit a positive reaction to *Malassezia* allergens. In Korea, Han *et al.* [201] reported that *Malassezia globosa* and *Malassezia restricta* were prevalent in healthy individuals and AD groups, whereas *Malassezia slooffiae* and *Malassezia dermatis* are characteristics of AD. In another study [75], *Malassezia* species were identified in 20.9% (44/211) of AD patients, while only 8.7% (2/23) of healthy individuals presented positive results. A study utilized the ALEX 2-Allergy Explorer to assess the relationship between sensitization to molecular components of yeasts and moulds and the severity of AD. The results indicated that sensitization to Mala s 6, Mala s 11, Sac c, Asp f 6, Cla h 6, and Cla h 8 correlates with AD severity. In severe AD patients, a

high level of specific IgE to Mala s 11 (36%) and Asp f 6 (12%) was observed [202]. Sensitization to *Malassezia* is more relevant in adults than in children, with serum *Malassezia*-specific IgE present in 30% of children with AD and 70% of adult patients, particularly those with head and neck lesions [203]. *Malassezia* has enhanced adaptation in barrier-damaged and metabolically dysregulated skin, which is associated with lipid dependence, potentially explaining the higher susceptibility rates in adults than in children having incomplete development of sebaceous glands. Furthermore, a recent prospective cohort study found a significant positive correlation between baseline *Malassezia*-specific IgE levels and the incidence of dupilumab-associated head and neck dermatitis (DAHND). The authors suggested that baseline *Malassezia*-specific IgE could be a biomarker to predict DAHND before dupilumab treatment [204].

Candida albicans is a frequent commensal colonizer of human mucocutaneous surfaces that functions as an opportunistic pathogen and contributes to AD exacerbation. Savolainen *et al.* reported a significant correlation between *Can. albicans* sensitization and AD symptoms in patients, although there was no correlation between *Can. albicans*-specific IgE and AD severity in patients without gastrointestinal colonization [205]. Similarly, Javad *et al.* [206] found that *Candida* species, particularly *Can. albicans*, were isolated from the skin and oral cavity of 31% of 100 AD patients and 12% of 50 healthy individuals. PCR analysis of 211 AD patients revealed that 10.9% (23/211) had *Candida* species, with *Can.albicans* (5.2%) and *Can. parapsilosis* (3.79%) being the most frequently detected species [75]. Ren *et al.* [207] found increased skin infections in emergency department visits of adult and pediatric AD patients. Pediatric and adult AD was associated with significantly higher odds of candidiasis, dermatophytosis of the skin/nails, and vulvo/urogenital and other skin infections. Moreover, studies have linked dermatophyte infections to allergic disorders and increased sensitivity to dermatophytes in AD patients. In a cross-sectional study comparing the prevalence of tinea pedis in AD patients, a relatively high prevalence of tinea pedis was observed in both groups. *Trichophyton rubrum* was the most common species in both AD and psoriasis patients. Recently, we reported that in children with tinea capitis, AD/eczema and AR were the most common allergic diseases [208], and male gender, KC, zoophilic pathogens, and animal contact history as risk factors for tinea capitis with allergic complications. Another nationwide multicenter study by Deng *et al.* revealed that AD, animal contact, endothrix infection, and geophilic pathogens were associated with KC.

The pathogenetic mechanisms and relationships between the AD-associated fungal genera and immune defense remain unclear despite evidence of their contributions

to AD development. *Malassezia* may trigger AD development through IgE-dependent, IgE-independent, and T-cell-independent mechanisms. For example, *Malassezia sympodialis* allergens Mala s1, 5, 6, and 9 can stimulate IgE-mediated mast cell degranulation via the TLR2/MyD88 signaling pathway [209, 210]. Additionally, patients with AD have shown hypersensitivity to MGL_1304 and its homologs produced by *Malassezia globosa*, which have been identified as major histamine-releasing antigens in human sweat [211]. *Malassezia* species interact with keratinocytes, altering cytokine release profiles and survival when keratinocytes absorb them. Additionally, *Malassezia sympodialis* secretes nanovesicles that activate dermal MCs and cutaneous DCs, releasing cytokines that worsen the condition [212]. Recent research has demonstrated that *Malassezia* selectively induces IL-17 and related cytokines, which play crucial roles in preventing fungal overgrowth on the skin. Under conditions of impaired skin integrity, resembling a characteristic feature of AD, the presence of *Malassezia* significantly exacerbates cutaneous inflammation, which is dependent on IL-23 and IL-17.

Urticaria

Urticaria, a common inflammatory skin disorder characterized by wheals and / or angioedema, has a global lifetime prevalence of up to 20%, imposing a significant burden on individuals and society [213, 214]. The etiology of urticaria is complex and involves transient exogenous and persistent endogenous factors. Common fungus-related factors include mushroom food, respiratory mould allergens, and superficial fungal infections [215].

Aeroallergens are well-established triggers of asthma and AR, and recent studies have highlighted their significant role in cutaneous reactions such as chronic urticaria and AD [216]. A review of hypersensitivity reactions to fungal aeroallergens in Iran revealed that sensitization to *Aspergillus fumigatus* and *Alternaria alternata* was the most common allergic sensitization among patients with allergic disorders. Sensitization to *Asp. fumigatus* was particularly prevalent in patients with urticaria and AD [217]. A retrospective study in China estimated the clinical data of patients with various allergic diseases, revealing that 266 out of 1367 patients had mixed fungal antigen positivity. The positive rate of fungal aeroallergens was 13.4% among 404 urticaria patients [218]. In India, a survey of fungal sensitivity among 100 urticaria patients revealed a 3% prevalence of skin prick test (SPT) reactivity to *Asp. flavus* and to *Fusarium* species [219].

Latent infections have long been considered a potential cause of chronic urticaria. In the 1930s, Wise *et al.* (1930) first reported hypersensitivity to *Trichophyton* in urticaria, hay fever, and asthma, suggesting widespread ringworm infections might contribute. Palma-Carlos *et al.* [220] reported that 57 out of 89 dermatophytosis patients also had chronic urticaria or angioedema, with a high percentage testing positive for trichophytin and increased *Trichophyton*-specific IgE levels. Interestingly, 18 of these patients were cured after antifungal treatment. Similarly, Godse *et al.* [221] reported four cases of chronic urticaria in patients with dermatophytosis and noted that antihistamine treatment was ineffective but that the urticaria resolved after antifungal treatment. Zhang *et al.* [222] observed a high positivity rate for fungal antigens in patients with chronic urticaria and onychomycosis, suggesting a potential link between fungal infection and *Trichophyton* hypersensitivity. Zawar *et al.* [223] reported improvement in chronic spontaneous urticaria patients after antifungal and antihistamine treatment for *Malassezia* infections. Additionally, recent studies have shown a potential association between *Malassezia* and cholinergic urticaria, with specific components of *Malassezia* triggering histamine release. The etiology of chronic urticaria is multifaceted, with some patients having concomitant allergies to other allergens and exogenous fungal allergens. While antifungal therapy may not be effective for all chronic urticaria patients with fungal-specific IgE, determining fungal-specific antibodies may offer insights into the etiology and new avenues for clinical treatment.

Allergic cross-reactivity between fungi and foods

Fungal food allergy syndrome (FFAS) is induced by cross-reactivity between consumed fungi (or fungal fermented foods) and fungal allergens from exposome, involving one or more target organs, including oral mucosa, skin, gastrointestinal tract, and respiratory tract. There are many studies on pollen food allergy syndrome (PFAS), but FFAS is still largely neglected in basic research as well as in clinical practice [103].

Alternaria alternata is a ubiquitous soil-borne fungus capable of causing diseases in various plants and occasionally in humans. It is also one of the most common moulds associated with allergic diseases, and *Alternaria* contamination is also documented to trigger food allergies. The *Alternaria*-spinach syndrome is one of the typical examples of FFAS and is characterized by mould-sensitized patients with food allergic reactions after mushroom (*Agaricus bisporus*) or spinach ingestion [224, 225]. Gabriel *et al.* [226] reported a case of severe urticaria and shock after consumption of *Agaricus bisporus* in a patient who was SPT-positive for airborne

moulds, such as *Alternaria*, *Cladosporium*, and *Aspergillus* species, and the authors identified a high degree of homology of MnSOD and MtDH proteins from *Asp. bisporus* and Asp f 6, Alt a 14, Alt a 8 and Cla h 8 of the mould as highly homologous. A 30 kD protein in spinach and mushroom extracts has a molecular weight and structure similar to those of Alt a 1 of *Alternaria* and Cla h 1 of *Cladosporium*. *Alternaria* contamination is also known to trigger food allergies. Researchers have shown that the co-sensitization phenomenon between Alt a 1 and Act d 2, a major allergen and PR5 of kiwifruit, is caused by the consumption of kiwifruits infected with *Alternaria* but apparently in good condition [227].

Accordingly, food contamination with fungal spores may be the cause of some adverse reactions in patients, who were apparently not sensitized in the past and obtained negative results in previous SPT for the diagnosis of a food allergy. It was reported that 17.8% of patients who consumed meat substitutes made with the *Fusarium venenatum* protein source may have suffered tachyphylactic hypersensitivity reactions, including urticaria and gastrointestinal symptoms [228]. The authors emphasize that sensitization to mould allergens by the respiratory tract and subsequent oral ingestion of cross-reactive proteins may lead to severe food-allergic reactions. Thus, the presence of 60S acidic ribosomal protein P2 of *Fusarium venenatum* is likely the reason for the described severe hypersensitivity reactions of the patient to Quorn-mycoprotein, because of its potential cross-reactivity with the *Fusarium culmorum* allergen Fus c 1. There have been many case reports of allergies in patients presenting with aggregated respiratory IgE allergies after ingestion of beer, red wine, cheese, and sauces containing crossover substances, and relative fungi including *Aspergillus fumigatus, Cladosporium herbarum, Alt. alternata, Penicillium notatum, Penicillium chrysogenum* and *Saccharomyces cerevisiae* [229, 230].

To date, a total of seventeen proteins are characterized as allergens in *Alt. alternata* (http://www.allergen.org/). Most of them have homologues in the other three relevant mould genera involved in allergies: *Cladosporium*, *Penicillium*, and *Aspergillus*, with the exceptions of Alt a 1 and Alt a 13, the most relevant allergens. The main mechanism leading to cross-allergy to various fungi and foods is the breakdown of the immune system and clinical tolerance of the body to the food ingested, resulting in IgE-mediated reactions or non-IgE-mediated diseases. Sensitization to food allergens occurs by fungi through the gastrointestinal tract, skin, and respiratory tract. The default response to food antigens is often immune tolerance. This response is mediated by antigen presentation by $CD103^+$ DCs in the gastrointestinal tract, $CD11b^+$ dermal DCs, and Langerhans cells in the skin. These antigen-presenting cells travel to the regional and mesenteric lymph nodes, where

they stimulate the production of T cells. Food allergy sufferers are thought to have impaired Treg cell induction, which is replaced by the production of distinct antigen specific Th2 cells that promote IgE class switching and the growth of allergic effector cells [231].

Component resolved diagnosis of fungal allergies

Accurate clinical history forms the basis for the diagnosis of fungal allergies. Allergen testing focuses on IgE-mediated type I hypersensitivity and T-cell-mediated type IV hypersensitivity. Allergen tests are divided into two categories: (i) *in vivo* allergen tests, including SPT, intradermal test, patch test, and provocation test; and (ii) *in vitro* allergen tests, including serum allergen sIgE, total IgE, and BAS activation test. To date, crude fungal allergen extracts remain the primary diagnostic tools for *in vivo* allergen tests used in clinical practice. However, the number of available molecular fungal allergen tests is limited, and the clinical implementation of these methods varies significantly, lacking standardized protocols.

The SPT is currently the preferred *in vivo* allergen test recommended by the International Organisation of Allergy and Reactions (IOAR) and is characterized by minimal skin damage, low levels of prick fluid, a high degree of safety, fewer side effects, and suitability for use in infants and young children, with a negative predictive value of 95% and a positive predictive value of 50–60% [232]. Skin prick test of patients with chronic rhinitis has revealed differences in the clinical history and the severity of symptoms [233]. The SPT is also an important tool in the diagnosis of chronic idiopathic urticaria and AD [233]. Kits for SPT are mainly based on traditional crude protein extracts, but they show extensive cross-reactivity with unrelated fungal allergens. There are almost no specialized SPT kits on the market, and component-resolved diagnosis (CRD) based SPT kits are rarely available. In recent years, Stallergenes Greer and ALK-Abelló manufacturers have also begun to use standardized, high-purity allergen extracts for SPT that are more purified than traditional crude extracts, which reduces cross-reactivity. Although they do not reach the molecular level of CRD, they improve diagnostic specificity to some extent. Additionally, the accuracy and reliability of both *in vivo* and *in vitro* tests are highly dependent on the quality of the materials used. Significant variability exists in SPT results when using antigens from different manufacturers are used. This issue is particularly prominent in fungal allergy diagnostics, where the lack of standardized, high-quality extracts poses a significant challenge. Several studies have reported that SPT sensitivity and reproducibility are lower than those of automated IgE tests, and the shortage of commercially available fungal extracts

further impedes the development of component-resolved diagnosis of fungal allergies.

Serum tests for fungal allergen specific IgE and IgG are used to detect type I hypersensitivity reactions and type II/III reactions, respectively, often serving as a supplementary diagnostic tool to skin tests. Molecular allergy diagnostics [234], or CRD, represents a method that uses purified natural or recombinant allergenic molecules to replace crude allergen extracts in allergy diagnostics [235]. Currently, commercially available recombinant fungal proteins are classified as 'markers' or 'cross-reactive components', which are used to distinguish proper sensitization. However, only few allergens have been developed thus far. The greatest challenges in developing diagnostic and therapeutic tools based on fungal recombinant allergens are estimating the size of the library required to replace allergen extracts and elucidating the role of cross-reactive structures to reduce the number of allergens required to achieve a universal allergy diagnosis. Recombinant or naturally purified allergenic components can be used for CRD, and sIgE can be measured using single or multiple platforms. Multiplex CRD methods should be used in complex cases of multiple allergen sensitization and in patients with severe allergic reactions. The multiplex platform is capable of simultaneous IgE testing of small amounts of serum (100 µL) components from more than 200 allergens but is not sufficiently sensitive compared to single testing. Several *Aspergillus fumigatus* specific antigens (Asp f 1, f 2, f 3, f 4, and f 6) can be obtained through recombinant techniques [236]. Recombinant *Asp. fumigatus* (rAsp) antigens can be used to identify true *Asp. fumigatus* sensitization [237, 238]. Two independent studies have demonstrated that IgE specific to rAsp antigens (Asp f 1, f 2, and f 4) is highly specific for ABPA [239, 240]. Li *et al.* [241] reported that IgE specific to rAsp f 6 is helpful for diagnosing ABPA, but it lacks specificity and may result in false-positive results in patients with AD, potentially due to cross-reactivity with *Malassezia* species. In a study by Tanimoto *et al.* [240] among 53 patients meeting the diagnostic criteria for ABPA, 11 had negative serum IgE results for both Asp f 1 and f 2. Based on molecular allergy diagnostic principles, IgE reactivity to Asp f 1 and f 2 is thought to result from exposure to airway-secreted *Asp. fumigatus* allergens. In contrast, patients who meet the diagnostic criteria for ABPA but have negative IgE results for Asp f 1 and f 2 may indicate that positive IgE reactivity to crude *Asp. fumigatus* extracts is due to sensitization to other fungal allergens and that the true allergenic fungus may be something else than *Asp. fumigatus*. These labeled allergens enable a highly specific diagnosis of fungal allergies, but although they represent the major allergens of a single species or genus, they do not achieve the sensitivity to fungal extracts because they are only recognized by 60–90% of all allergens that are sensitized to the corresponding fungi. Here, microarrays

containing all major species- or genus-specific fungal allergens supplemented with known cross-reactive structures may meet or exceed the *in vitro* diagnostic specificity of fungal extracts [242].

Conclusions and perspectives

Fungi have a distinctive propensity for inducing allergic reactions in the host, driven by a multifactorial interplay involving the species of fungi, the route of exposure, host genetic factors, immune status, and potential evolutionary selection. Both thermotolerant and non-thermotolerant fungi are potent inducers of type 2 immune responses, with innate and adaptive immunity playing critical roles in this process. Despite advancements in identifying and diagnosing fungal allergens, current research continues to face significant limitations:

i. Insufficient epidemiological data: current studies predominantly target specific regions or populations, and they need more extensive epidemiological data across diverse geographic areas and demographics. This insufficiency hampers a comprehensive understanding of the global prevalence and distribution of fungal allergy.
ii. Complex immune response mechanisms: fungal allergies involve intricate immune responses encompassing innate and adaptive immunity. The specific mechanisms underlying these responses have not yet been fully elucidated, limiting the development of targeted therapeutic interventions.
iii. Challenges in specific antigen detection: the vast diversity of fungal species, each presenting a wide array of antigens, significantly complicates detecting and identifying specific allergens. This diversity makes accurate diagnosis of fungal allergies particularly challenging.
iv. Cross-reactivity issues: the significant cross-reactivity among fungal allergens and between fungal and other environmental allergens increases diagnostic complexity. This often leads to misdiagnosis or undiagnosed cases, complicating effective management and treatment strategies.
v. Lack of standardization: the absence of globally recognized standards for fungal allergen detection results in variability and inconsistency in clinical diagnoses and research findings. This lack of uniformity affects the reliability and comparability of results across different studies and clinical settings.
vi. Environmental control and prevention: there is a critical need to control fungal exposure in both living and working environments. Reducing

allergen exposure and increasing public awareness about fungal allergies and preventive measures are essential in mitigating these conditions' impact.

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Author contributions

XZ: conceptualisation; writing, original draft preparation; writing, review and editing. PYF: conceptualisation; writing, review and editing. YQ: resources, reference collection and collation. SAA: writing, original draft preparation; writing, review and editing. MI: writing, original draft preparation; writing, review and editing. WTL: writing, original draft preparation; table and editing. XYZ: writing, original draft preparation; writing, review and editing.

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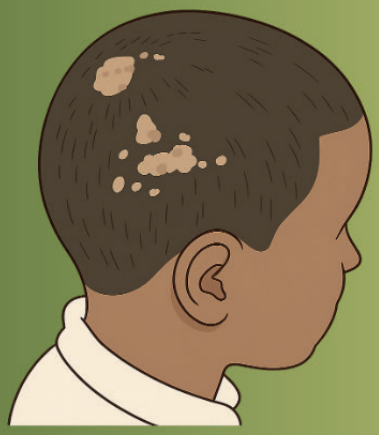
Competing interests

None



CHAPTER 3

EXPLORING THE CLINICAL FEATURES AND RISK FACTORS FOR CHILDREN IN TINEA CAPITIS COMPLICATED WITH ALLERGIC DISEASES

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ABSTRACT

Tinea capitis, atopic dermatitis, and allergic rhinitis are the most common disorders endured by prepubescent children. Dermatophyte infections have been linked to allergic disorders, such as increased sensitivity to dermatophytes in patients with atopic dermatitis, and, conversely, more difficult management of allergic asthma in patients with chronic dermatophyte infections. To explore the correlation between tinea capitis and allergic diseases in children and to analyse their risk factors. This study monitored epidemiological changes in childhood tinea capitis and risk factors for whom with allergic disease in a single centre in three consecutive five-year intervals by reviewing clinical data and multivariate logistic data analysis. Between 2007 and 2022, there were 127 pediatric patients with tinea capitis, and the mean age was 4.83 years, the male-to-female ratio was 1.76:1. Zoophilic *Microsporum canis* and *Trichophyton mentagrophytes* were the most prevalent pathogens and the proportions remained relatively constant every five years. There were 34 (26.8%) children with tinea capitis complicated with allergic disease, among them 14 children with atopic dermatitis/eczema, 13 with allergic rhinitis, 8 urticaria, 6 food allergies and 1 allergic asthma. Male, kerion, zoophilic species infections and animal contact history were prevalent features in allergic disease combined with tinea capitis. Patients with tinea capitis plus allergic disease mostly had a family history with similar complications. *Microsporum canis* and *T. mentagrophytes* were the most prevalent pathogens of tinea capitis in the last 15 years; atopic dermatitis/eczema and allergic rhinitis are the most complicated allergic diseases for which males, kerion, zoophilic pathogens and animal contact history are risk factors.

INTRODUCTION

Tinea capitis is the most common dermatophyte infection of the scalp affecting mainly preadolescent children. This remains an important public health concern. The epidemiology of tinea capitis varies across different geographical areas and has changed over time. Although *Microsporum* and *Trichophyton* dermatophytes are the major etiological agents of tinea capitis, there is a gradual shift from anthropophilic pathogens to zoophilic pathogens enhanced by increased domestic pet keeping. Recently, a multicentre prospective study of tinea capitis in China showed that the zoophilic species *M. canis* was the most common pathogen (65.2%), only in central China was the antropophilic *T. violaceum* (69.0%) predominant [13].

Kerion is a severe inflammatory subtype of tinea capitis, and generally caused by zoophilic and geophilic fungi. Children with kerion are more likely to develop dermatophytid reactions (Id reaction) maily characterized by disseminated pruritic eczematous eruptions [67, 187, 243]. A recent prospective study from Turkey revealed that 13 of 19 patients (68%) with kerion developed Id reactions. The main clinical manifestations of these reactions are eczematous eruptions (36.8%), pruritic papules (15.8%), excoriated papules and pustules (10.5%) and angioedema-like reactions (5.3%) [67].

A wealth of evidence supports the association between dermatophytosis and allergic diseases such as atopic dermatitis (AD), urticaria, allergic rhinitis (AR) and allergic asthma (AS). Many cases reported positive immediate response to species of *Trichophyton* and *Microsporum* [78, 220, 221, 244-247]. AD is associated with altered skin barrier, changed microbiome, and immune dysregulation which increases the risk of having higher odds of multiple bacterial, viral, and fungal skin infections [207]. In a 13.7-year study of underage AD patients, a significantly higher rate of infection with dermatophytosis was found compared to non-AD patients, the fungal infections were more severe and more difficult to eradicate in AD patients. George *et al.* [78] reported 12 patients with allergic respiratory disease who had a positive immediate reaction to *Trichophyton*, were 10 of them gave immediate bronchial reactions to an extract of *T. tonsurans*. Patients with chronic urticaria complicated by tinea unguium had a significantly higher positive rate of dermatophytes antigen prick tests than other fungal controls [248].

Dermatophytes are pathogenic fungi as well as important allergens, which is one of the crucial risk factors for atopic allergic disease development or exacerbation. However, only a few papers reported an association between tinea capitis and

allergic disease, which suggests that this combination may have been overlooked. The aim of this study was to analyse the clinical features and risk factors of children with tinea capitis complicated with allergic diseases and to contribute to accurate clinical management and prevention.

PATIENTS AND METHODS

Study population

We conducted a retrospective study at the Department of Dermatology and Department of Allergy, the Third Affiliated Hospital, Sun Yat-Sen University, Guangzhou, China from August 2007 to August 2022. This study included tinea capitis patients (< 14 years old), who had a clinical presentation of tinea capitis and confirmed by mycological tests, including direct microscopic examination and fungal culture from scalp hair specimens. Causative fungal species were identified based on the morphological characteristics of colonies on Sabouraud dextrose agar, and PCR using primers targeting internal transcribed spacer regions for species with uncertain phenotypic identification.

Return survey

One hundred fifty-one pediatric patients with tinea capitis were seen between 2007 and 2022. The process of returning information is illustrated in figure 1. A questionnaire survey was conducted on all patients and 127 responded. Questions mainly included demographic details, characteristics of tinea capitis, exposure to animals, personal history, family history of tinea, treatment, and outcome. For respondents with an allergic history, a structured questionnaire with follow-up questions according to international guidelines on atopic dermatitis [249, 250], allergic rhinitis [251], asthma [252], urticaria [253] and food allergy was sent.

Children with tinea capitis and accompanying allergic diseases that did not reach a conclusive diagnosis were excluded. Informed consent was obtained from all participants. This study complied with the ethical policies required by the journal and was approved by the ethics committee of the Third Affiliated Hospital, Sun Yat-Sen University (approval number 2019-01-578-01).

Statistical analysis

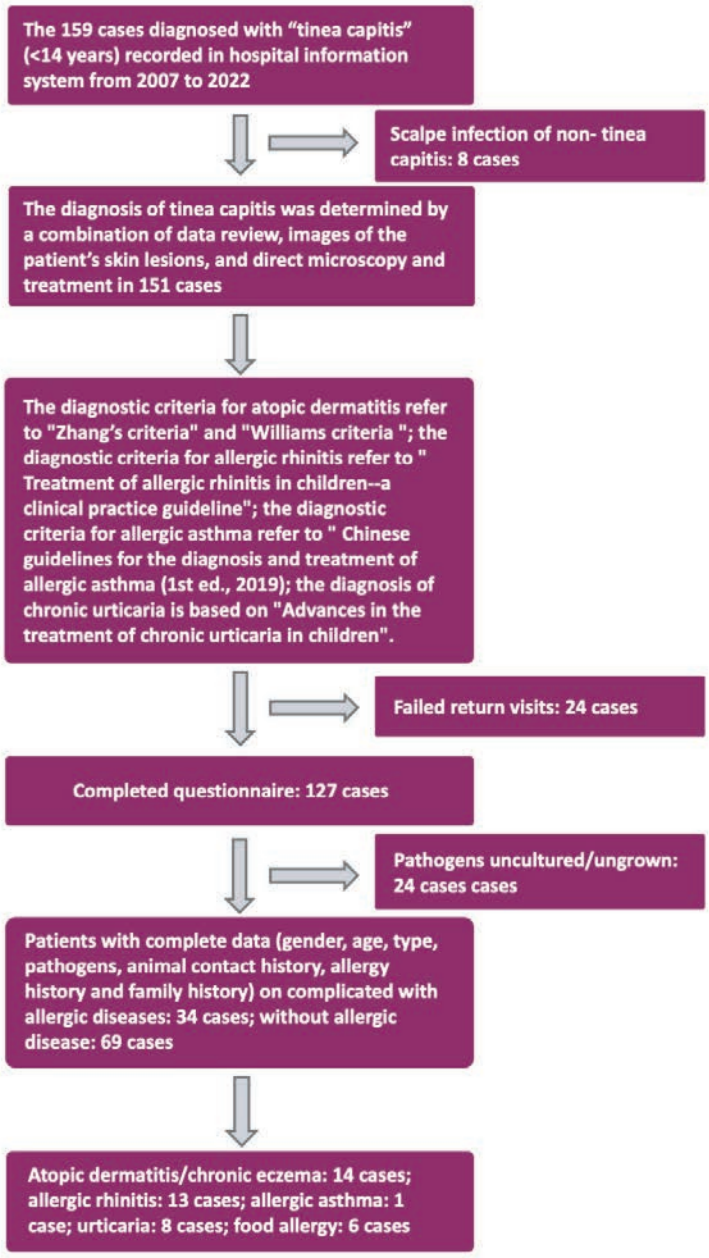

Figure 1. Conceptual flowchart of this study's investigation, including the number of cases included in the study at each step and the reasons for exclusion.

All data were statistically analysed in SPSS Statistics v27.0. The chi-square test was used to compare the differences in risk factors between groups, and multivariate logistic analysis was used to analyse the risk factors for tinea capitis combined with allergic disease. $P<0.05$ was considered statistically significant.

RESULTS

Epidemiological survey of tinea capitis

There were 127 patients with tinea capitis, of which 81 (63.8%) were male, with a male-to-female ratio of 1.76:1. The mean age was 4.83 years, with 2-7 years (74%) being the common age of our patients, age 5 (18.1%) being the highest incidence, and with 5 patients (3.9%) older than 11 years (Figure 2). Grey patch was the most common type of pediatric tinea capitis (56.7%, 72/127), followed by kerion (33.9%, 43/127) and black dot (9.4%, 12/127).

From 2007 to 2022, the number of tinea capitis were 56, 33, 38 per five-year period, respectively, and the mean age were 5.3 years, 4.43 years, 4.96 years, respectively. The children with tinea capitis were mainly boys, counting for 62.5%, 54.5%, 73.7% per five-year period. There were 17 tinea capitis cases that were caused by *M. canis* between 2007 and 2011, follow by *T. mentagrophyte* (n = 7), *T. violaceum* (n = 6), *M. ferrugineum* (n = 2), *N. gypsea* (n = 2), *T. tonsurans* (n = 2) and *T. rubrum* (n = 1), and 19 cases were without culture results. Between 2012 and 2016, there were *M. canis* (n = 16), *T. mentagrophytes* (n = 6), *T. violaceum* (n = 4), *T. rubrum* (n = 1), and *N. gypsea* (n = 1), and 5 cases without culture results. Between 2017 and 2022, there were *M. canis* (n = 18), *T. mentagrophyte* (n = 10), *T. violaceum* (n = 4), *M. ferrugineum* (n = 3), *N. gypsea* (n = 2) and *T. tonsurans* (n = 1) (Figure 3). Zoophilic *M. canis* and *T. mentagrophytes* have been the most prevalent infections over the previous 15 years and the proportions remained relatively constant every five years, with 45.9% of *M. canis* and 18.9% of *T. mentagrophytes* in 2007-2011, 57.1% *M. canis* and 21.4% *T. mentagrophytes* in 2012-2016, and 47.4% *M. canis* and 26.3% *T. mentagrophytes* in 2017-2022.

Comparison of tinea capitis with and without complication by allergic disease

There were 34 (26.8%) children with infections complicated with allergic disease (Table 1). Their mean age was 5.23 years and the male-to-female ratio 2.78:1. There were 14 children with AD or eczema, 13 children with AR, 8 children with urticaria,

6 children with food allergy and 1 child with AS (Figure 4). Parents or family members of 7 children with tinea capitis also had a history of allergic diseases, 5 of whom had AD or eczema, 5 had AR, 2 had food allergies and 1 had AS (Table 1). Of these 34 children, 44.1% had grey patch tinea capitis (n = 15), 41.2% had kerion (n = 14) and 14.7% had black dot (n = 4). 55.9% of the patients had a history of animal contact. In 9 (26.5%) children, Id reactions developed during tinea capitis infection, and all of these occurred in kerion. The causative agents were isolated in 17 cases of *M. canis*, 10 of *T. mentagrophytes*, 5 of *T. violaceum* and 2 of *M. ferrugineum*. Zoophilic species accounted for 79.4% of patients complicated with allergic diseases. The mean age of the 69 children in tinea capitis without allergic disease was 4.24 years and 43 were males, the male-to-female ratio was 1.65:1. Among these patients, 60.9% (n = 42) of tinea capitis was gray patch, 29% (n = 20) was kerion and 8.7% (n = 7) was black dot. 26% of the patients had a history of animal contact, of which 2 were human-to-human infections. 15.9% of patients were found to have Id reaction during the infection. The pathogenic species were *M. canis* in 34 cases, follow by *T. mentagrophytes* (n = 13), *T. violaceum* (n = 9), *N. gypsea* (n = 5), *M. ferrugineum* (n = 3), *T. tonsurans* (n = 3) and *T. rubrum* (n = 2). Zoophilic *M. canis* and *T. mentagrophytes* together accounted for 68.1% of patients without allergic diseases.

Table 1 . Clinical features of tinea capitis complicated with allergic disease

No	Gender	Age (year)	Type of tinea capitis	Pathogen	Contact with animals	Id reactions	Allergy history	Family history
1	M	12	Kerion	*M. canis*	Cat	Yes	Eczema	
2	F	5	Gray patch	*M. canis*	Cat	No	FA	Mother had mango allergy
3	M	4.2	Kerion	*M. canis*	Cat/dog	Yes	Urticaria	Elder brother had oat allergy and mother had AR
4	M	5.8	Kerion	*M. canis*	Cat	No	AR	
5	M	2	Gray patch	*M. canis*		No	AD, AS, AR and FA	Mother had AD
6	F	3	Black dot	*T. violaceum*	Cat/dog	No	AR, FA	Both grandmother and father had AD and tinea capitis history
7	M	8	Gray patch	*T. mentagrophytes*	Cat	No	Eczema and AR	Father had AR and neurodermatitis
8	M	5	Kerion	*M. canis*		No	Urticaria	
9	M	11	Gray patch	*M. canis*	Cat/dog	No	AR	

Table 1 . (continued)

No	Gender	Age (year)	Type of tinea capitis	Pathogen	Contact with animals	Id reactions	Allergy history	Family history
10	M	4	Black dot	*T. violaceum*		No	FA	Father had AR
11	M	7	Gray patch	*M. canis*	Cat/dog	No	AR	Two elder brothers had AS and eczema respectively
12	M	6	Gray patch	*T. mentagrophytes*	Cat/dog	No	Urticaria	
13	F	6	Kerion	*T. mentagrophytes*		No	Urticaria	Mother had tinea pedis
14	M	6	Kerion	*M. canis*	Cat	Yes	AD	
15	M	3	Black dot	*T. violaceum*	Cat/dog	No	AD	
16	M	4	Kerion	*T. mentagrophytes*	Cat	Yes	AD, AR	
17	M	6	Kerion	*T. mentagrophytes*		No	AR	
18	F	4	Gray patch	*T. mentagrophytes*		No	AR	
19	M	3	Gray patch	*M. ferrugineum*	Dog	No	AD	Cousin had tinea capitis
20	M	4	Gray patch	*M. canis*		No	Urticaria	
21	F	3	Kerion	*M. canis*		Yes	Eczema	
22	F	5	Kerion	*T. mentagrophytes*		Yes	AR, FA	
23	F	6	Gray patch	*T. mentagrophytes*		No	Urticaria	
24	M	6	Gray patch	*M. canis*	Cat	No	AD, AR	
25	M	6	Kerion	*M. canis*		No	Eczema	
26	M	2	Gray patch	*M. ferrugineum*	Dog	No	AR, AD, AS	Father had AR
27	M	6	Kerion	*M. canis*	Cat	Yes	AD	Mother had tinea capitis
28	M	8	Black dot	*T. violaceum*		No	Urticaria	
29	M	7	Kerion	*M. canis*	Cat/dog	Yes	AD, AR	Mother had AD
30	M	5	Kerion	*T. mentagrophytes*	Cat	No	AR	

Table 1 . (continued)

No	Gender	Age (year)	Type of tinea capitis	Pathogen	Contact with animals	Id reactions	Allergy history	Family history
31	F	5	Black dot	*T. violaceum*		No	Eczema	
32	M	1	Gray patch	*M. canis*		No	Urticaria	
33	M	3	Gray patch	*T. mentagrophytes*	Rabbit	No	AD	
34	F	6	Gray patch	*M. canis*		No	FA	

M, male; F, female; AD, atopic dermatitis; AR, allergic rhinitis; AS, allergic asthma; FA, food allergy

Risk factor assessment in tinea capitis complicated with allergic disease

The complete data of 103 patients included 34 cases with tinea capitis complicated with allergic disease and 69 patients without allergic disease. We compared the clinical characteristics of children with tinea capitis complicated with allergic disease with those without allergic disease (Table 2). The effect of these risk factors on tinea capitis was initially analysed using tinea capitis combined with allergic disease as the dependent variable and age, gender, type of tinea capitis infection, history of animal contact, type of pathogens and family history of allergic disease as independent variables. Among children with combined allergic diseases, the proportion of males, kerion and black dot, history of animal contact, family history of allergic disease and zoophilic species infection was higher than in children with normal tinea capitis (Table 2). An exploratory multivariate logistic analysis using gender, type of tinea capitis, history of animal contact and type of pathogens was conducted to investigate the impact of these clinical characteristics on disease. In our study, cases with a family history of allergic disease were only present in the group of patients complicated with allergic disease, and to avoid complete separation in the logistic analysis, we excluded this indicator. The results showed that the area under the ROC curve was 0.67 (P=0.0059), the Pseudo R squared was 0.11, the Hosmer-Lemeshow hypothesis test P=0.8, indicates that the model was recognized for its predictive accuracy. In this model, the risk factors being assessed were male (OR = 2.338, 95% CI 0.8233 to 7.355), kerion type (OR = 1.462, 95% CI 0.4783 to 4.403), zoophilic pathogens (OR = 1.513, 95% CI 0.4713 to 5.455), history of animal contact (OR = 3.113, 95% CI 1.166 to 8.698). Of these 4 indicators, the history of animal exposure was significantly different between patients with and without allergic disease (P=0.0084) (Figure 5).

Table 2. Comparison of epidemiological factors for tinea capitis with and without allergic disease

		With allergic disease (n=34)		Without allergic disease (n=69)		P-value chi-square test	P-value Logistic regression
Epidemiological factors		**n**	**%**	**n**	**%**		
Age (year)		5.23		4.24		0.4807	
Gender	Male	25	73.5	43	62.3	0.2586	0.3987
	Female	9	36.5	26	37.7		
Type	Kerion	14	41.2	20	29	0.216	0.8765
	Gary patch	15	44.1	42	60.9	0.1078	
	Black dot	4	14.7	7	8.7	0.8024	
Id reaction		9	26.5	11	15.9	0.204	
Animal contact		19	55.9	18	26	0.003	0.0084
Family history	Tinea	4	11.8	2	2.9	0.0708	
	Allergic history	9	26.5	0	0	<0.0001	
Pathogens	Zoophilic	27	79.4	47	68.1	0.1067	0.4238
	Anthropophilic	7	0.20588	17	24.6	0.3622	
	Geophilic	0	0	5	7.2	0.1076	

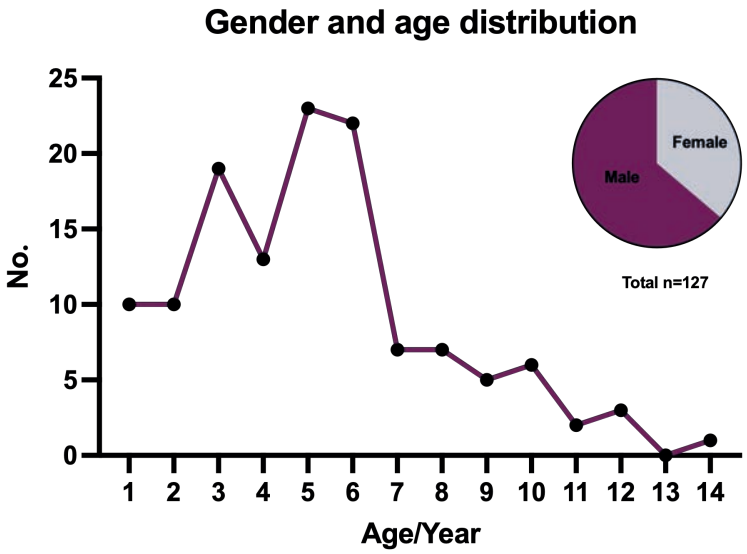

Figure 2. Gender and age distribution of patients with tinea capitis

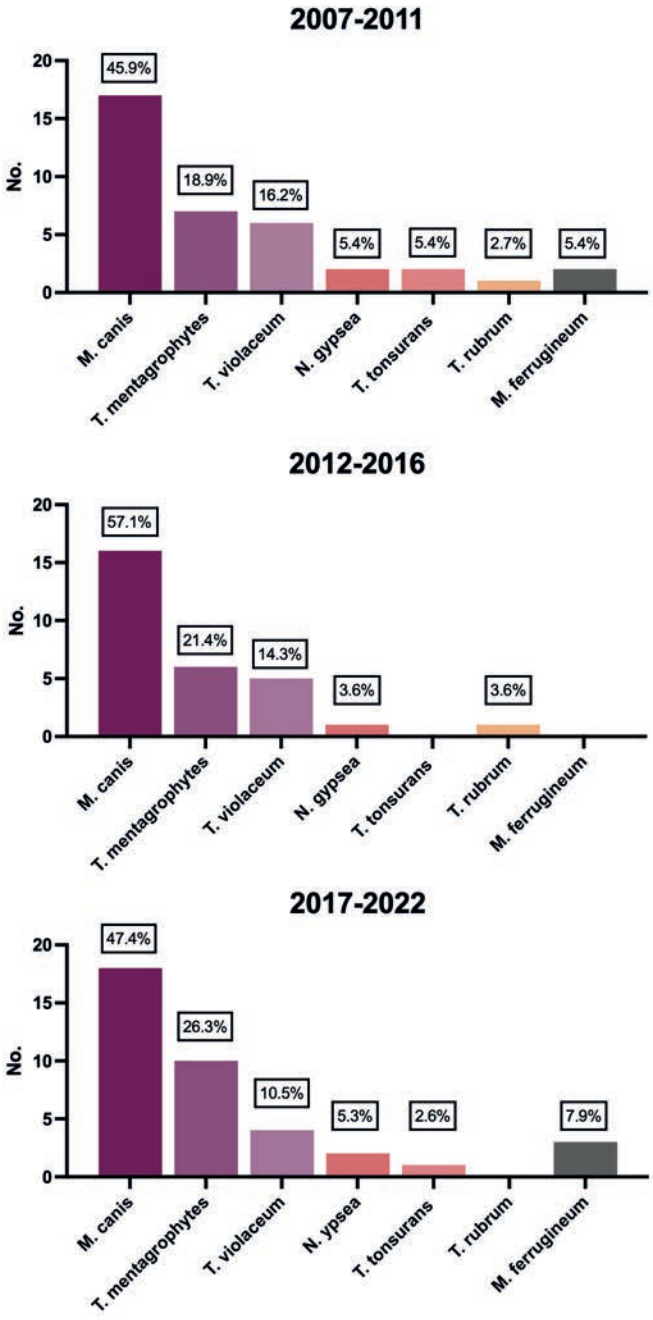

Figure 3. Proportion of change in tinea capitis pathogen spectrum per five-year period from 2007 to 2022

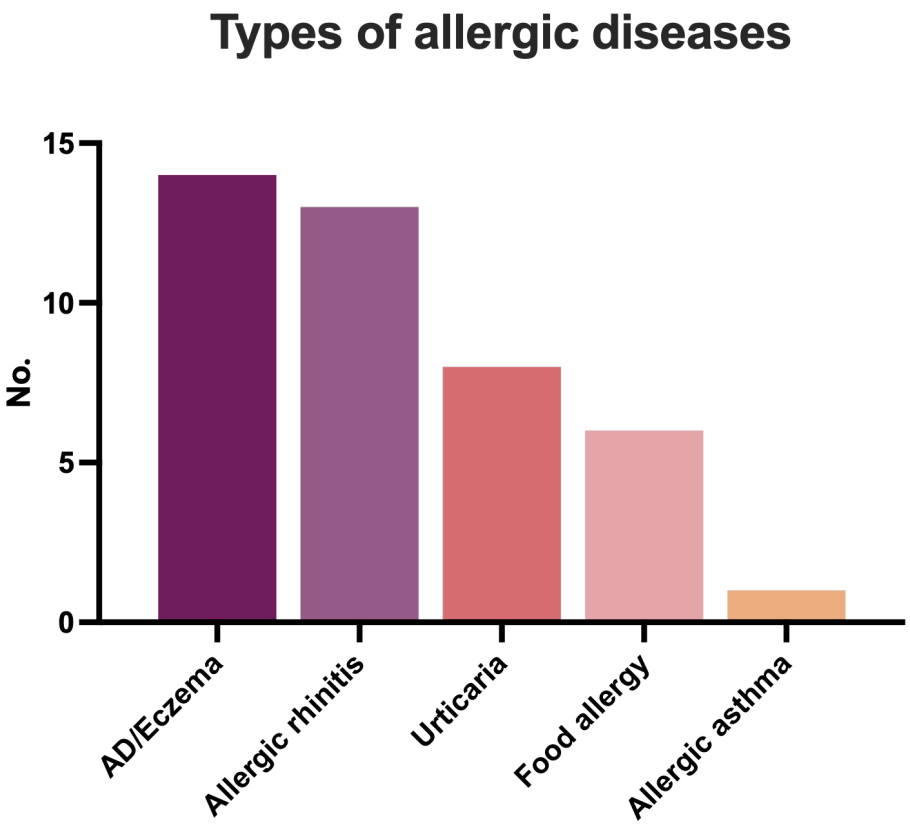

Figure 4. Types of allergic diseases in tinea capitis patients complicated with allergic diseases

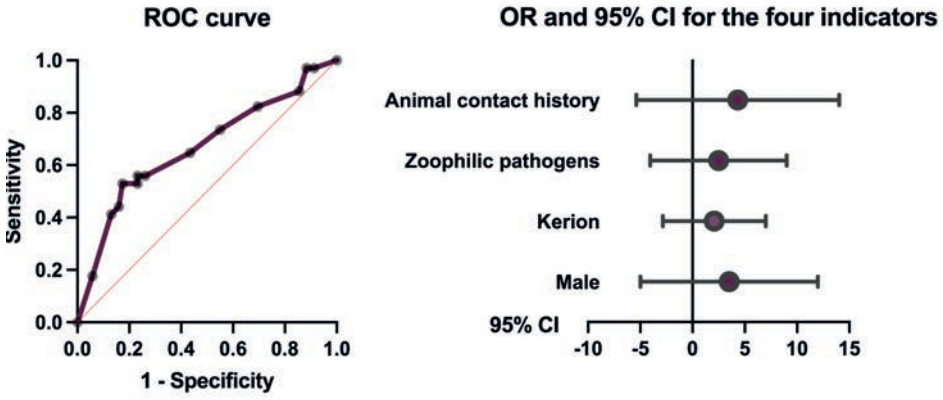

Figure 5. ROC curves and ORs for the four risk factors

DISCUSSION

In this study, we first retrospectively studied the epidemiological characteristics and pathogen spectrum of children with tinea capitis over the past 15 years. We observed that the average age of children with tinea capitis in our hospital was 4.83 years, which is consistent with the recent a retrospective study tinea capitis in China that most patients were prepubertal children aged approximately 5 years [254]. From 2007 to 2011, the proportion of patients with tinea capitis aged 2 to 5 years old was 39.3% (22/56), compared to 51.5% (17/33) in 2012-2016 and 68.4% (26/38) in 2017-2022. Of the 127 children with tinea capitis in this study, 81 (63.8%) were male and 46 (36.2%) were female, with a male-to-female ratio of 1.76:1. This is higher than the ratio of 1.28:1 found in a previous study from China, where the percentage of female patients was 43.8% [13]. Gender plays an important role in immune responses, including susceptibility to fungal infections, possibly due to sex-specific genetic architecture, sex-differentiated responses to disease susceptibility may be caused by direct and indirect effects of sex hormones as well as non-hormonally biased effects of the X and Y chromosomes [255].

Kerion caused zoophilic pathogens are reported more common in children than in adults [256], Wang *et al.* reported that the incidence of kerion in childhood was 13.9% (10/72), which was significantly higher than that in adults (3.8%, 4/104) [256]. In our study, gray patch (59%, 75/127) was the most common, followed by kerion (36.2%, 46/127) and black dot (9.4%, 12/127). Though the annual incidence of tinea capitis varied over the survey period, with 56 patients in 2007-2011, 33 patients in 2012-2016, and 38 patients in 2017-2022, the percentage of the main pathogen *M. canis* every five years remained relatively stable, i.e., 45.9%, 57.1%, and 47.4%, respectively. The predominant pathogens were the zoophilic species *M. canis* (49.5%, 51/103) and *T. mentagrophytes* (22.3%, 23/103), accounting for more than 70% of cases, with *T. violaceum* (13.5%,14/103) predominating among the anthropophilic species. Animal contact history was associated with zoophilic pathogen infection, and the percentage of children with a history of animal contact was 35.9% (32/103) in this study, *M. canis* 59.4% (19/32) and *T. mentagrophytes* 18.8% (6/32) being the main pathogens in this patient cohort, which is consistent with previous studies [13, 254, 257].

The frequency of allergic diseases has increased over the last century. Allergic diseases including AD, AR, AS, and food allergy affect about 20% to 35% of all children, and many of these conditions share similar risk factors. In our study, the prevalence of tinea capitis in children with allergic disease was 26.8%, which was similar with the worldwide report [258]. The results of the logistic analysis suggest

that male, kerion, animal contact history and zoophilic dermatophyte infection are risk factors. The male-to-female ratio in tinea capitis in the allergic disease group is 2.78:1, which is much higher than that of the group without allergic diseases (1.65:1). Although the reason for sex disparity in many diseases remains unclear, sex has been identified as an important factor influencing the clinical presentation of infection in allergic diseases. Male children have been reported to be more susceptible to childhood wheezing [259], asthma [260], AD [261] and food allergy [262]. Epidemiological findings indicated that tinea capitis caused by *M. canis* is more common in males, while there is no major gender difference in tinea capitis caused by *T. violaceum* [263, 264]. With the rise of the pet ownership boom, cases of tinea capitis caused by zoophilic species are rapidly increasing, animal fur and dander may act as dual risk factors for both tinea capitis and allergic diseases.

Kerion is a severe inflammatory subtype of tinea capitis, caused by a hypersensitivity reaction of the host to dermatophyte antigens [265]. Outside of the primary lesion site, the Id reaction has also been shown to generate a secondary inflammatory response to dermatophytosis, caused by an immediate, cytotoxic, immune complex or delayed-type hypersensitivity reaction mediated by host T lymphocytes. Dermatophytids that accompany tinea capitis are characterized by diffuse pruritic papules located mainly on the trunk, which are mainly caused by a Th2 cell-mediated delayed immune response of type IVb. There have been consistent reports associating allergic diseases with dermatophyte infection. In our results, AD/chronic eczema and allergic rhinitis were the most common combined allergic diseases in children with tinea capitis. Interactions among genetic, skin barrier dysfunction [266] and reduced production of epidermal antimicrobial peptides [267] in AD patients have been suggested to predispose to susceptibility factors leading to skin infections. It has been suggested that superficial fungal infections induce a Th2 response that exacerbates atopy, Th1/Th2 immune dysregulation may further lead to persistent infection [268]. Th2 cytokines induce B cell recruitment of eosinophils and IgE class switching, leading to a worsening of atopic conditions, from rhinitis and asthma to atopic dermatitis [269]. The use of *M. canis* to construct a mouse model of skin infection revealed that IL-17 controls *M. canis* infection and downregulates the Th1 inflammatory response [270], and that the downregulation of the Th1 immune response may further cause the imbalance towards Th1/Th2 response [271, 272]. In addition, there is cross-reactivity between fungal allergens of different genera/species, e.g., a patient allergic to airborne moulds can also have a hypersensitivity reaction when exposed to dermatophytes [273-278]. The possible cause of the pathogenic role of dermatophytes in allergic rhinitis or asthma is the long-term colonisation of atopic individuals by the fungus. Systemic exposure to allergens may promote the binding of allergens to antigen-

presenting cells (APC) in the blood, which preferentially localise at the site of allergic inflammation and induce the production of Th2 cytokines by naive T cells [279], and the properties of dermatophyte antigens may also promote the migration of T cells to the site of infection, leading to allergic disease [268]. In addition, there is cross-reactivity between fungal allergens of different genera/ species, e.g., a patient allergic to airborne moulds can also have a hypersensitivity reaction when exposed to dermatophytes [273-278]. Zoophilic dermatophytes are responsible for inflammatory reactions probably due to their poor adaptation to the human host. There has been little investigation on the immune responses of hosts infected with zoophilic and anthropophilic dermatophytes, but it is now evident that they differ significantly in genotype, capacity to adhere to and break down host keratin, and virulence [280].

In conclusion, *M. canis* and *T. mentagrophytes* have been the predominant tinea capitis pathogens in our hospital for the past 15 years, with 5 years being the age of high incidence, and the male-to-female ratio 1.76:1. Male gender, inflammation, zoophilic species, and history of animal contact were features prevalently observed in patients with allergic disease and tinea capitis. Although there has been much literature supporting the association between dermatophyte infection and allergic disease, this is the first study to analyse this topic systematically, which can provide a reference for improvement of clinical management and prevention. Timely analysis of the possible progression of the disease upon the patient's initial visit, particularly in children with a history of allergic disease, is essential to consider a combination of antifungal and anti-allergic treatment to reduce the risk of causing irreversible damage. For some refractory allergic diseases, clinicians also need to consider the potential risks associated with fungal infection. Future prospective studies with larger sample sizes are needed to further confirm this relationship, focusing on the measurement of atopic indicators in tinea capitis patients and the therapeutic implications of this diagnosis.

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Competing Interests

The authors have no competing interests to declare that are relevant to the content of this article.

Author Contributions

XZ: conceptualisation; methodology; Writing, original draft preparation; writing, review and editing. WTL: return survey and investigation. Sulian Yang: data collection. AW: return survey and investigation. PRC: return survey and investigation. SCY: conceptualisation. SDH: writing, review and editing; supervision. MRL: methodology; data collection. PYF: conceptualisation; funding acquisition; supervision; writing—review and editing.

Ethics approval

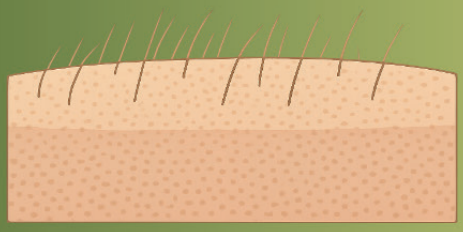
This study complied with the ethical policies required by the journal and was conducted in compliance with the protocol approved by the ethics committee of the 3rd affiliated hospital of Sun Yat-sen University (approval number 2019-02-578-01). Written informed consent was obtained from the patients (for patients aged >8 years) and their guardians (for patients aged <18 years) before enrollment.

Consent to participate

Informed consent was obtained from all individual participants and their parents participants included in the study.

Consent to publish

No other individual person's data was used in this manuscript.



CHAPTER 4

AETIOLOGY OF TINEA OF VELLUS HAIR AND SUBSTRATE SPECIFICITY IN *MICROSPORUM* SPECIES

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ABSTRACT

Tinea of vellus hair is a dermatophyte infection with or without localised infection of adjacent skin. The epidemiology and pathogenesis of this disorder have insufficiently been established. To gain insight into the epidemiology and analysis dissection of patterns of vellus hair infection. A comprehensive examination of our series of cases and existing literature, clinical information was performed. *Microsporum* species were assayed for levels of keratinase and *SUB1-3* level after different keratin-induced cultures. The infection affects both children and adults, with a male-to-female ratio of 1:1.3. The most afflicted areas are the face, neck, and upper limbs. Both typical (ringworm-like) and atypical (tinea incognito) tinea lesions can be observed. The disorder is caused primarily by the zoophilic species *Microsporum canis* (40.39%). Topical antifungal drugs alone are ineffective; mycological cure is achieved in combination with systemic antifungal therapy for 4-8 weeks. *In vitro*, *M. canis* exhibited significantly elevated levels of secreted protease and *SUB1* and *SUB3* transcripts in cat hair cultures. Conversely, the findings of anthropophilic dermatophytes *M. audouinii* and *M. ferrugineum* demonstrated the opposite trend, suggesting that there are associations between keratinase activity and expression of *SUB1* and *SUB3* induced by different keratin substrates among *M. canis* complex members. The epidemiological characteristics of tinea of vellus hair help us to understand its transmission patterns and develop effective prevention strategies. There are associations between keratinase activity and expression of *SUB1-3* induced by different substrates, which may explain the host shift and adaptation from pet animals to particular micro-habitats on the human host.

INTRODUCTION

Dermatophytosis is a disease of worldwide importance and a major public health problem, being one of the most common fungal infections among contagious patients [281]. The dermatophytes compose a unique group of fungi, able to infect keratinous tissues and invade hair, skin, and nails of humans and other mammals. Dermatophytosis is usually classified according to the site of infection. Tinea capitis refers to dermatophyte infection of the scalp and hair shafts (https://www.osmosis.org/learn/Development_of_the_integumentary_system), while agents of tinea barbae affect facial hair follicles of bearded individuals. Both tinea capitis and tinea barbae are infections of terminal hairs. These hairs can be recognized by their coarse and hard structure and intense coloration. In contrast, vellus hair is fine and soft, with light pigmentation. Vellus hair is predominantly distributed over the glabrous skin, such as face, trunk, and limbs [282-284]. In case of tinea of vellus hair, the hairs are affected by hypha and/or spores internally and on the outside, and concomitant localised skin infection can be observed. Despite the widespread presence of vellus hair on the human body, limited data is available regarding the epidemiology of vellus hair infection, compared to that of the more pronounced presence of tinea capitis; tinea of vellus hair has therefore long been regarded as a rarely encountered clinical entity.

A variety of reasons can lead to underdiagnosis and misdiagnosis of the disorder. Frequent washing and application of topical steroids tend to lead to loss of its typical features of the tinea. Inappropriate recognition of vellus hair infection may result in misdiagnosis as tinea faciei, tinea corporis, or other forms of glabrous skin dermatophytosis. The first case of tinea of vellus hair was reported in 1971 in a 3-year-old girl who with an inflammatory facial infection caused by *Nannizzia incurvata* [16]. Later, series cases were reported from Italy [17], Spain [18, 285], China [20, 21], and Thailand [286]. Gómez-Moyano *et al.* [18] prospectively analysed 13 cases of tinea of vellus hair infection and noted poor resolution after topical antifungal treatment, but all cases healed properly after oral antifungal treatment, with no recurrence. The authors suggested that systemic antifungal therapy is necessary for patients with vellus hair infection, whereas topical antifungal therapy is sufficient for glabrous skin infections that only invade the stratum corneum.

Subtilisins (SUBs) are extracellular serine endopeptidases which play crucial roles in the virulence and adhesion capabilities of dermatophytes. Earlier studies have provided evidence of a noteworthy association between the level of keratinase activity and both the clinical presentation and prognosis of infections by

Microsporum canis and relatives [287]. Strains exhibiting elevated *in vitro* keratinase activity have been observed to induce acute infections in experimental animals. In *M. canis*, the Subtilisin family is known to be encoded by *SUB1*, *SUB2* and *SUB3*. These proteases are produced by *M. canis* during invasion of keratinized structures, but it has not been possible to directly link the *SUB*s keratinase activity of the fungus [39].

The present study aims to provide an overview of tinea of vellus hair with a series of 23 cases, to review the literature, to evaluate the clinical presentation and the spectrum of fungi implicated in causing the infection. Subsequently, the keratinase activity of *SUB1-3* and the effect of different substrates on the *M. canis* complex keratinase production in various hair types and nail were analysed, to reveal the peculiarities of tinea of vellus hair.

PATIENTS AND METHODS

Case series and literature review

Patients from outpatient clinics of the Department of Dermatology & Allergy, third Affiliated Hospital, Sun Yat-Sen University, who were diagnosed with tinea of vellus hair from September 2021 to October 2023, were enrolled. Upon the first visit to our department, patients were subjected to dermoscopic (Dermosopy-II, Beijing), the skin flakes were scraped and vellus hair was plucked for direct microscopic examination in potassium hydroxide (KOH) and fluorescence (Shuokang, Beijing). Isolates were incubated in Potato Dextrose Agar (PDA, Oxoid, Germany) at 28°C for 14 days and identified by sequencing using the ITS1/ITS4 rDNA region [192]. Patients with vellus hair infection were treated with oral itraconazole for 4-8 weeks and topical antifungal medication for 4 weeks. Clinical information including demographic details, characteristics of tinea of vellus hair, exposure to animals, personal history, family history of tinea, treatment and outcome were also collected.

Published literature on tinea of vellus hair was reviewed in the China Academic Journal Network Publishing Database (CNKI), PubMed and the Web of Science from 2000 until September 2023, using search terms “tinea of vellus hair” OR “tinea vellus hair infection”. Articles describing tinea of vellus hair but without fungal microscopic or histopathological findings were excluded. The resulting literature was manually analysed, and demographic details, characteristices of tinea of vellus hair, history of animal exposure, and family history of tinea were extracted from the literature.

Strains and keratin-induced fermentation culture

Strains of *Microsporum canis*, *M. audouinii* and *M. ferrugineum* were obtained from the Radboud University Medical Center mycological collection. Cat hair, children's scalp hair, vellus hair and nail were ground into keratin powder and lyophilised, which was later used in keratinase-inducing medium (KH_2PO_4 2 g, $MgSO_4 \cdot 7H_2O$ 1 g, peptone 5 g, glucose 20 g, keratin powder 2.5 g, dissolved in 1000 mL distilled water); keratin powder was omitted from the control group. Each 20 mL fermentation medium was dispensed separately into 50 mL triangular flasks and autoclaved. *Microsporum canis*, *M. audouinii* and *M. ferrugineum* were grown on PDA for 14 days. Conidia/mycelial fragments were eluted from culture plates with sterilized saline and suspensions at concentrations of (0.5-1) × 10^7 CFU/mL were prepared using the modified EUCAST broken mycelium inoculation method [288]. Four isolates of each species were selected, inoculated separately into three triangular flasks, then 1 mL of suspension was added to each flask, and cultures were incubated for 4 days at 28°C at 130 r.p.m.

Keratinase activity assay

After incubation, flasks were centrifuged at 3000 r.p.m. for 15 min and the supernatant was aspirated and filtered through a 0.22 μm filter; 1 mL of filtrate was collected and added to 1.5 mL of keratin buffer (Keratin Azure 830 mg, 1 M Tris-HCl 5 mL, $CaCl_2$ 1.11 g, pH 8.0, dissolved in 100 mL distilled water) [289]. The mixture was incubated at 180 r.p.m. for 72 h at 37°C. The reaction was terminated after 5 min, centrifuged at 3000 r.p.m. for 10 min and the absorbance of the supernatant was measured at λ 595 nm (UV-Vis Spectrophotometers, Thermo Scientific, U.S.A.).

RNA extraction and quantitative real-time PCR

The keratinase medium was centrifuged at 3000 r.p.m. for 10 min to remove excess supernatant, and mycelia were dried at 75°C for 24 h. Mycelia were quickly ground to powder under liquid nitrogen, and ground mycelia powder was added to in 1 mL of TRIzol (Invitrogen, U.S.A.) and vortexed vigorously for 1 min. After incubation at room temperature for 5 min, 200 μL chloroform was added to extract the total RNA, shaken vigorously for 15 s, and incubated again at room temperature for 3 min. After centrifugation at 12,000 r.p.m. for 15 min at 4°C, the supernatant was aspirated into a new tube, and 200 μL of chloroform was added to perform a second extraction. To the new supernatant, 600 μL of isopropanol was added, followed by incubation at −20°C overnight. Subsequently, the pellet was centrifuged at 12,000 r.p.m. for 15 min at 4°C, the supernatant discarded, and 1 mL

70% EtOH was added to wash the RNA pellet. Subsequently, the pellet was centrifuged at 7,500 r.p.m. for 5 min at 4°C discarding the supernatant, and air-dried under the hood for 15 min. Finally, the RNA pellet was suspended in 50 µL of enzyme-free water and incubated in a water bath at 65°C for 5 min until the RNA pellet was completely dissolved. cDNA synthesis was retrotranscribed with RNA and Transcriptor Universal cDNA Master mix (Roche, Mannheim, Germany). RT-qPCR transcription reactions were performed with the *SUB1-3* primers [290] and LC 480 SYBR Green I Master (Roche, Mannheim, Germany).

Statistical analysis

Statistical significance was conducted using SPSS Statistics v26. The two-way ANOVA with Tukey corrected multiple comparison test was used to compare the expression differences among the keratin-induced culture groups. The resultant graphs are produced using GraphPad Prism 9.0. Samples from each group are presented using mean ± SEM. The value of $P < 0.05$ was considered statistically significant.

RESULTS

Clinical manifestations

The 23 patients under study (Table 1, Figure 1), included 11 children and 12 adults, with a mean age of 24.13 years and a male-to-female ratio of 1:1.3. The duration of the lesions ranged from 10 days to 1 year, of which 17 within 1 year and 6 more than 1 year. The presentation of lesions was dominated by pruritic scaly erythema with scattered small papules. In 17 (73.91%) patients, the lesions formed erythematous and desquamative plaques with well-defined or relatively well-defined borders. In 2 (8.69%) of the cases, the erythema covered with yellow scales, and in 2 (8.69%) cases, hypopigmented spots and mild atrophy were present. Nineteen patients (82.60%) had used topical steroids, and 10 (43.47%) had used topical antifungal agents before consultation, of which 7 (30.43%) had previously been diagnosed with ‘tinea faciei’ or ‘tinea corporis’. The face and neck were the most frequent single site of involvement (n=16, 69.56%). Notably, all children's lesions appeared on the face (n=11, 47.82%), followed by the upper extremities (n=5, 21.73%) and anterior chest (n=5, 21.7%), only a single patient having lesions on the lower extremities. Fifteen (65.21%) patients had a single lesion, 4 (17.39%) patients had more than 2 sites involved, 4 (17.39%) patients had

primary tinea corporis/cruris, and then new-onset tinea of vellus hair appeared on the facial area.

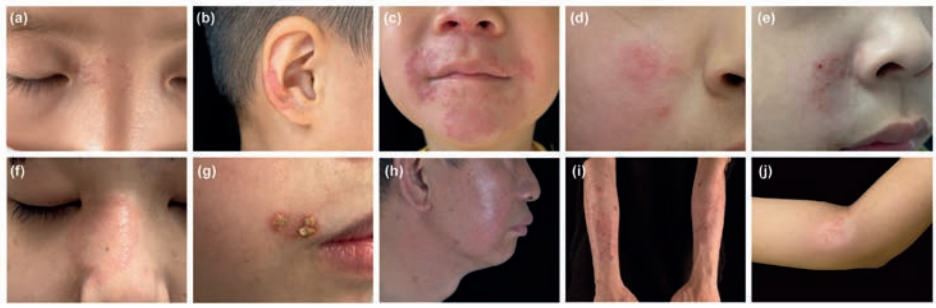

Figure 1. (A–E) Typical tinea faciei-like rash; (F–J) atypical lesions, (G) yellow scabs on erythema, (H) rosacea-like lesions with ill-defined erythema, (I) eczematous lesion, (J) hypopigmented spots.

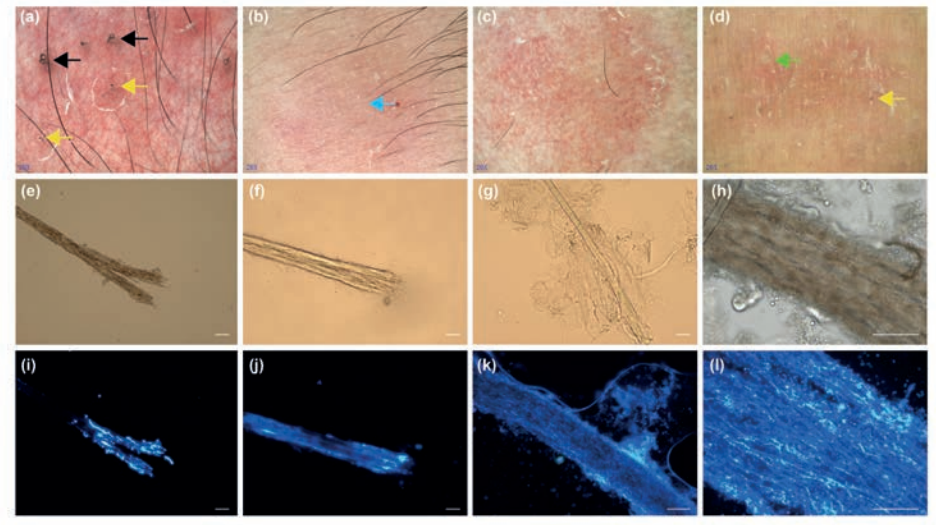

Figure 2. (A–D) Dermoscopy examination. (A) scaly plaque, scales encircling the root area and reticular blood vessels; (B, C) scaly plaques and punctate blood vessels; (D) absence of vellus hair and small pustules around hair follicles. Black arrow: spiral hair; yellow arrow: black spot sign; blue arrow: absence of vellus hair; green arrow: small pustules around hair follicles. (E–H) KOH examination. (E, F, H) Endothrix infection of vellus hair, hair shaft structure is loose, broken and split; (G) ectothrix infection of vellus hair, keratinocytes and mycelium around hair shafts. (I–L) fluorescence examination. (I–J, L) endothrix infection; (K) ectothrix infection.

Diagnosis and treatment

Under dermoscopy, we observed scaly erythema (93.63%), scales encircling the hair root (62.52%), broken hair (81.25%), spiral hair (6.25%), absence of vellus hair

(50.11%), morse code hair (37.56%), reticular blood vessels (25.23%), striated blood vessels (31.25%), punctate blood vessels (31.25%), and pustules (18.75%). No hyphae or spores were found in the dander of 5 (21.73%) patients under the microscope, but vellus hair infection was involved in all of them. Ectothrix and endothrix infections were present in most of the cases (Table 1, Figure 2).

Fourteen (60.86%) patients of tinea of vellus hair were infected by *M. canis*, 7 (30.43%) by *Trichophyton mentagrophytes* complex and 2 (8.69%) by *T. rubrum*. Fifteen cases (65.21%) had a definite history of animal contact, mainly cats. The two patients (8.69%) with *T. rubrum* infection were all over 60 years of age.

Patients with tinea of vellus hair were administered oral itraconazole 3-5 mg/kg/d (children) or 100-200 mg/d (adults) for 4-8 weeks, combined with topical antifungal medication. Regular phone follow-ups were performed on all patients, and none of the reported cases have developed new eruptions at the site of the primary lesion.

Review of the literature

A comprehensive review of 21 publications included 141 cases (Table 2). There were 114 (80.85%) cases in children and 27 (19.15) cases in adults. The male-to-female ratio was 1/1.53. The duration of lesions in patients ranged from 4 days to 10 years; 103 (73.05%) patients had received treatment with topical steroids after the onset of the disease, forty-four (31.21%) patients were treated with topical antifungal therapy for a period ranging from 4 weeks to 6 months without cure or providing mild relief of symptoms at most, followed by relapse. Typical tinea of vellus hair rash is clinically similar to tinea corporis, with ring-shaped lesion edges which are reddish and slightly elevated; the tinea has a tendency to self-healing in the centre. Sometimes the infection comprises a variety of lesions: unclear borders, edematous erythema, hypopigmented patches, folliculitis, papules, and pustules. The face and neck (n=118, 83.69%) and upper arms (n=16, 11.35%) were the most frequently affected sites, followed by the trunk (n=9, 6.38%), lower limbs (n=7, 4.96%) and buttocks (n=3, 2.13%), 8 (5.67%) cases with unclear reported sites. Eighty-nine (63.12%) had a definite animal contact history, and one had a history of family contact with a patient with tinea corporis; anamnesis was not mentioned in 18 (12.77%) cases.

The causative agent of infection was reported in 129 cases. *Trichophyton mentagrophytes* complex was the most common (n=49, 37.98%), followed by *M. canis* (n=47, 36.43%), *T. rubrum* (n=8, 6.20%), *N. gypsea* (n=17, 13.18%), *T. tonsurans* (n=4, 3.10%), *T. violaceum* (n=3, 2.33%) and *T. schoenleinii* (n=1, 0.78%).

Keratinase activity and transcript level of *SUB1-3*

We examined the keratinase activity and the transcript levels of *SUB1-3* of the zoophilic species *M. canis* and its anthropophilic siblings *M. audouinii* and *M. ferrugineum* upon incubation in different keratin substrates. The highest absorbance of protease secreted by *M. canis* was found in cat hair-induced culture, followed by scalp hair and vellus hair-induced culture, and the lowest value in the nail-induced culture ($P < 0.05$). The anthropophilic species *M. audouinii* and *M. ferrugineum* showed obvious activity with scalp hair-induced culture; only very low levels of absorbance were detected in the cat hair-induced culture. Keratinase activity levels in both anthropophilic species were slightly higher in scalp hair-induced culture than in vellus hair-induced culture; however, differences between scalp hair, vellus hair and nail-induced culture were statistically insignificant (Figure 3).

In cat hair and scalp hair-induced culture, the *SUB1* transcript level of *M. canis* was significantly higher than that of vellus hair and nail-induced culture. The *SUB1* transcript levels of *M. audouinii* and *M. ferrugineum* were lower in the cat hair-induced culture, consistent with the absorbance level of keratinase activity. For *M. audouinii*, *SUB1* transcript levels were higher in both scalp hair and vellus hair-induced culture than in cat hair-induced culture. We did not find statistically significant differences in *SUB2* transcript levels across keratin-induced cultures and between species. In the anthropophilic species *M. audouinii* and *M. ferrugineum*, *SUB3* transcript levels were significantly higher in scalp hair, vellus hair and nail-induced culture than in cat hair-induced culture (Figure 4).

DISCUSSIONS

Tinea of vellus hair is considered a rare form of dermatophytosis, in which hyphae and spores can be found not only in the stratum corneum but also within the vellus hair. In clinical practice, the appearance of this entity can be misleading. Most cases receive correct treatment only with delay and with low response to topical antifungal treatment. In this study, we present 23 cases of tinea of vellus hair, which is the second-largest documented case series to date (Table 1). We observed that tinea of vellus hair involved children, adolescents, and adults at more or less equal frequency (47.82% children, 52.18% adults), with the total patient cohort having a mean age of 24.13 years. Earlier reports from Spain and Italy [17-19, 285] showed a preponderance of children under 10 years, while in Taiwan, most reported cases were from adults [22]. Females were slightly more affected than

males, with a male-to-female ratio of 1: 1.3. Our results indicate that the sex distribution differed between children and adults, tinea of vellus hair mainly appearing in boys aged under 18 years and women aged over 18 years, which is consistent with that of tinea capitis [256]. The predominance of females among adults may be related to the degeneration of sebaceous glands due to hormonal changes and decreasing blood estrogen levels [291].

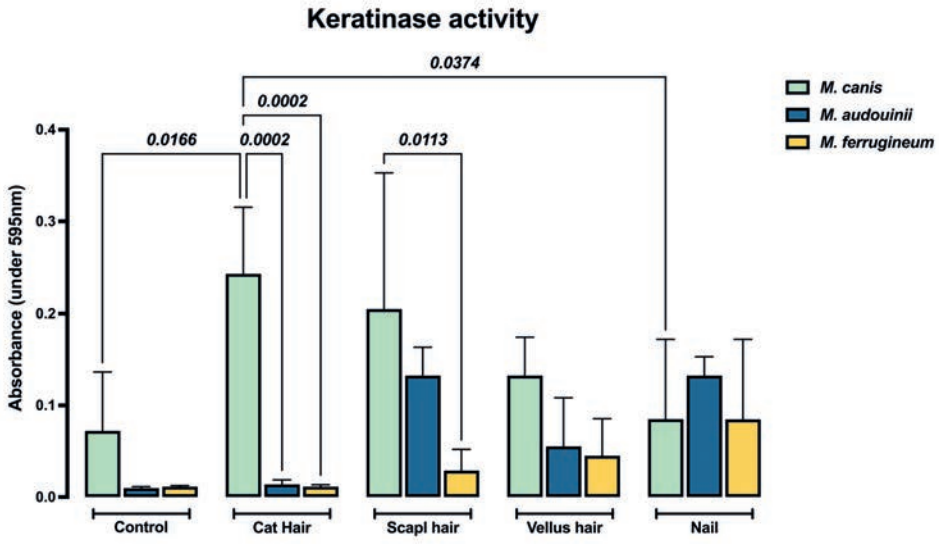

Figure 1. Absorbance level at λ 595 nm demonstrates the keratinase activity. Keratin activities of zoophilic and anthropophilic *M. canis* species were compared under different keratin substrate-induced cultures, respectively. Values are expressed as the means ± SEM deviation. n= 3–4 strains per group. Significantly different *P*-values between or within groups are shown in the figures.

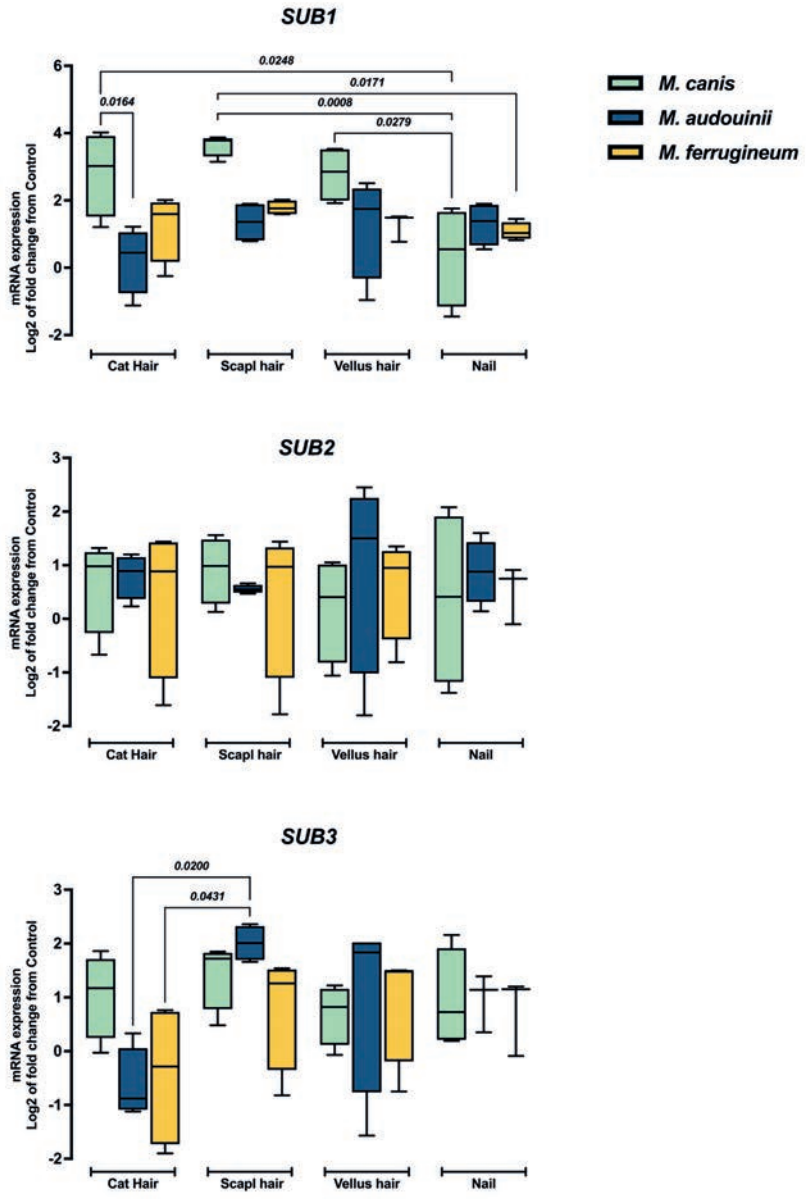

Figure 2. Relative messenger RNA (mRNA) expression (log2 fold change from Control group) of *SUB1-3* in response to different keratin substrate-induced cultures. Values are expressed as the means ± SEM deviation. n=3–4 strains per group. Significantly different *P*-values between or within groups are shown in the figures.

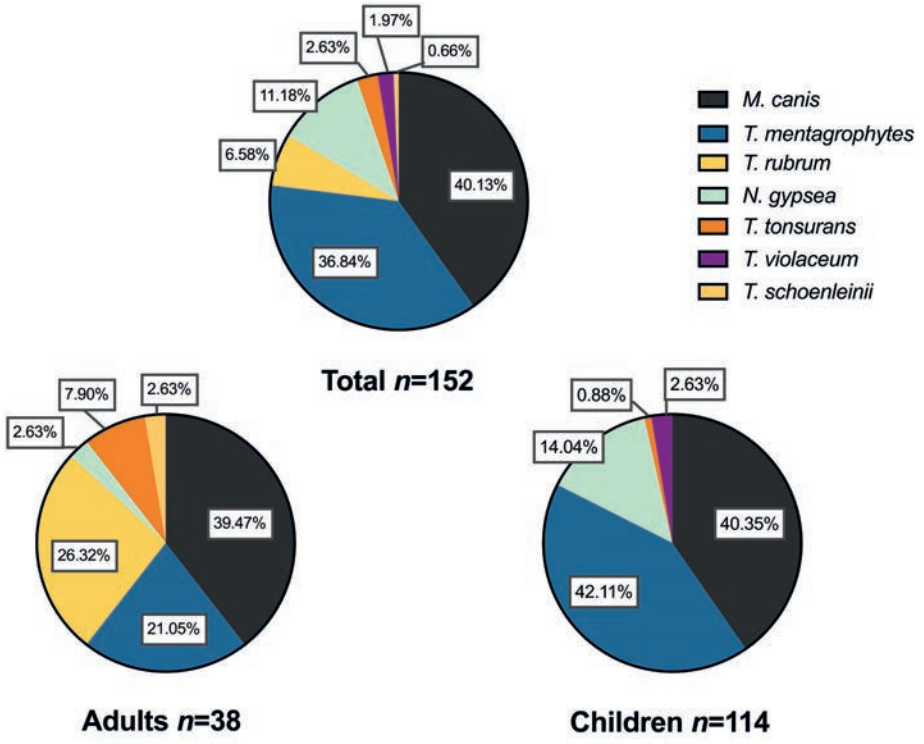

Figure 3. Distribution of tinea of vellus hairs pathogen spectrum in adults and children.

The clinical manifestations of tinea of vellus hair were quite variable. Most cases presented as typical tinea corporis or tinea faciei, having one or more round or oval erythematous scaly plaques. Other types of lesions, such as poorly demarcated erythematous plaques, telangiectasia or follicular micropustules, were also noted in some patients, presenting as tinea incognita or eczematous dermatitis; these lesions were frequently observed in patients with preceding topical steroid treatment. The use of corticosteroids may cause a local immunosuppression facilitating hair infection. As known in tinea capitis, dermoscopy proved to be a useful bedside assessment tool that helps clinicians diagnose tinea of vellus hair by the observation of broken hairs, black dots, corkscrew hair, translucent hairs, and morse code hair [292]. Short broken hairs were the most frequent dermoscopic feature, and is usually followed by black dots [293]. In addition, the use of topical antifungal medication results in a high percentage of patients (61.53-74.00%) where no fungal elements could be seen in the scales, the pathogen being limited to the hair [18, 294]. In this study and reviewed cases, 39.13% (9/23) and 30.71% (43/140) of patients, respectively, had used topical antifungal treatment prior to consultation, but the lesions continued to relapse. The duration of our cases

ranged from 10 days to 2 years, and in some published cases even as long as 10 years [22]. Therefore, it is necessary to examine both the scales and vellus hairs with direct microscopy and combined with dermoscopy. Calcofluor fluorescence microscopy served as a highly efficient and precise diagnostic tool. Gómez-Moyano & Crespo-Erchiga [9] suggested that the presence of affected vellus hairs upon direct examination in lesions of tinea of the glabrous skin would be indicative for systemic antifungal therapy. Consequently, the recommended treatment strategy for tinea of vellus hair is like that of tinea capitis [18, 286, 295].

Combining the literature review and our report, pathogens were listed in 152 cases (Table 1-2, Figure 5). Zoophilic *M. canis* (61, 40.13%) was the primary causative agent, followed by *T. mentagrophytes* complex (56, 36.84%), Geophilic *Nannizzia gypsea* (17, 11.18%) was also common, while anthropophilic species such as *T. rubrum*, *T. tonsurans* and *T. violaceum* were less common. However, there were geographic and age differences in the distribution of the dominant pathogens. Among the patients in our hospital, the predominant pathogens were the zoophilic species *M. canis* (14/23) and *T. mentagrophytes* complex (7/23), accounting for more than 90% of the cases, with only two cases caused by the anthropophilic species *T. rubrum*. In Spain, the main pathogen is *N. gypsea* followed by *T. mentagrophytes* complex and *M. canis* [18, 19, 285], while in eastern China [294], *T. mentagrophytes* complex predominates, followed by *M. canis* and *N. gypsea*. *Trichophyton rubrum* is the most common agent in Taiwan, where it was found to be the causative agent in 43.75% of the cases, closely followed by *M. canis* (37.50%) [22]. There were also differences in the distribution of pathogen spectrum in adults and children. In children, zoophilic and geophilic species of dermatophyte infections accounted for more than 96% of the cases; however, in adults, in addition to 60.52% of patients being infected with zoophilic species, infections with anthropophilic species accounted for 36.85% (Figure 5). This distribution is somewhat similar to that of the tinea capitis pathogen spectrum in adults and children [256], though zoophilic species are the dominant pathogens at all ages of tinea capitis. This proportion is significantly higher in children than in adults, where infection by the anthropophilic species *T. violaceum*, *T. rubrum*, and *T. tonsurans* is more common [296].

Tinea of vellus hair is a contagious fungal skin infection, which often spreads by direct skin-to-skin contact with an infected animal, or person or even by autoinoculation. Our results and literature review suggest that patients present with lesions on different body parts, with the most involved sites being the face and neck, followed by the upper extremities and trunk area. In pediatric patients, the lesions were mostly solitary on the face and neck. Multiple sites are often involved in adults, and although pet contact is also a common source of infection,

self-inoculation from pre-existing tinea cruris/tinea corporis may also be a common route. Interestingly, none of our cases or reported cases had a combination of vellus and scalp hair (tinea capitis) infections. It remains unclear whether the affinity of zoophilic dermatophytes varies for different kinds of hairs. The percentage of a history of animal contact was over 65.21% (15/23) in our study, and thus pet holding is an obvious risk factor for zoophilic pathogen infection [208]. In Italy, 46 cases of tinea faciei due to *M. canis* were reviewed, 39.13% (18/46) of the cases involving the vellus hair follicle. Among these patients, 91.30% (42/46) of the children had a contact history of domestic animals such as cats and dogs, while four cases resulted from contact with children exhibiting tinea capitis due to *M. canis* [17]. In general, the pathogen spectrum of tinea of vellus hair is similar to that of tinea capitis, and the zoophilic agents *M. canis* and *T. mentagrophytes* complex are among the predominant dermatophytes.

Keratinase, including proteases and peptidases, are important virulence factors and allergens of dermatophytes [42] and are essential for their ability to infect keratinous tissues [287]. The difference in keratinase activity of dermatophytes at different environmental conditions is an attribute of adaptation to parasitism [297]. Keratinase activity of *M. canis* isolated from symptomatic dogs and cats was significantly higher than that of samples isolated from asymptomatic animals [69]. Experimental infection in guinea pig model showed that strains of *M. canis* with high *in vitro* keratinase activity often cause acute, severe infections characterized by erythema, desquamation, and alopecia, whereas strains of *M. canis* with low *in vitro* keratinase activity were associated with less severe skin lesions but with longer duration of inflammation [287]. The relationship between keratinase and hair type remains to be determined. Zhou *et al.* [192] revealed an evolutionary adaptation of strains of *M. canis* from cat to humans, leading to a host shift with *M. ferrugineum* as derived anthropophilic sister species. The closely related and genomically similar species provide an excellent model for study of dermatophyte evolution that leads to adaptation to niches on the human body. Our results revealed differences in keratinolytic activity between *M. canis* showing intense keratinase production on cat hair *versus* much lower amounts produced by the anthropophilic counterparts *M. audouinii* and *M. ferrugineum*. Although lacking statistical support, a difference in the intensity of keratinase production between the species was observed with vellus hair. Possibly, preference for hair compared to glabrous skin or nail [30] is an ancestral condition in the dermatophytes.

Subtilisins are a group of serine proteases which are encoded by gene members of the *SUB* family and play an important role in dermatophytosis by decomposing keratinous structures. *SUB1-3* have been identified in *M. canis* [287] and in its sister

anthropophilic species, *M. audouinii* and *M. ferrugineum*. They are likely to play a crucial role in the anthropophilic evolution in *Microsporum*. We revealed the presence of *SUB1-3* amplicons in all strains of *M. canis* and its siblings, with significant differences in expression when different keratin substrates were offered. When cat hair was used as a substrate, the expression levels of *SUB1* and *SUB3* in *M. canis* strains were higher than those in *M. audouinii* and *M. ferrugineum*. The most derived anthropophilic species *M. ferrugineum* showed lowest expression levels for all *SUB* genes; adaptation to the human host seems to be associated with loss of keratinase activity. In experimentally infected guinea pigs, high keratinase activity was associated with increased severity of *M. canis* infections, and mRNA expression of *SUB3* was detected in the hair of the animal model, indicating a role in initial phases of *M. canis* hair infection [298]. The adherence of *M. canis* to repaired interfollicular cat epidermis was found to be greatly reduced in the presence of a serine protease inhibitor and a monoclonal antibody targeting *SUB3*, suggesting *SUB3* plays a crucial role in the adhesion process [298]. There were no significant differences in the expression of *SUB2* among different species and substrates. Ordinal level phylogenetic analysis of *SUB*s of dermatophyte species reveal a possible ancestral lineage that includes *SUB2* immediate homologs and *Aspergillus* ALP1, whereas other coding *SUB*s, such as *SUB1* and *SUB3*, are dermatophyte-specific and have emerged only through recent sequential gene duplication events [71, 299, 300].

Our study shows that tinea of vellus hair is probably an underdiagnosed entity. The epidemiological characteristics of the disease help us to understand its transmission patterns and develop effective prevention strategies. Pets are likely to be the most important infectious sources in pediatric patients, while autoinoculation might be more common in adults. Dermoscopy and Calcofluor fluorescence microscopy are useful bedside assessment tools that helps clinicians to diagnose tinea of vellus hair precisely and efficiently and may help in therapeutic management. There are associations between keratinase activity and expression of *SUB1-3* induced by different substrates among *M. canis* and its anthropophilic relatives, which may explain the host shift and adaptation from pet animals to micro-habitats on the human host.

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Author Contributions

XZ: conceptualisation; methodology; writing, original draft preparation; writing, review and editing. WTL: methodology; writing, review and editing. MRL, LW and GXZ: data collection. RW and AW: return survey and investigation. PRC: conceptualisation. STQ and HTL: methodology; data collection. SDH: writing, review and editing; supervision. PYF: conceptualisation; funding acquisition; supervision; writing, review and editing.

Ethics Statement

The authors confirm the ethical policies of the journal, as noted on the journal's author guidelines page; this study complied with the ethical policies required by the journal and was approved by the ethics committee of the Third Affiliated Hospital, Sun Yat-Sen University (approval number II2023-025-03).

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent to publish

No other individual person's data was used in this manuscript.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Table 1 . Summary of 23 cases with tinea of vellus hair

Age/ sex	Sites	Lesion duration	Manifestations	Pruritus	Previous treatment with topical steroids	Previous treatment with topical antifungals	Animal contact history	Concurrent dermatophytosis	Family exposure history	Microscopy	Pathogen
24 y/M	Bilateral upper limbs	1 y	Irregular erythema with scattered papules	Yes	Yes	Yes	Cat	No	No	Endothrix & ectothrix	*M. canis*
2 y/F	Nose bridge	2 w	Papules, erythema, well-defined borders	Yes	Yes	No	No	No	No	Endothrix & ectothrix	*M. canis*
2 y/M	Right cheek	3 m	Erythematous and finely scaly with relatively clear borders	Yes	No	Yes	No	No	Father has tinea pedis	Endothrix & ectothrix	*M. canis*
6 y/F	Right cheek	4 m	Erythematous and finely scaly with relatively clear borders	Yes	Yes	Yes	Cat	No	No	Endothrix & ectothrix	*M. canis*
19 y/F	Bilateral upper limbs	3 m	Well-defined hypopigmented spots, mild atrophy, dark spot sign	Yes	Yes	No	Cat	No	No	Endothrix & ectothrix	*M. canis*

Table 1 . (continued)

Age/ sex	Sites	Lesion duration	Manifestations	Pruritus	Previous treatment with topical steroids	Previous treatment with topical antifungals	Animal contact history	Concurrent dermatophytosis	Family exposure history	Microscopy	Pathogen
60 y/F	Face, neck	5 m	Diffuse irregular erythema, papules with fine scales	Yes	Yes	Yes	No	No	Grandson with tinea capitis	Endothrix & ectothrix	*T. mentagrophytes* complex
4 y/M	Right cheek	3 m	Pale erythema, papules with hypopigmented margins	Yes	Yes	No	Cat	No	No	Endothrix & ectothrix	*M. canis*
2 y/M	Left corner of mouth, left eyelid	10 d	Well-defined erythema, papules	Yes	Yes	Yes	Cat	No	No	Endothrix & ectothrix	*M. canis*
37 y/F	Left shoulder, right lumbar region, lower limbs	2 m	Well-defined pale red/brown spots with fine scales	Yes	Yes	No	Cat	No	No	Ectothrix	*M. canis*

Table 1 . (continued)

Age/ sex	Sites	Lesion duration	Manifestations	Pruritus	Previous treatment with topical steroids	Previous treatment with topical antifungals	Animal contact history	Concurrent dermatophytosis	Family exposure history	Microscopy	Pathogen
24 y/F	Face, neck, anterior chest, right upper limb	3 w	Rounded erythematous plaques with profuse yellow scales	Yes	Yes	No	Cat	No	No	Endothrix & ectothrix	*M. canis*
19 y/F	Nose bridge	1 w	Rounded, scaly erythematous plaques with elevated margins and black spot signs	Yes	No	No	Cat	No	No	Endothrix & ectothrix	*T. mentagrophytes* complex
62 y/M	Face, neck	2 y	Erythematous and finely scaly with relatively clear borders	Yes	Yes	Yes	No	No	No	Endothrix & ectothrix	*T. rubrum*
8 y/F	Face, upper limb	1 m	Rounded erythematous plaques with profuse yellow scales	Yes	Yes	Yes	Cat	No	No	Endothrix & ectothrix	*M. canis*

Table 1 . (continued)

Age/ sex	Sites	Lesion duration	Manifestations	Pruritus	Previous treatment with topical steroids	Previous treatment with topical antifungals	Animal contact history	Concurrent dermatophytosis	Family exposure history	Microscopy	Pathogen
6 y/F	Face	2 m	Erythematous and finely scaly with relatively clear borders	Yes	Yes	No	Rabbit	No	No	Endothrix & ectothrix	*T. mentagrophytes* complex
52 y/M	Hand, upper limb	3 w	Diffuse irregular erythema, papules with fine scales	Yes	Yes	No	Dog	No	No	Endothrix & ectothrix	*T. mentagrophytes* complex
5 y/M	Face	2 w	Erythematous and finely scaly with relatively clear borders	Yes	Yes	No	Cat	No	No	Endothrix & ectothrix	*M. canis*
2 y/M	Face	1 w	Erythematous and finely scaly with relatively clear borders	Yes	Yes	No	Cat	No	No	Endothrix & ectothrix	*T. mentagrophytes* complex
24 y/F	Face, neck	1 w	Rounded erythematous plaques with profuse yellow scales	Yes	Yes	No	Cat	No	No	Endothrix & ectothrix	*M. canis*

Table 1 . (continued)

Age/ sex	Sites	Lesion duration	Manifestations	Pruritus	Previous treatment with topical steroids	Previous treatment with topical antifungals	Animal contact history	Concurrent dermatophytosis	Family exposure history	Microscopy	Pathogen
5 y/F	Face	2 w	Diffuse irregular erythema, papules with fine scales	Yes	Yes	No	Rabbit	No	No	Endothrix & ectothrix	*M. canis*
67 y/F	Face, abdomen, groin	1 y	Dark red patches with clear boundaries	Yes	Yes	Yes	No	Tinea cruris	No	Endothrix & ectothrix	*T. rubrum*
19 y/M	Face, chest, groin	2 y	Well-defined scaly erythema	Yes	Yes	Yes	Yes	Tinea cruris	No	Endothrix & ectothrix	*M. canis*
78 y/F	Left ear, abdomen, vulva, buttocks	1 y	Well-defined scaly erythema, papules	Yes	Yes	No	No	Tinea corporis	No	Endothrix & ectothrix	*T. mentagrophytes* complex

Table 1 . (continued)

Age/ sex	Sites	Lesion duration	Manifestations	Pruritus	Previous treatment with topical steroids	Previous treatment with topical antifungals	Animal contact history	Concurrent dermatophytosis	Family exposure history	Microscopy	Pathogen
15 y/M	Face, groin, feet	2 y	Well-defined rounded, arcuate scaly dark red rash; plantar erythematous blisters	Yes	No	No	No	Tinea pedis, tinea cruris	No	Ectothrix	*T. mentagrophytes* complex

M, male; F, female; y, years old; m, month; d, day; w, week

Table 2. A comprehensive review of 19 publications on tinea of vellus hair

Year	Area	Patient number	Species	Author
2001	China	1	*T. tonsurans*	Li JB, *et al.* [301]
2008	China	1	*T. mentagrophytes*	Zhang RF, *et al.*
2010	Spain	13	*N. gypsea* (5), *M. canis* (3), *T. mentagrophytes* (5)	Gómez Moyano E, *et al.* [18]
2012	Italy	18	*M. canis* (18)	Atzori L, *et al.* [17]
2015	Spain	1	*M. canis*	Knopfel N, *et al.* [285]
2016	Spain	1	*T. rubrum*	Moyano EG, *et al.* [302]
2016	Spain	6	*M. canis* (2), *T. tonsurans* (2), *N. gypsea* (1), *T. mentagrophytes* (1)	Gómez-Moyano E, *et al.* [292]
2018	Thailand	1	*T. mentagrophytes*	Eksomtramage T, *et al.* [286]
2018	Taiwan, China	16	*T. rubrum* (7), *M. canis* (6) *and T. mentagrophytes* (3)	Sun PL, *et al.* [22]
2019	Uruguay	1	*T. rubrum*	Turra N, *et al.* [303]
2019	Japan	1	*T. schoenleinii*	Iwasa K, *et al.* [304]
2020	China	1	*M. canis*	Wei F, *et al.* [20]
2020	China	1	*N. gypsea*	Tang J, *et al.* [305]
2020	China	73	*T. mentagrophytes* (38), *M. canis* (12), *N. gypsea* (10) *and T. violaceum* (2)	Yan W, *et al.*
2021	Spain	1	*T. tonsurans*	Lopez Riquelme I, *et al.* [19]
2022	China	1	*M. cains*	Zhi HL, *et al.* [295]
2022	China	1	*M. canis*	Hu W, *et al.* [21]
2022	China	1	*M. canis*	Xu X, *et al.* [215]
2022	China	1	*M. canis*	Liu ZH, *et al.* [306]
2022	China	1	*T. violaceum*	Ge S,*et al.*

CHAPTER 5

HUMAN ADAPTATION AND DIVERSIFICATION IN *MICROSPORUM CANIS* COMPLEX

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« *Microsporum canis,* PDA, 28°C

ABSTRACT

The *Microsporum canis* complex consists of one zoophilic species, *M. canis*, and two anthropophilic species, *M. audouinii* and *M. ferrugineum*. These species are the most widespread zoonotic pathogens causing dermatophytosis in cats and humans worldwide. To clarify the evolutionary relationship between the three species and explore the potential host shift process, this study used phylogenetic analysis, population structure analysis, multispecies coalescent analyses, determination of *MAT* idiomorph distribution, sexual crosses, and macromorphology and physicochemical features to address the above questions. The complex of *Microsporum canis*, *M. audouinii* and *M. ferrugineum* comprises 12 genotypes. *MAT1-1* was present only in *M. canis*, while the anthropophilic entities contained *MAT1-2*. The pseudocleistothecia were yielded by the mating behaviour of *M. canis* and *M. audouinii*. Growth rates and lipase, keratinolysis and urea hydrolytic capacities of zoophilic *M. canis* isolates were all higher than those of anthropophilic strains; DNase activity of *M. ferrugineum* exceeded that of *M. canis*. The optimum growth temperature was 28°C, but 22°C favoured the development of macroconidia. Molecular data, physicochemical properties and phenotypes suggest the adaptation of zoophilic *M. canis* to anthropophilic *M. ferrugineum*, with *M. audouinii* in an intermediate position.

INTRODUCTION

The dermatophytes comprise one of the most prevalent groups of zoonotic fungal pathogens [6, 307]. Infections are typically superficial, involving keratinized structures such as skin, nails, and hair. Currently, seven genera of dermatophytes are accepted, of which members of *Trichophyton*, *Epidermophyton*, and *Microsporum* are commonly associated with human dermatophytosis [308]. From an ecological and clinical perspective, three approximate groups are distinguished, i.e., geophilic, zoophilic, and anthropophilic species. Classically, it has been supposed that the evolution from geo- to anthropophilic lifestyles has taken thousands of years. This refers to the evolution from geophily in *Arthroderma* to anthropophily in *Trichophyton*. However, Tang *et al.* [26] suggested that this may also occur at a smaller scale within single species complexes. For example, *Trichophyton mentagrophytes* represents an interbreeding cloud of genotypes with a preponderantly zoophilic lifestyle, while *T. indotineae* and *T. interdigitale* are anthropophilic clonal offshoots [26]. Similar taxonomic structures can be observed in the prevalently zoonotic *T. benhamiae* species complex [309].

The zoophilic species *Microsporum canis* is flanked by anthropophilic clones, *M. audouinii* and *M. ferrugineum*. The group is phylogenetically remote from all other dermatophytes. *Microsporum canis* is prevalent in cats and dogs, and when transmitted to humans, causes tinea capitis, tinea faciei and tinea corporis. Tinea capitis may show an often-severe inflammatory host response, presenting pruritic, scaly areas of alopecia. In contrast, the anthropophilic species *M. audouinii* and *M. ferrugineum* are generally transmitted from human to human, causing tinea capitis, mainly presenting with limited inflammation and minimal symptoms. *Microsporum audouinii* has occasionally been detected in animals and in the environment [310-313], while also some cases of severe inflammation have been reported [314-316]. From the early 1930s to the fifties, *M. audouinii* pandemics were a major cause of tinea capitis in Europe and the U.S.A. [54]. With the tinea capitis eradication campaign in the 1950s, *M. audouinii* decreased significantly and was eventually confined to Africa [317]. However, pet ownership grew in popularity from the 1990s, the zoophilic *M. canis* became the predominant agent of tinea capitis in Europe and Asia, and during the last decades, *M. audouinii* reemerged in school children [318-321]. *Microsporum ferrugineum* is considered endemic between the Balkan, East Asia and Nigeria [322] and has been reported in Europe and the Americas with the flow of migrants [323]. It is worth mentioning that *M. equinum*, an obsolete name for *M. canis* strains causing horse tinea, has been reported from several locations during the last century [324-326]. The identity of *M. equinum* remains controversial, as it differs morphologically and physicochemically

from *M. canis*, but molecularly it is an infraspecific taxon and was synonymised with *M. canis* [327]. Kano *et al.* (2001) reported that in the chitin synthase 1 gene, *M. equinum* was close to *Arthroderma otae*. Still, when crossed with tester strains of *A. otae*, *M. equinum* failed to produce ascomata [326].

Significant phenotypic and ecological variation exists between the three members of the *M. canis* complex, but evidence of sexual reproduction underlines a strong connection between the species. Fertile gymnothecia have been observed in mating experiments [56], and by nomenclatural rules of that time, the sexual state was described as *Arthroderma otae* (= *Nannizzia otae*). Monoascospore cultures were demonstrated to be heterothallic [56]. Since then, an imbalance of the *MAT* idiomorph was shown in *M. canis*, with the predominance of *MAT* (-). *Microsporum audouinii* and *M. ferrugineum* were considered to be strictly clonal.

Whether the above anthropophilic and zoophilic species should be considered members of a single biological species represented by the sexual state '*Arthroderma otae*' is still an open question. To better delineate species identities in the *M. canis* complex and to understand the shift process from animal to human hosts, the present study combines multiple approaches, including molecular phylogenetic and population genetic analysis, using a global set of isolates with a focus on human strains. We screened the variability of phenotypic, physiological, and genetic properties of the three taxonomic entities and determined the distribution of *MAT* idiomorphs.

MATERIAL AND METHODS

Strains

Microsporum canis, *M. audouinii*, and *M. ferrugineum* were isolated from patient tinea capitis, tinea corporis, and animal dermatophytosis. The 183 strains obtained from the collection conservation of the Department of Dermatology, the Third Affiliated Hospital of Sun Yat-sen University (n=20); Clinical Microbiology, University of Liège, Belgium (n=15); Faculty of Medicine, University of Ljubljana (n=28); Department of Dermatology, UH Cleveland Medical Center (n=37); Labor für Medizinische Mikrobiologie - Partnerschaft (n=30); Microbiology, Parasitology and Pathology Graduate Program, Federal University of Paraná (n=13); Department of Veterinary Medicine, University of Bari Aldo Moro (n=28); and Department of Veterinary Sciences, University of Turin (n=12). The Westerdijk Fungal Biodiversity (Utrecht, The Netherlands) provided the tester strains CBS 495.86 and CBS 496.86. Detailed information on the strains' origin is listed in Additional table 1.

Molecular studies

A rapid DNA extraction method was used to extract DNA from 185 strains. The strains were incubated in Potato Dextrose Agar (PDA, Oxoid, U.K.) at 28°C for 14 days. Conidia or hyphae were picked from colonies and dissolved in 300 µL Breaking buffer (containing 2.14% w/v Triton X-100, 1% w/v SDS, 0.585% w/v NaCl, 0.1575% w/v Tris-HCL, 0.0292% w/v EDTA). Samples were shaken at 1,400 rpm for 45 minutes at 60°C incubation. After adding 250 µL 25:24:1 phenol-chloroform-isoamyl alcohol (Sigma, U.S.A.), the mixture was centrifuged at 13,000 rpm for 10 min at room temperature and the supernatant was collected. The rapid DNA extraction method bypassed the detection of DNA concentration and went directly to thermal cycling.

ITS1/ITS4 or ITS1F/NL4 [328] were used to amplify the ITS (ITS1-5.8S-ITS2) rDNA region. The partial β-tubulin II (*tub2*) was amplified using the primers BT-2a/T2 [329]. EF-DermF/EF-DermR [330] amplification of translation elongation factor 1 (*tef-1α*) was done. DNA topoisomerase I (*topI*) and II (*topII*) were amplified using the TOP1 501-F/TOP1 501-R [331], *TOPII*-F1/*TOPII*-Mic [332] primer sets, respectively. The *60S L10 (L1)* region was amplified using 60S-908R/60S-506F [331].

The 25 µL PCR reaction system contained 0.25 µL high-fidelity DNA polymerase (Thermo Fisher Scientific, U.S.A.), 0.5 µL DNA, 0.5 µL dNTPs, 1 µL forward and reverse primers, 5 µL HF buffer and 17.75 µL nuclease-free water. DNA purification was carried out according to the instructions of the QIAquick gel Extraction Kit (QIAquick, Germany).

Phylogenetic analyses

The harvested DNA forward and reverse sequences were assembled in SnapGene v6.0.2 [333], and multiple sequence alignments were performed using Clustal Omega. The maximum likelihood (ML) method was used to construct phylogenetic trees for a single locus and six loci concatenation with *Lophophyton gallinae* CBS 300.52 as the root in MEGA v10.2. The concatenated multi-locus sequences totaled 3299 bp in length. For *tub2*, ITS, *topI*, *tef-1α*, *topII*, *60S L10 (L1)* and tandem sequences, the best substitution models were K2, HKP+G, JC, K2, K2, JC, and K2+I, respectively. The first 25% of trees were discarded as burn-in after 1000 bootstrap replicates. The evolutionary tree was further edited and annotated in iTtol (https://itol.embl.de/) and Adobe Illustrator 2020.

The R (v4.0.2) package phylogram/dendextend was used to visualize each locus evolutionary tree relative to the others. Matching taxa were connected using auxiliary lines to minimize the number of reticulations in any biconnected network components.

Analyses of nuclear haplotypes

Nucleotide polymorphism analysis of concatenated multilocus sequences was performed in DNASP v6.12.03 [334]. Population expansion was analyzed using Tajima's D and Fu's Fs neutrality tests. Populations of 185 sequences (without outgroups) were grouped by species type, origin, and *MAT* type in Arlequin (v3.5.2.2). Visualization of haploid networks was achieved in PopART v1.7 [335] using the TCS network method.

Multispecies coalescent analyses

Based on the haplotype results, the corresponding numbered strains were selected as representatives in each grouping. The Beast (v2.6.7 StarBeast3) [336] template was used to accomplish efficient multi-species joint inference using parallel gene tree operators. The dataset was partitioned by the 6 genes. HKY for substitution Model, Strict Clock molecular clock model, and Empirical for Frequencies were applied. The Yule model was used to set the tree species prior, and the clock rate priors for all loci were set to Exponential with a mean of 1.0. The chain length was set to 5,000,000, the tree was sampled every 5,000 steps, the tracelog was set to 5,000, and the screenlog was adjusted to 10,000. After running Beast v2.6.7, the results were analyzed with Tracer v1.7 [337] to assess the convergence of the model parameters. The burn-in percentage was set to 10, and the posterior probability (PP) was limited to 0.75 in TreeAnnotator v1.10 and the PP support for the specified branches was displayed in the DensiTree v2.6.7.

Phenotypic and physiological studies

The 185 strains were grown on Potato dextrose agar (PDA, Oxoid) at 28°C for 14–21 days. Colony morphology was described with reference to the Atlas of Clinical Fungi, 4th edition (de Hoog *et al.* 2020), using a hexadecimal color code to indicate the colour of the colony (https://coolors.co/).

For the preparation of the Tween-80 agar (1% w/v Bacto Peptone, 0.5% w/v NaCl, 0.01% w/v $CaCl_2$, 1.5% w/v agar and 0.5% w/v Tween-80) Petri dish is referred to [26], the diameter of the halo around the colony was recorded after incubation at

28°C for 15 days. The Deoxyribonuclease (DNase) test was performed by first inoculating the isolates on DNase Test Agar (Oxoid, U.S.A.) and cultured at 28°C for 15 days, after which 18.25% w/v HCL was poured on the surface of the medium and the diameter of the ring-clear zone was measured after 10 minutes [338]. The protocol of the keratin azure test was based on [339]. Each tube contained 5.5 ml of medium, which was divided into two layers, with the lower layer of 5 ml consisting of 2.5% w/v agar, 0.05% w/v $MgSO_4 \cdot 7H_2O$, 0.05% w/v KCl, 0.05% w/v K_2HPO_4, 0.01% w/v $ZnSO_4 \cdot 7H_2O$, 0.01% w/v $FeSO_4 \cdot 7H_2O$, 0.003 % w/v $CuSO_4$, and the upper layer of 0.5 ml consisting of 1% w/v agar, 0.05% w/v $MgSO_4 \cdot 7H_2O$, 0.05% w/v KCl, 0.05% w/v K_2HPO_4, 0.01% w/v $ZnSO_4 \cdot 7H_2O$, 0.01% w/v $FeSO_4 \cdot 7H_2O$, 0.003% w/v $CuSO_4$, 0.4% w/v keratin azure. Mycelia were transferred to the upper medium and grown at 28°C for 4 weeks, then the degree of blue of the lower layer represented the strength of the keratin decomposition. The urea hydrolysis tests were performed using Urea Agar Base (Oxoid, Hampshire, U.K.) and incubated at 28°C for three days, after which the colour change of the medium was observed. The results of keratinase activity and urea hydrolysis Tween opacity test were given a score ranging from 5 (strongly positive) through 4 (positive), 3 (weak), 2 (weak/negative) and 1 (negative). The effect of *M. canis* complex on hair was studied by culturing the strain in distilled water containing 0.06% w/v yeast extract solution (BD, Bacto, U.S.A.) and blond children's hair. After 6 weeks of culturing at 28°C, structural alterations in the hairs were observed under the microscope (Zeiss, Germany).

The strains were inoculated in triplicate on Sabouraud Dextrose Agar (SDA; Oxoid), PDA, and Malt Extract Agar (MEA; Oxoid) and cultured at 22, 28, and 37°C for 14 days. Colony diameters were measured to assess temperature tolerance. Three angles were chosen for each colony to measure the diameter to take the average. At least three slides per Petri dish were made to record the spore abundance, septa number, length, and width of macroconidia under the microscope. Principal component analysis of morphological characteristics was constructed in GraphPad Prism 9.0.

MAT idiomorph determination

To detect the presence of *MAT1-1* or *MAT1-2* regions in *M. canis*, the primer pairs Mc alpha F/Mc alpha R and Ab HMG F/Ab HMG R were used, respectively, while the primer pair HMG for 1/HMG rev 1 [340] was used to amplify the *MAT1-2* region in *M. audouinii* and *M. ferrugineum*. Thermal cycling procedure and conditions are shown in the above molecular studies. The mating type of 183 strains was identified using the reference strains CBS 495.86 (*MAT1-2*) and CBS 496.86 (*MAT1-1*). On agarose gel plates, the results of the bands were determined, and numerous bands for each

species were chosen for sequencing to verify the correctness of the amplified sequences.

Sexual crosses

Sexual crosses were performed on Niger Seed agar (5% w/v niger seed, 0.1% w/v glucose, 0.1% w/v yeast extract, 0.05% w/v MgSO4·7H2O, 0.1% w/v KH2PO4, 2% w/v agar) and Oatmeal Agar (2% w/v Oatmeal, 0.1% w/v yeast extract, 0.1% w/v NaNO3, 0.1% w/v MgSO4·7H2O, 0.05% w/v KH2PO4, 1% w/v agar). Based on MAT idiomorphs determination, each isolate was tested for heterothallic crosses with CBS 495.86 (MAT1-2) or CBS 496.86 (MAT1-1), respectively. Small pieces of fresh, vigorously growing cultures were cut from the colonies and placed approximately 5 mm apart in the centre of the Petri dish. All crosses were incubated in the dark at 23–25°C for 4–6 weeks and were periodically checked for cleistothecia or pseudocleistothecia.

Statistics

Statistical significance was conducted using SPSS Statistics v26. Tween-80 opacity, keratin azure, urea hydrolysis and tests were performed using the Kruskal-Wallis test with Dunn's multiple comparisons tests. The growth rate and characteristics of macroconidia were conducted using two-way nonparametric, Scheirer-Ray-Hare test. The resultant graphs were produced using GraphPad Prism 9.0. Each dot in the figure represents a sample, and each group's standard error of the mean (SEM) is displayed. The value of $P<0.05$ was considered statistically significant. The significance layout appears as follows: *$P<0.05$; **$P<0.01$; ***$P<0.001$; ****$P<0.0001$.

RESULTS

Phylogeny of *Microsporum canis* complex

Phylogenetic analysis of the *M. canis* complex was performed by reconstructing an evolutionary tree combining six loci. Genealogical concordance of single strains in the different loci was visualized using a tanglegram (Figure 1), showing consistency in the topology of sequences in supported clades. Clades containing the type strains of *Microsporum canis*, *M. audouinii* or *M. ferrugineum*, respectively, were identified in the *tef-1α* and *topII* datasets. Each clade was supported by bootstrap (bs) >70%, except for CBS 495.86, which remained separate from *M. audouinii* and *M. ferrugineum* only based on the *tub2* region. CBS 496.86 had significant sequence

identity with the major genotype known as *M. canis* in the *topI* and *tef-1α* regions but had multiple nucleotide substitutions at all four other loci compared to the remaining strains. In *topI*, CBS 495.86 and CBS 496.86 shared the same nucleotide sequence and were classified in the *M. canis* group. Linkage lines are drawn (Figure 1) relative to the phylogeny of the *tub2* gene fragment in the tanglegram. The relationships between the three groups were consistent across the six loci of bs-supported groupings, but some isolates deviated. In *tub2*, *60S L10 (L1)*, *tef-1α*, *topII* and *topI*, *M. audouinii* and *M. ferrugineum* clustered in the same group, whereas in the ITS region, *M. canis* and *M. ferrugineum* belonged to the same group. Both CBS 495.86 and CBS 496.86 were assigned to the *M. canis* group in the *topI* area, while the *M. audouinii* strain 204 was a member of the *M. ferrugineum* cluster.

Phylogenetic analysis of the above six concatenated loci revealed three supported main groups (Figure 2). *Microsporum canis* emerged as a separate clade (99% bs), and the paraphyletic clades *M. audouinii* and *M. ferrugineum* each had >90% bs support. CBS 495.86 deviated, being intermediate between *M. canis* and *M. audouinii* (65% bs). CBS 496.86 was individualized and affiliated with the *M. canis* clade (>80% bs).

Genetic diversity and population structure of the *Microsporum canis* complex

Nucleic acid diversity and population structure based on DNA sequences were used to determine the genotypic and evolutionary relationships of groups within the complex. The six DNA makers were concatenated to multiple sequence alignments of 3322 bp, which included 76 variable sites, 14 singleton variable sites, 62 parsimony informative sites, and 11 indel events.

The haplotype network comprised 12 multilocus genotypes (Figure 3, Hap1-12), of which Hap1-7 genotypes belonged to *M. canis*, Hap8-11 to *M. audouinii*, and Hap12 to *M. ferrugineum*. Hap1 was the predominant genotype in *M. canis*. Compared to Hap1, Hap2 and Hap3 isolates had single nucleotide site substitutions in the *topII* and ITS sections, Hap4 exhibited a single substitution in the *topI* region, a single substitution of Hap5 was found in the *topII* area, and the ITS region of Hap6 included five extra substitutions. Hap7 (CBS 496.86) was genetically distinct from the remaining genotypes. Eight base substitutions occurred between CBS 496.86 and Hap1. Hap9 was the predominant genotype of *M. audouinii*, with one site substitution in *topII* being Hap10 and three nucleotide substitutions producing Hap11. Hap8 (CBS 495.86) was situated in the middle of the network diagram and

had greater variability with Hap1 and Hap9. *Microsporum ferrugineum* had only one (Hap12) genotype and no intragroup variation.

Analysis of nucleotide diversity and population genetic differentiation indicated that the complex has evolved stably over a long period (Pi = 0.00513, Hd = 0.571). Hap1 was a shared haplotype and an interior clade haplotype, implying that Hap1 might be the ancestral genotype within the entire complex. The complex has yet to undergo significant population expansion or experience a bottleneck event (Tajima's D = 0.781, Fu's Fs = 0.11943). The minimum recombination event Rm was 5. There has been significant genetic divergence (Fst > 0.25) between the three clades within the complex (Additional table 2).

Coalescence analysis

The species limits of the *M. canis* complex were re-examined by multi-species coalescence (MSC) analysis. StarBeast3 is a template for efficient Bayesian inference under the MSC model using the Markov chain Monte Carlo algorithm. The difference with the phylogenetic analysis of multiple sequence motifs is that MSC does not perform concatenation of the loci and proceeds directly to the analysis. Based on the 12 haplotypes, we selected representative sequences from each Hap group: Hap1 included *M. canis* 10, 50, 65, 68 and 228, Hap2 included *M. canis* 138 and 244, Hap3 was *M. canis* 3, Hap4 included *M. canis* 9, 12 and 21, Hap5 was *M. canis* 73, Hap6 included *M. canis* 167, 168, 213 and 224, Hap7 was CBS 496.86, Hap8 was CBS 495.86, Hap9 was *M. audouinii* 178, Hap10 included *M. audouinii* 88, 93 and 99, Hap11 was *M. audouinii* 204, and Hap12 included *M. ferrugineum* 19 and 190. Trimming the sequences to equal length resulted in a total of 25 individuals with complex genotypes. *Lophophyton gallinae* was included as the root. Figure 4A shows the tree for 1000 random calculations, and Figure 4B displays the tree with the highest posterior probability product of nodes. Posterior probability (PP) support greater than 0.7 was shown next to the branches. In the MSC tree, *M. ferrugineum* is a separate taxonomic entity and *M. canis* and *M. audouinii* are delimited in the same branch. Strain CBS 495.86 and remaining *M. audouinii* strains are considered to be paraphyletic (PP=1). Both Hap9 and Hap10 are monophyletic (PP=1) strain sequences that are reciprocal to one another. The Hap6 and Hap7 genotypes and the Hap1–5 genotypes constitute branches that are parallel to each other (PP=1). Of the Hap1–5 genotypes, Hap2 differs from the other four (PP=1) and the remaining typing is not supported by sufficient PP (Figure 4).

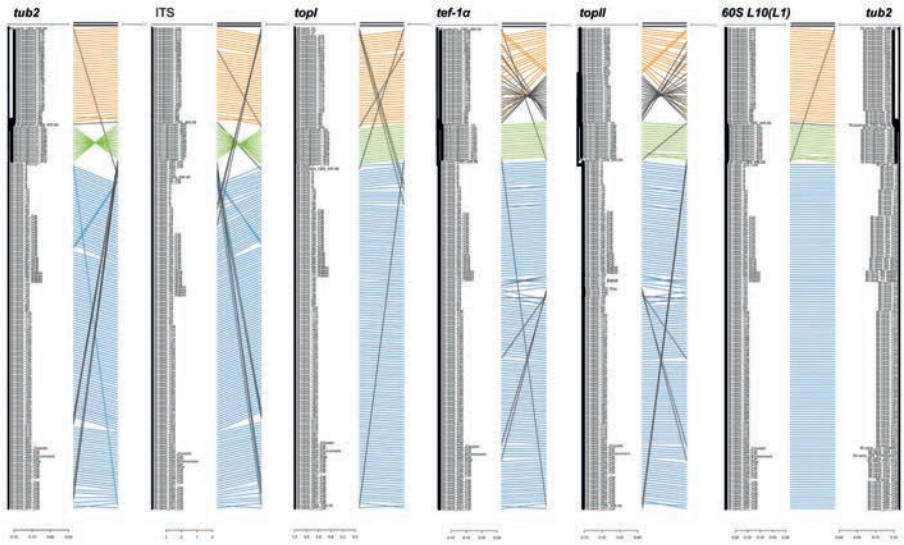

Figure 1. Tanglegram of six DNA regions. The diagram shows the evolutionary tree of each locus about the others. Matching taxa are connected using auxiliary lines of the same colour, the blue line for *M. canis*, the green line for *M. ferrugineum* and the orange line for *M. audouinii*. The grey line connects incongruent strains

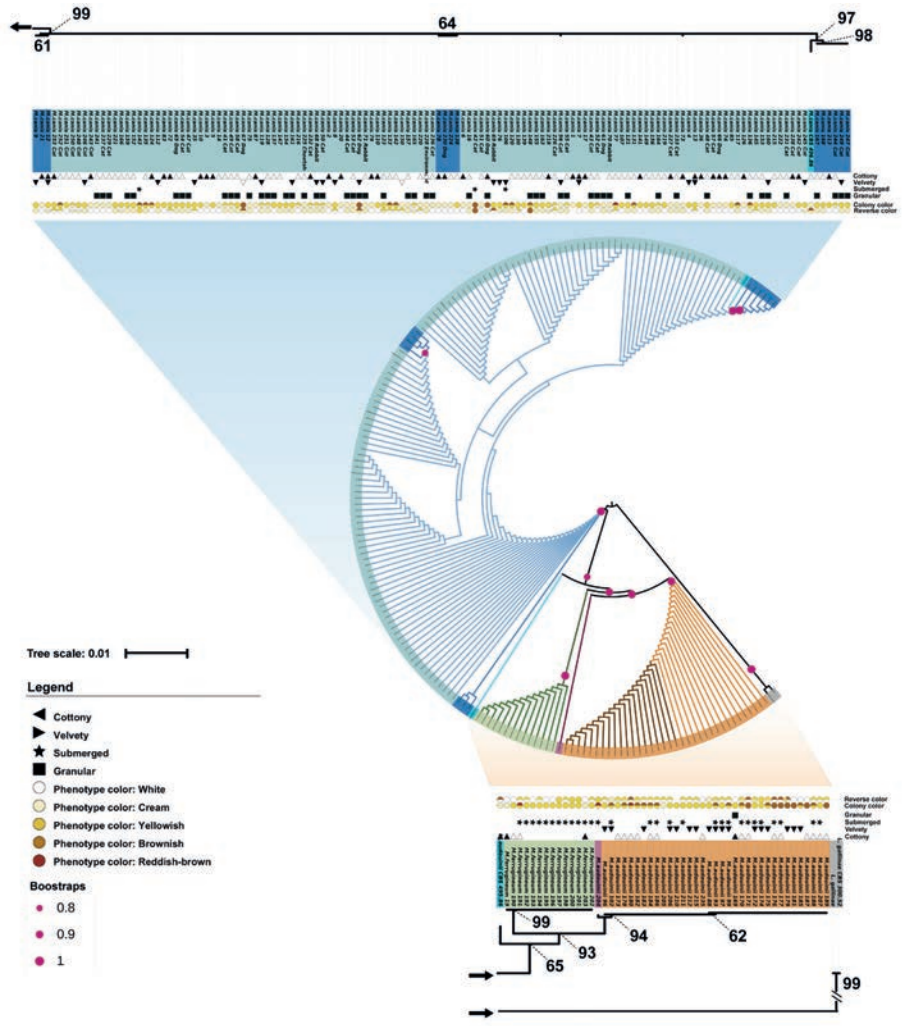

Figure 2. Multilocus phylogeny of *M. canis* complex. A maximum likelihood tree was constructed using tub2, ITS, topI, tef-1α, topII, 60S L10 (L1) concatenated loci to analyse the multilocus phylogeny of the *M. canis* complex. The tree is annotated with the morphology, colony colour and reverse colour of 185 strains

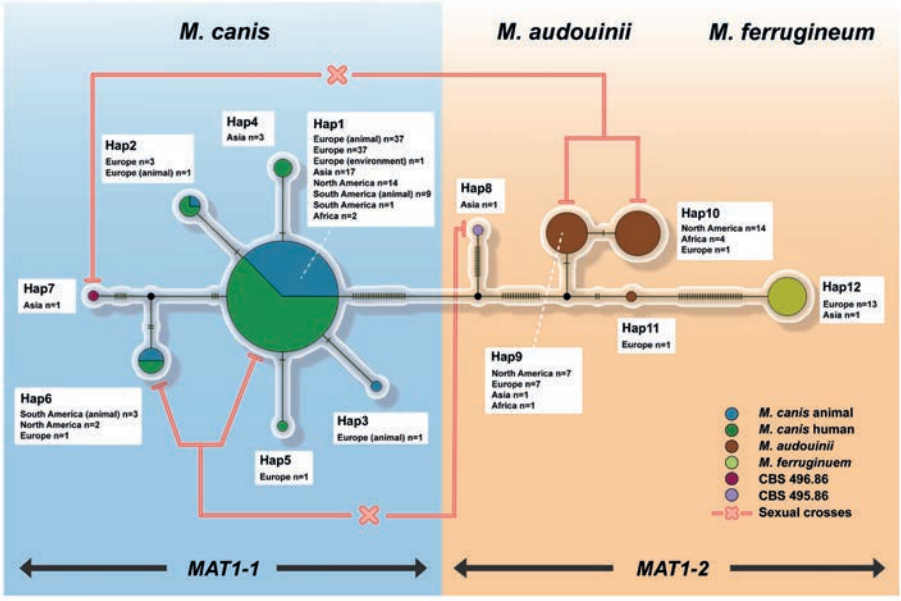

Figure 3. Haplotype network analysis based on multilocus data. The haplotype network map of the diferent species and host sources, the relationship between geographical origin and genotype, and the distribution of MAT idiotypes and sex crosses are shown. Strains of animal origin are marked in the haplotype network; the rest are strains of human origin

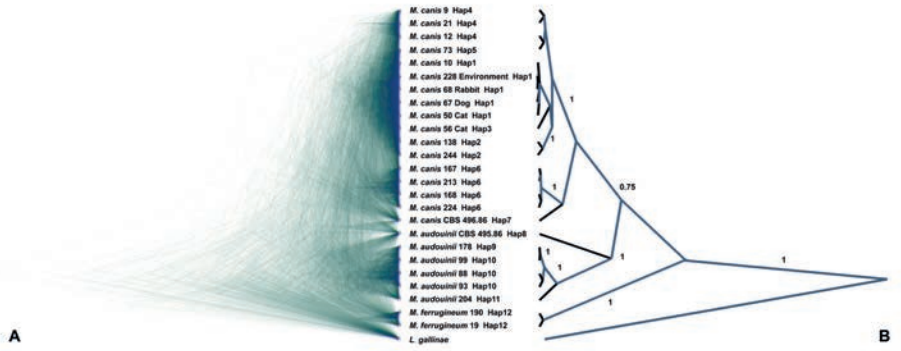

Figure 4. Multispecies coalescent tree constructed from six loci. Posterior probability support of >75% is shown next to the node

Geography and host origin

We further analyzed the geographic and host origins of the different Hap genotypes to understand the distribution and prevalence of members of the *M. canis* complex (Figure 3). Hap1 (n=118) included isolates from Belgium, Brazil, China, Germany,

Guinee, Italy, Slovenia, and the U.S.A. Of these strains, 47 were from animals (both symptomatic and asymptomatic), and the remaining strains (n=71) were from humans. In Hap1, concerning the animal origins, 35 isolates were from cats, 6 from dogs, 1 from a cheetah kept in captivity and 1 from the environment; of the human-derived isolates, 10 strains were isolated from smooth skin, and 51 from the scalp, while for some isolates no data were available. Hap2 (n=4) originated from Europe (Belgium, Germany, Italy, Slovenia), Hap3 (n=1) and Hap4 (n=1) were from Italy and Belgium, respectively, and Hap4 was only found in China. Hap6 (n=6) was mainly from Brazil, followed by the U.S.A. and Germany. The animal isolates in Hap2 were from dogs, and those in Hap3 and Hap6 were all from cats. Thirty-six strains of *M. audouinii* and 14 strains of *M. ferrugineum* were isolated from humans. Hap9 (n=16) was from the U.S.A., Germany, and Senegal; Hap10 (n=19) was mainly isolated in the U.S.A., followed by African countries (Congo, Cameroun, Guinee). Hap12, *M. ferrugineum,* originated in China and Germany.

Physiology

For these tests, we divided the main groups defined above into six groups for comparison, viz. *Microsporum canis* cat (cat-derived *M. canis* isolates), *M. canis* dog (dog-derived *M. canis* isolates), *M. canis* rabbit (rabbit-derived *M. canis* isolates), *M. canis* human (human-derived isolates), *M. audouinii* and *M. ferrugineum* groups.

Compared to isolates of human origin, all three animal groups showed higher lipolytic capacity, especially in the *M. canis* cat group ($P<0.001$), but there were no statistically significant differences among the cat, dog and rabbit origins. The *M. audouinii* and *M. canis* human groups had similar lipase catabolic abilities, with 72.7% and 75.3% positivity, respectively. Only 14.2% of *M. ferrugineum* strains were positive in the Tween-80 opacity test. The keratinolytic capacity of the four *M. canis* groups was higher than that of both the *M. audouinii* and *M. ferrugineum* groups ($P<0.05$), and no difference was observed in *M. canis* from human and animal origin. The *M. canis* rabbit group showed the highest scores in keratinase activity and urea hydrolysis tests compared to *M. ferrugineum* ($P<0.05$). Urea catabolic capacity did not differ between the *M. canis* animal, *M. canis* human, and *M. audouinii* groups, but all were stronger than in *M. ferrugineum*. The DNase activity of *M. ferrugineum* was the strongest among the six groups, higher than that of *M. canis* ($P<0.01$) (Figure 5A).

Hair perforations perpendicular to the hair shaft were not observed. All three species caused ectothrix, but hairs in the *M. canis* group showed different levels of damage at 4–6 weeks, which appeared after 8–10 weeks in *M. audouinii* and *M.*

ferrugineum cultures. The hairs showed mild damage of brush-like changes in cuticle scaling, moderate damage in the form of continuous or interrupted medullae, and finally severe hair breakage (Additional figure 2). In all *M. canis* animal groups, 56.5% of the hairs showed severe damage to the hair structure with breakage, 30.4% were dominated by mild hair damage, and 13% were unchanged. In the *M. canis* rabbit, cat, and dog groups, 75%, 42.9%, and 28.5% of the strains showed severe hair breakage, respectively. This manifestation was followed by 40.9%, 50% and 9% in the *M. canis* human group. In the *M. audouinii* and *M. ferrugineum* groups, 20% and 10.5% of the hairs were broken, 40% and 42.1%, respectively of the hairs remained structurally normal, and the others showed only mild damage.

The fastest growth was observed at 28°C, followed by 22°C (Figure 6A). At 37°C, only a few *M. canis* strains (n=7) could grow. *Microsporum audouinii* and *M. ferrugineum* did not grow at 37°C. *Microsporum canis* showed the fastest expansion, with no difference between strains of animal and human origin, followed by *M. audouinii*, while *M. ferrugineum* grew very slowly. Growth rates were not statistically correlated with haplotypes of *M. canis*. *Microsporum audouinii* of Hap9 grew faster than *M. audouinii* of Hap10 (P<0.05). Hap11 and Hap12 had the lowest growth rates under all conditions (P<0.01).

We further compared the state of development of macroconidia on PDA at three temperatures, including the length, width, and septa of macroconidia. These indicators showed significant differences at 22°C of cultivation in the animal/human-derived *M. canis* isolates and *M. audouinii* isolates (Figure 6B). At 22°C, the length, width, and the septa number of macroconidia of *M. canis* were much higher than at 28°C, especially in the animal-derived *M. canis* isolates (Figure 6B). *Microsporum ferrugineum* 191 strain was observed to have very few macroconidia which were small and without septa, upon incubation at 22°C, while all other strains of this species were entire without conidia. Principal component analysis (PCA) made use of a mixed model of growth rate, macroconidia abundance, length, width, and septation (Figure 6C). The results of PCA confirmed the above differences and the principal component 1 extended X-axis distribution could separate *M. canis* and *M. audouinii* micromorphologically at 22°C incubation, but not at 28°C.

Macromorphology

The clusters were grouped according to haplotype diversity, and no significant morphological differences within the groups were found, but differences between

groups were observed (Additional figure 1). When strains were cultured on PDA at 28°C for 14–21 days, *M. canis* colonies showed white to cream, cottony to velvety, raised, radially furrowed, and granular morphology. Fifty-two isolates of *M. canis* on PDA had a granular-powdered surface; 26 were of animal origin. The reverse was white to cream in 73.68% of the human-derived *M. canis* isolates and 48.72% of the animal-derived isolates (Figure 2). The isolates *M. canis* 13, 18, 60, 67, 69, 150, 156 and 248 showed dysgonic colony. The dysgonic type colony is like that of *M. ferrugineum*, filamentous or heaped brown thallus. Macroconidia were usually absent, but microconidia were still visible.

Colonies of *M. ferrugineum* were white to yellow, filamentous, flat, with hyphae appressed to and submerged in the medium; two strains (2/14) had a cottony surface. The reverse colour of *M. ferrugineum* was predominantly yellow (78.57%) (Figure 2).

CBS 495.86 showed a white, velvety-like colony, with cream-coloured reverse, and produced numerous macroconidia and densely associated microconidia (Figure 2). Colonies of *M. audouinii* were white to brown, being predominantly downy to densely suede-like; in 17/36 strains the colonies were white in the centre, raised and downy, with the edges extending into the medium, and the hyphae were white to brown. The reverse colour was yellow to brown (96.3%), white and cream colours being uncommon.

Mating behaviour

We examined the distribution of *MAT* idiomorphs in the complex to examine the selection of *MAT* gametes on different hosts. Of the Hap1–7 genotypes of *M. canis*, which originated from different countries and different hosts, mating types are all *MAT1-1* idiomorph. In contrast, Hap8–11 of *M. audouinii* and Hap12 of *M. ferrugineum*, from human tinea capitis, are *MAT1-2* idiotypes (Figure 3).

After 8 weeks in culture, positive responses were noted in Hap1 × Hap8, Hap6 × Hap8, Hap9 × Hap7 and Hap10 × Hap7 in a total of 11 isolates (Figure 3), i.e., in *M. canis* 44, 74, 112, 156, 213, 246, *M. audouinii* 99, 169, 177, 186 and 211, respectively. A small amount of white powdery thallus was visible at the junction on the plates. Microscopically, sterile cleistothecia were observed, globose and light brown. The peridium of *M. audouinii* 177 included the peridial hyphae, and the peridium borders of the other strains were clear. However, no mature ascospores were observed in these crosses (Figure 7).

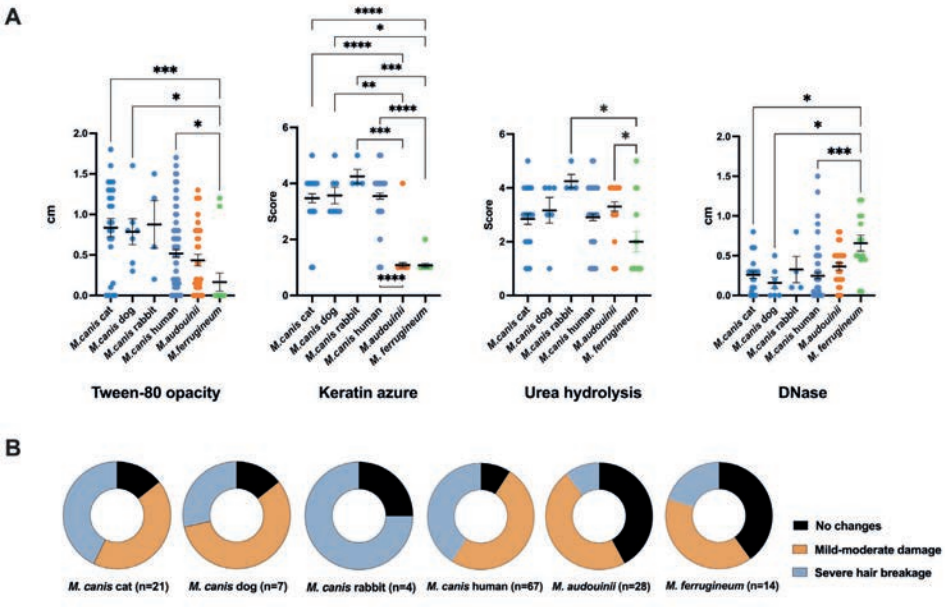

Figure 5. Physiological characteristics of *M. canis* complex. A Results of scores for the Tween-80 opacity test, keratin azure test, urea hydrolysis tests and in the *M. canis* animal group, *M. canis* human group, *M. audouinii* and *M. ferrugineum* group; B Hair changes in four groups. The mean+SEM of each group is indicated. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001

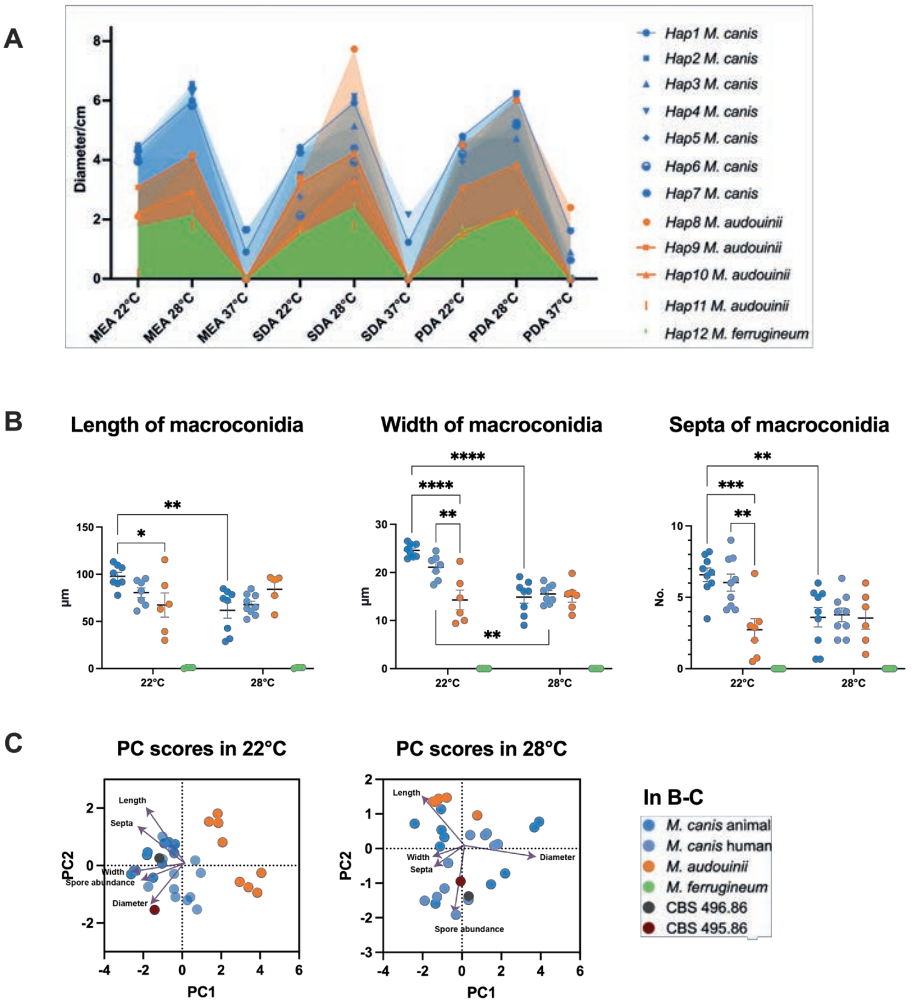

Figure 6. . Morphological characteristics of *M. canis* complex at diferent temperatures. A Growth rate of isolates within the whole complex based on 12 genotypes, with the area shaded below the fold line indicating the magnitude of the rate; B Length, width and septa number of macroconidia in *M. canis* and *M. audouinii* under incubation at 22 and 28 °C; C Principal component analysis of morphological characteristics at 22 °C and 28 °C of incubation, including growth rate, macroconidia abundance, length, width and the septa number of macroconidia. The mean+SEM of each group is indicated. **P*<0.05, ***p*<0.01, ****p*<0.001, *****p*<0.0001

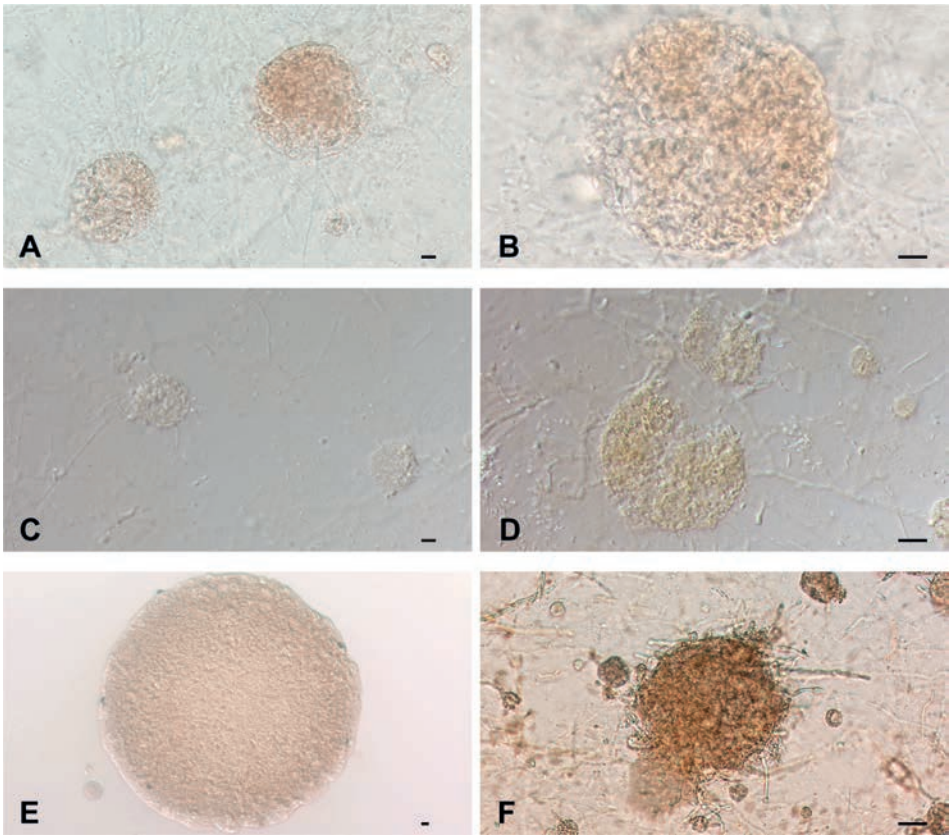

Figure 7. Cleistothecia production of *M. canis* complex. A–B Cleistothecia were yielded by *M. canis* 74 (Hap1)×CBS 495.86; C–D Cleistothecia were yielded by *M. canis* 213 (Hap6)×CBS 495.86; E Cleistothecia were yielded by *M. audouinii* 186 (Hap9)×CBS 496.86; F Cleistothecia were yielded by *M. audouinii* 177 (Hap10)×CBS 496.86 with irregular peridial hyphae visible of the peridium. Scale bars=10 µm

DISCUSSION

The variability of phenotypic and clinical parameters in the *Microsporum canis* complex urges critical determination of species identity and barcoding markers and investigate the transition from animal to human hosts. Based on the molecular overview of dermatophytes by [308], we analysed ITS, *tub2* and *60S L10 (L1)* supplemented with *tef-1α*, *topI* and *topII* molecular markers. In our results, the *tef-1α* and *topII* proved to be optimal to distinguish the three species, and the ITS and *topII* offered the greatest nucleotide diversity. Ciesielska *et al.* [341] proposed *velB* as a marker to differentiate *M. canis* from other dermatophytes, but *M. ferrugineum* was not mentioned. Rezaei-Matehkolaei *et al.* [330] suggested that *tef-1α* may be used for rapidly screening of Iranian *M. ferrugineum* isolates. The

novel *topI* gene showed great promise for ascomycetes through the Pfam approach [331] and has not been explored previously in *M. canis*. Shamsizadeh *et al.* [332] found that the number of polymorphic sites in the *topII* region of dermatophytes was similar to that of ITS.

The topology of the phylogenetic tree suggested an evolutionary trajectory from zoophilic to anthropophilic species. The haplotype network combined with species origin analysis and multispecies coalescence analysis was applied to define the genetic structure of the complex further. A total of 12 haplotypes were recognized, Hap1–7 being *M. canis*, 8–11 being *M. audouinii* and 12 being *M. ferrugineum*. This demonstrates that the observed phenotypic differences are more than just a matter of expression. Hap1 is the prevalent *M. canis* genotype, and it is widely dispersed on four continents: Europe, Asia, America, and Africa. Hap2, 3, and 5 are all native to Europe, while Hap4 is found only in Asia. However, the position of Hap2–4 in the multilocus evolutionary tree lacks sufficient bs (70%) to support a separate subgroup. Hap6 has two subgroups (bs>90%), primarily containing North and South American isolates. Within the *M. canis* group, there appears to be no correlation between host origin and genotype. The human isolates in Hap1 most likely concern recent direct infections from animals. *Microsporum canis* is known to cause small, self-limiting epidemics, e.g., in school children [342-344], indicating that zoophilic strains can reside on human hosts, at least temporarily. It may be surmised that host adaption in these strains remains inadequate [62].

Subgroups Hap9 and 10 were supported by 65% bs in the phylogenetic tree. European isolates mainly have the Hap9 genotype, while African isolates predominantly Hap10. North American strains are present in but are more prevalent in Hap10; possibly, North American strains may result from recent African immigration. During the last 20 years, an increase in the prevalence of *M. audouinii* infections has been reported worldwide on all continents, particularly in Belgium, France and Switzerland, and there in cities heavily populated by immigrants from African countries [54, 345]. *Microsporum ferrugineum*, the most derived clonal entity that lacks intraspecific diversity and has only a single genotype, Hap12, is limited geographically. A study by Zhan *et al.* [322] on 60 years of change in tinea capitis in China showed that *M. ferrugineum* remained highly endemic in Xinjiang and Yunnan in an era of great epidemiological change in tinea capitis. Both mating tester strains, CBS 496.86 and CBS 495.86, diverge from all other isolates in the haplotype network. CBS 496.86 and CBS 495.86 derived from the F1 progeny of two feline isolates (VUT 73015 × VUT 74001). As offspring of crosses, sexual reproduction always leads to higher population diversity; they acquire different genotypes from

the parents in the process of genetic recombination, thus causing positional deviations.

Microsporum canis was *MAT1-1* in all our strains, while *M. audouinii* and *M. ferrugineum* were all *MAT1-2* idiomorph. Mating is still possible in the complex, but it is challenging to produce fertile offspring. The distribution of the *MAT* idiomorphs also indicated the possibility of animal-to-human host shift. Anthropophilic and zoophilic strains have opposite sex distributions, with the *MAT1-2* gamete apparently being better adapted to humans. Although clonal reproduction facilitates the spread of the fungus, without the adaptability and plasticity achieved through genetic recombination, the organism would not be able to cope with long-term changes in host resistance or other natural or anthropogenic environmental changes [346]. The extinction of a *MAT* gamete in dermatophyte populations may be caused by the preferential transmission of strains exhibiting favourable combinations of alleles associated with higher virulence/transmission potential [347]. Loss of mating ability may be triggered by epigenetic factors, but the underlying mechanism is unknown. An association of different *MAT* idiomorphs and reproducing sexually with virulence, ecology and pathogenicity has been demonstrated in a variety of dermatophytes and non-dermatophytes [348-350]. Only a single *MAT* idiomorph was identified per isolate, but thus far, sexual reproduction with fertile gymnothecia has been observed exclusively in the tester strains, which are molecular mavericks. The sexual *MAT1-1* and *MAT1-2 M. canis* strains may be the ancestors of the entire complex, with *M. ferrugineum* as the most recent, still invariant human-adapted clone. Gradually, two distinct anthropophilic species evolved because of the human host adaptation of one sexual type (*MAT1-2*), with *MAT1-1* remaining prevalent in the cat.

Phylogeny, population structure analyses and mating idiomorph distributions reveal a transition in the *M. canis* complex from zoophilic to anthropophilic lifestyles. The host shift is reflected in differences in physicochemical capabilities, resulting in reduced virulence in the anthropophilic species. The statistical significance in ecologically relevant parameters suggests gradual host adaptation, which is more than just phenotypic in nature. The non-sporulating phenotype of *M. ferrugineum* occurs occasionally in cat-derived *M. canis* and is then known as ‘dysgonic’; it seems that this phenotype is more suitable for infection of the human host and thus might be selected during evolution. Animal-derived *M. canis* isolates caused more direct hair damage than the isolates derived from humans and compared to *M. canis*, *M. audouinii* and *M. ferrugineum* caused structural damage to the hair of blond children twice as slowly and with lesser symptoms. Viani *et al.* [351] showed that *M. canis* from symptomatic dogs and cats exhibited statistically

higher keratinase activity than those isolated from asymptomatic dogs and cats and caused acute inflammatory responses in guinea pigs. Interestingly, Cafarchia *et al.* [338] reported that *M. canis* isolated from healthy rabbits had higher keratinase activity than isolates from rabbits with skin lesions, whereas the latter had greater lipase activity. In our results, the lipolytic capacity of *M. canis* isolated from animals was higher than that of those isolated from humans. Lipase catabolism and keratinolytic capability of *M. ferrugineum* and *M. audouinii* were significantly lower than those of *M. canis*, matching with hair decomposition in fur by the latter species. The urease activity of dermatophytes was highly variable, as proven by the similar urea hydrolysis ability of *M. audouinii* and *M. canis*. Notably, we found that the keratinolytic and ureolytic capacities of *M. canis* isolated from rabbits may be stronger than those of strains isolated from cats and dogs. *Microsporum ferrugineum* scored the lowest of all four groups regarding lipase and keratolytic and urea hydrolytic capacities. The low virulence of *M. ferrugineum* may result in persistence provoking mild inflammatory response, leading to a peaceful coexistence of the anthropophilic fungus with its host, like *T. rubrum* [352]. DNase activity has been shown to facilitate the evasion of the innate immune system by *Paracoccidioide*s [353], *Cryptococcus* [354] and *Trichosporon* [355]. It may have a similar role in *M. ferrugineum.*

DNA hydrolysis has been observed in other anthropophilic dermatophytes. López-Martínez *et al.* [356] showed that all 47 analyzed *T. rubrum* strains produced DNase. The function of DNAse in *M. canis* is unknown; it does not vary across symptomatic and asymptomatic animal strains [356]. The composition of the host's skin may impact the pathogenic effect of each dermatophyte in a given host [338]. The optimal temperature for *in vitro* growth rate of the *M. canis* complex was 28°C, but at 22°C, sporulation and macroconidial size of animal-derived *M. canis* were greater than those of *M. canis* and *M. audouinii* of human origin; at 28°C, no difference was observed. It is unclear why temperature changes affect the formation of macroconidia, which may be related to changes in virulence and the shift of energy to the host during infection. A lower temperature may also indicate a distance to the host's body increasing the need for dispersal by conidia. For example, Song *et al.* (pers. comm.) identified changes in gene expression that accompany the shift from aerobic residence in cat fur to invasion of hairless human skin by analyzing the genome of *M. canis* strains that cause deep invasion. In summary, in terms of physicochemical properties, the characteristics accompanying the animal to human shift are loss of sporulation in human tissue compared to the residence in fur, higher pigment (xanthomegnin) production, lower keratinolysis, and lower lipolysis.

There are some limitations in the present study, including that the animal-derived *M. canis* strains were mostly isolated from cats and dogs and were restricted to Europe and South America. Additionally, the study did not include any *M. canis* from horses, we cannot ignore the possibility that this is a transitional genetic event in the complex similar to cat-to-human adaptation. Consequently, the host shift from animal fur to naked human skin in the *M. canis* complex shows that the dysgonic phenotype is more suitable for human infection, but the question of whether this is an evolution requires a more in-depth study of wild and domesticated animals. To further explore the selective role and virulence differences between *M. canis* evolution in animals and humans, a more diverse panel of animal isolates is required.

CONCLUSIONS

Phylogeny, population genetics and multispecies coalescent analyses clearly distinguished the three species, not only phenotypically but also genetically. Combined with physicochemical properties and *MAT* idiomorph distribution analysis, we could reconstruct the host shift to the human within the *M. canis* complex. *Microsporum canis* (*MAT1-1*) was by far the most dominant population, still maintaining high virulence and not adapted to humans; *M. audouinii* (*MAT1-2*) and *M. ferrugineum* (*MAT1-2*) are widespread in humans, with slow growth, progressive loss of conidia, weak lipolytic and keratolytic capacity, but increased DNA hydrolysis. Mating still occurs in the complex, exemplified by *MAT1-1* and *MAT1-2* tester strains. *Microsporum audouinii* appears to be intermediate in all respects, while *M. ferrugineum* is the most invariable compared with *M. canis*.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All sequence data generated for this study (Additional table 1) can be accessed via GenBank: https://www.ncbi.nlm.nih.gov/genbank/.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Study conceptualization and design of experiments, XZ; molecular sequencing, XZ, CT and MEG; data analysis, KW and AP; morphological description and microscopy, SAA and FGW; technical and methodological support, TM and YQK; original manuscript preparation, XZ, PYF and SDH, comments and corrections, SU, CC, MPH and RS; supervision, PYF and SDH. All authors read and approved the final manuscript.

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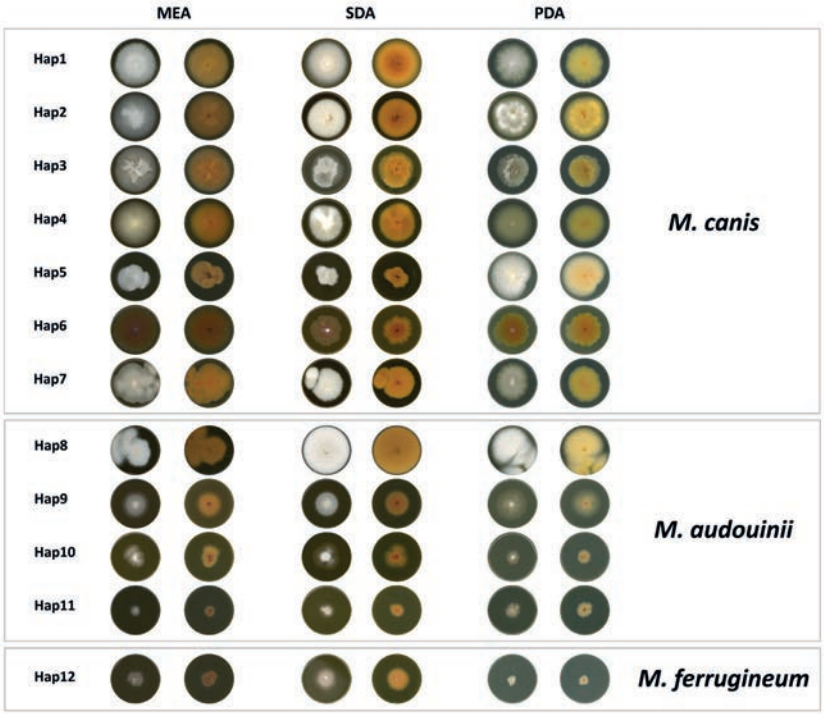
SUPPLEMENT INFORMATION

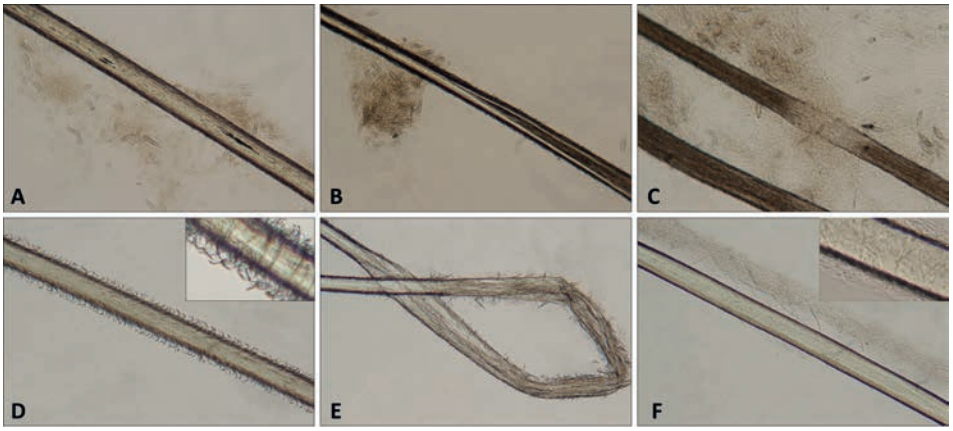
Additional table 2. Genetic differentiation among populations with mutiloucus

Assemblages		HS	KS	Kxy	Gst	DeltaSt	GammaSt	Nst	Fst	Dxy	Da
L. gallinae	*M. audouinii*	0.563	2.074	898.054	0.192	0.027	0.977	0.999	0.999	0.280	0.279
L. gallinae	*M. canis*	0.223	0.669	887.291	0.192	0.008	0.975	0.999	0.999	0.276	0.276
L. gallinae	*M. ferrugineum*	0.000	0.000	891.000	1.00	0.060	1.00	1.00	1.00	0.278	0.278
M. audouinii	*M. canis*	0.293	1.000	37.782	0.410	0.003	0.925	0.962	0.962	0.011	0.013
M. audouinii	*M. ferrugineum*	0.419	1.586	27.540	0.413	0.003	0.872	0.960	0.960	0.008	0.008
M. canis	*M. ferrugineum*	0.204	0.614	40.350	0.434	0.002	0.918	0.991	0.991	0.012	0.012

Hs: haplotype-based statistic;
Ks: statistic based on nucleotide sequences;
Kxy: average proportion of nucleotide differences between populations;
Gst: genetic differentiation index based on the frequency of haplotypes;
Dxy: average number of nucleotide substitutions per site between populations;
Da: net nucleotide substitutions per site between populations.



Additional figure 1. Morphological performance after 14 days of incubation on three media at 28 °C



Additional figure 2. Hair alteration after 4-6 weeks of co-culture with the strain.
(A-E) Hair alteration cultured with *M. canis*; (A-B) Continuous or interrupted medullae parallel to the hair shaft; (C) Entire structural damage and breakage; (D) The hair cuticle were damaged and the hair shaft shows brush-like changes; (E) Softened hairs, easily broken; (F) Ectothrix hyphae and no structural changes of hair.

Additional table 1.

Specie name	Specimen ID	Orign	Host	Sites	Haplo type	GenBank accession numbers					
						ITS	*BUT2*	*TEF-1α*	*TOPI*	*TOPII*	*60S L10 (L1)*
Dept of Dermatology, 3rd Affiliated Hospital, Sun Yat-Sen University											
M. c	1	China	Human	Tinea capitis	1	OQ160236	OQ160088	OQ159533	OQ159718	OQ159903	OQ159348
M. c	2	China	Human	Tinea capitis	1	OQ160237	OQ160089	OQ159534	OQ159719	OQ159904	OQ159349
M. c	3	China	Human	Tinea capitis	1	OQ160238	OQ160090	OQ159535	OQ159720	OQ159905	OQ159350
M. c	4	China	Human	Tinea capitis	1	OQ160239	OQ160091	OQ159536	OQ159721	OQ159906	OQ159351
M. c	5	China	Human	Tinea capitis	1	OQ160240	OQ160092	OQ159537	OQ159722	OQ159907	OQ159352
M. c	6	China	Human	Tinea capitis	1	OQ160241	OQ160093	OQ159538	OQ159723	OQ159908	OQ159353
M. c	7	China	Human	Tinea capitis	1	OQ160242	OQ160094	OQ159539	OQ159724	OQ159909	OQ159354
M. c	8	China	Human	Tinea capitis	1	OQ160243	OQ160095	OQ159540	OQ159725	OQ159910	OQ159355
M. c	9	China	Human	Tinea capitis	4	OQ160244	OQ160096	OQ159541	OQ159726	OQ159911	OQ159356
M. c	10	China	Human	Tinea capitis	1	OQ160245	OQ160097	OQ159542	OQ159727	OQ159912	OQ159357
M. c	12	China	Human	Tinea capitis	4	OQ160246	OQ160098	OQ159543	OQ159728	OQ159913	OQ159358
M. c	13	China	Human	Tinea capitis	1	OQ160247	OQ160099	OQ159544	OQ159729	OQ159914	OQ159359
M. c	14	China	Human	Tinea capitis	1	OQ160248	OQ160100	OQ159545	OQ159730	OQ159915	OQ159360
M. c	16	China	Human	Tinea capitis	1	OQ160249	OQ160101	OQ159546	OQ159731	OQ159916	OQ159361
M. c	17	China	Human	Tinea capitis	1	OQ160250	OQ160102	OQ159547	OQ159732	OQ159917	OQ159362
M. f	19	China	Human	Tinea capitis	12	OQ160251	OQ160222	OQ159667	OQ159852	OQ160037	OQ159482
M. c	20	China	Human	Tinea capitis	1	OQ160252	OQ160103	OQ159548	OQ159733	OQ159918	OQ159363

Additional table 1. (continued)

Specie name	Specimen ID	Orign	Host	Sites	Haplo type	GenBank accession numbers					
						ITS	*BUT2*	*TEF-1α*	*TOPI*	*TOPII*	*60S L10 (L1)*
M. c	21	China	Human	Tinea capitis	4	OQ160253	OQ160104	OQ159549	OQ159734	OQ159919	OQ159364
M. c	35	China	Human	Tinea capitis	1	OQ160254	OQ160105	OQ159550	OQ159735	OQ159920	OQ159365
M. c	36	China	Human	Tinea capitis	1	OQ160255	OQ160106	OQ159551	OQ159736	OQ159921	OQ159366
Department of Veterinary Medicine, University of Bari Aldo Moro											
M. c	41	Italy	Cat	Cutaneous lesions	1	OQ160256	OQ160107	OQ159552	OQ159737	OQ159922	OQ159367
M. c	42	Italy	Cat	Cutaneous lesions	1	OQ160257	OQ160108	OQ159553	OQ159738	OQ159923	OQ159368
M. c	43	Italy	Cat	Cutaneous lesions	1	OQ160258	OQ160109	OQ159554	OQ159739	OQ159924	OQ159369
M. c	44	Italy	Cat	Cutaneous lesions	1	OQ160259	OQ160110	OQ159555	OQ159740	OQ159925	OQ159370
M. c	47	Italy	Cat	Cutaneous lesions	1	OQ160260	OQ160111	OQ159556	OQ159741	OQ159926	OQ159371
M. c	48	Italy	Cat	Cutaneous lesions	1	OQ160261	OQ160112	OQ159557	OQ159742	OQ159927	OQ159372
M. c	49	Italy	Cat	Cutaneous lesions	1	OQ160262	OQ160113	OQ159558	OQ159743	OQ159928	OQ159373
M. c	50	Italy	Cat	Cutaneous lesions	1	OQ160263	OQ160114	OQ159559	OQ159744	OQ159929	OQ159374

Additional table 1. (continued)

Specie name	Specimen ID	Orign	Host	Sites	Haplo type	GenBank accession numbers					
						ITS	*BUT2*	*TEF-1α*	*TOPI*	*TOPII*	*60S L10 (L1)*
M. c	51	Italy	Cat	Cutaneous lesions	1	OQ160264	OQ160115	OQ159560	OQ159745	OQ159930	OQ159375
M. c	52	Italy	Cat	No symptoms	1	OQ160265	OQ160116	OQ159561	OQ159746	OQ159931	OQ159376
M. c	53	Italy	Cat	No symptoms	1	OQ160266	OQ160117	OQ159562	OQ159747	OQ159932	OQ159377
M. c	54	Italy	Cat	No symptoms	1	OQ160267	OQ160118	OQ159563	OQ159748	OQ159933	OQ159378
M. c	55	Italy	Cat	No symptoms	1	OQ160268	OQ160119	OQ159564	OQ159749	OQ159934	OQ159379
M. c	56	Italy	Cat	No symptoms	3	OQ160269	OQ160120	OQ159565	OQ159750	OQ159935	OQ159380
M. c	57	Italy	Cat	No symptoms	1	OQ160270	OQ160121	OQ159566	OQ159751	OQ159936	OQ159381
M. c	58	Italy	Cat	No symptoms	1	OQ160271	OQ160122	OQ159567	OQ159752	OQ159937	OQ159382
M. c	60	Italy	Dog	Cutaneous lesions	1	OQ160272	OQ160123	OQ159568	OQ159753	OQ159938	OQ159383
M. c	61	Italy	Dog	Cutaneous lesions	1	OQ160273	OQ160124	OQ159569	OQ159754	OQ159939	OQ159384
M. c	62	Italy	Dog	Cutaneous lesions	1	OQ160274	OQ160125	OQ159570	OQ159755	OQ159940	OQ159385
M. c	65	Italy	Dog	Cutaneous lesions	1	OQ160275	OQ160126	OQ159571	OQ159756	OQ159941	OQ159386
M. c	66	Italy	Dog	Cutaneous lesions	1	OQ160276	OQ160127	OQ159572	OQ159757	OQ159942	OQ159387
M. c	67	Italy	Dog	No symptoms	1	OQ160277	OQ160128	OQ159573	OQ159758	OQ159943	OQ159388

Additional table 1. (continued)

Specie name	Specimen ID	Orign	Host	Sites	Haplo type	GenBank accession numbers					
						ITS	*BUT2*	*TEF-1α*	*TOPI*	*TOPII*	*60S L10 (L1)*
M. c	68	Italy	Rabbit	Cutaneous lesions	1	OQ160278	OQ160129	OQ159574	OQ159759	OQ159944	OQ159389
M. c	69	Italy	Rabbit	Cutaneous lesions	1	OQ160279	OQ160130	OQ159575	OQ159760	OQ159945	OQ159390
M. c	70	Italy	Rabbit	No symptoms	1	OQ160280	OQ160131	OQ159576	OQ159761	OQ159946	OQ159391
M. c	71	Italy	Rabbit	No symptoms	1	OQ160281	OQ160132	OQ159577	OQ159762	OQ159947	OQ159392
M. c	72	Italy	Human	Cutaneous lesions	1	OQ160282	OQ160133	OQ159578	OQ159763	OQ159948	OQ159393
M. c	73	Italy	Human	Cutaneous lesions	5	OQ160283	OQ160134	OQ159579	OQ159764	OQ159949	OQ159394
Belgian National Reference Center, Clinical Microbiology, University Hospital of Liege											
M. c	74	Belgium	Human	Hair	1	OQ160284	OQ160135	OQ159580	OQ159765	OQ159950	OQ159395
M. c	75	Belgium	Human	Scalp	1	OQ160285	OQ160136	OQ159581	OQ159766	OQ159951	OQ159396
M. c	76	Belgium	Human	Scalp	1	OQ160286	OQ160137	OQ159582	OQ159767	OQ159952	OQ159397
M. c	77	Guinea	Human	Scalp	2	OQ160287	OQ160138	OQ159583	OQ159768	OQ159953	OQ159398
M. c	78	Belgium	Human	Scalp	1	OQ160288	OQ160139	OQ159584	OQ159769	OQ159954	OQ159399
M. c	79	Belgium	Human	Scalp	1	OQ160289	OQ160140	OQ159585	OQ159770	OQ159955	OQ159400
M. c	83	Romania	Human	Scalp	1	OQ160290	OQ160141	OQ159586	OQ159771	OQ159956	OQ159401
M. c	85	Belgium	Human	Scalp	1	OQ160291	OQ160142	OQ159587	OQ159772	OQ159957	OQ159402

Additional table 1. (continued)

Specie name	Specimen ID	Orign	Host	Sites	Haplo type	GenBank accession numbers					
						ITS	*BUT2*	*TEF-1α*	*TOPI*	*TOPII*	*60S L10 (L1)*
M. c	87	Belgium	Human	Scalp	1	OQ160292	OQ160143	OQ159588	OQ159773	OQ159958	OQ159403
M. a	88	Republic of the Congo	Human	Scalp	10	OQ160293	OQ160051	OQ159496	OQ159681	OQ159866	OQ159311
M. a	93	Cameroon	Human	Hair/Scalp	10	OQ160294	OQ160052	OQ159497	OQ159682	OQ159867	OQ159312
M. a	96	Turkey	Human	Scalp	9	OQ160295	OQ160053	OQ159498	OQ159683	OQ159868	OQ159313
M. a	97	Cameroon	Human	Scalp	10	OQ160296	OQ160054	OQ159499	OQ159684	OQ159869	OQ159314
M. a	99	Guinea	Human	Scalp	10	OQ160297	OQ160055	OQ159500	OQ159685	OQ159870	OQ159315
M. a	102	Senegal	Human	Hair/Scalp	9	OQ160298	OQ160056	OQ159501	OQ159686	OQ159871	OQ159316
Institute of Microbiology and Immunology, Medical Faculty, University of Ljubljana											
M. c	106	Slovenia	Human	Scalp scraping	1	OQ160299	OQ160144	OQ159589	OQ159774	OQ159959	OQ159404
M. c	107	Slovenia	Human	Skin scraping	1	OQ160300	OQ160145	OQ159590	OQ159775	OQ159960	OQ159405
M. c	110	Slovenia	Human	Skin scraping	1	OQ160301	OQ160146	OQ159591	OQ159776	OQ159961	OQ159406
M. c	112	Slovenia	Human	Skin scraping - arms and torso	1	OQ160302	OQ160147	OQ159592	OQ159777	OQ159962	OQ159407
M. c	117	Slovenia	Human	Skin scraping	1	OQ160303	OQ160148	OQ159593	OQ159778	OQ159963	OQ159408
M. c	119	Slovenia	Human	Skin scraping - face and chest	1	OQ160304	OQ160149	OQ159594	OQ159779	OQ159964	OQ159409
M. c	120	Slovenia	Human	Skin scraping	1	OQ160305	OQ160150	OQ159595	OQ159780	OQ159965	OQ159410

Additional table 1. (continued)

Specie name	Specimen ID	Orign	Host	Sites	Haplo type	GenBank accession numbers					
						ITS	*BUT2*	*TEF-1α*	*TOPI*	*TOPII*	*60S L10 (L1)*
M. c	121	Slovenia	Human	Skin scraping	1	OQ160306	OQ160151	OQ159596	OQ159781	OQ159966	OQ159411
M. c	123	Slovenia	Human	Skin scraping	1	OQ160307	OQ160152	OQ159597	OQ159782	OQ159967	OQ159412
M. c	124	Slovenia	Human	Skin scraping	1	OQ160308	OQ160153	OQ159598	OQ159783	OQ159968	OQ159413
M. c	126	Slovenia	Human	Skin scraping	1	OQ160309	OQ160154	OQ159599	OQ159784	OQ159969	OQ159414
M. c	128	Slovenia	Human	Skin scraping	1	OQ160310	OQ160155	OQ159600	OQ159785	OQ159970	OQ159415
M. c	130	Slovenia	Human	Skin scraping	1	OQ160311	OQ160156	OQ159601	OQ159786	OQ159971	OQ159416
M. c	131	Slovenia	Human	Skin scraping	1	OQ160312	OQ160157	OQ159602	OQ159787	OQ159972	OQ159417
M. c	133	Slovenia	Human	Skin scraping	1	OQ160313	OQ160158	OQ159603	OQ159788	OQ159973	OQ159418
M. c	135	Slovenia	Human	Skin scraping - arms and torso	1	OQ160314	OQ160159	OQ159604	OQ159789	OQ159974	OQ159419
M. c	136	Slovenia	Human	Skin scraping	1	OQ160315	OQ160160	OQ159605	OQ159790	OQ159975	OQ159420
M. c	137	Slovenia	Human	Skin scraping	1	OQ160316	OQ160161	OQ159606	OQ159791	OQ159976	OQ159421
M. c	138	Slovenia	Human	Skin scraping - arms and legs	2	OQ160317	OQ160162	OQ159607	OQ159792	OQ159977	OQ159422
M. c	139	Slovenia	Human	Skin scraping	1	OQ160318	OQ160163	OQ159608	OQ159793	OQ159978	OQ159423
M. c	141	Slovenia	Human	Skin scraping	1	OQ160319	OQ160164	OQ159609	OQ159794	OQ159979	OQ159424
M. c	142	Slovenia	Human	Skin scraping	1	OQ160320	OQ160165	OQ159610	OQ159795	OQ159980	OQ159425
M. c	143	Slovenia	Human	Skin scraping	1	OQ160321	OQ160166	OQ159611	OQ159796	OQ159981	OQ159426
M. c	145	Slovenia	Human	Skin scraping	1	OQ160322	OQ160167	OQ159612	OQ159797	OQ159982	OQ159427

Additional table 1. (continued)

Specie name	Specimen ID	Orign	Host	Sites	Haplo type	GenBank accession numbers					
						ITS	*BUT2*	*TEF-1α*	*TOPI*	*TOPII*	*60S L10 (L1)*
M. c	147	Slovenia	Human	Skin scraping	1	OQ160323	OQ160168	OQ159613	OQ159798	OQ159983	OQ159428
M. c	148	Slovenia	Human	Skin scraping - groins	1	OQ160324	OQ160169	OQ159614	OQ159799	OQ159984	OQ159429
M. c	149	Slovenia	Human	Skin scraping - neck	1	OQ160325	OQ160170	OQ159615	OQ159800	OQ159985	OQ159430
M. c	150	Slovenia	Human	Skin scraping - torso	1	OQ160326	OQ160171	OQ159616	OQ159801	OQ159986	OQ159431
				Department of Dermatology, UH Cleveland Medical Center							
M. c	152	USA	Human	Hair/Scalp	1	OQ160327	OQ160172	OQ159617	OQ159802	OQ159987	OQ159432
M. c	153	USA	Human	Hair/Scalp	1	OQ160328	OQ160173	OQ159618	OQ159803	OQ159988	OQ159433
M. c	154	USA	Human	Hair/Scalp	1	OQ160329	OQ160174	OQ159619	OQ159804	OQ159989	OQ159434
M. c	155	USA	Human	Hair/Scalp	1	OQ160330	OQ160175	OQ159620	OQ159805	OQ159990	OQ159435
M. c	156	USA	Human	Hair/Scalp	1	OQ160331	OQ160176	OQ159621	OQ159806	OQ159991	OQ159436
M. c	157	USA	Human	Hair/Scalp	1	OQ160332	OQ160177	OQ159622	OQ159807	OQ159992	OQ159437
M. c	159	USA	Human	Hair/Scalp	1	OQ160333	OQ160178	OQ159623	OQ159808	OQ159993	OQ159438
M. c	160	USA	Human	Hair/Scalp	1	OQ160334	OQ160179	OQ159624	OQ159809	OQ159994	OQ159439
M. c	161	USA	Human	Hair/Scalp	1	OQ160335	OQ160180	OQ159625	OQ159810	OQ159995	OQ159440
M. c	163	USA	Human	Hair/Scalp	1	OQ160336	OQ160181	OQ159626	OQ159811	OQ159996	OQ159441
M. c	164	USA	Human	Skin-Body	1	OQ160337	OQ160182	OQ159627	OQ159812	OQ159997	OQ159442

Additional table 1. (continued)

Specie name	Specimen ID	Orign	Host	Sites	Haplo type	GenBank accession numbers					
						ITS	*BUT2*	*TEF-1α*	*TOPI*	*TOPII*	*60S L10 (L1)*
M. c	165	USA	Human	Hair/Scalp	1	OQ160338	OQ160183	OQ159628	OQ159813	OQ159998	OQ159443
M. c	166	USA	Human	Hair/Scalp	1	OQ160339	OQ160184	OQ159629	OQ159814	OQ159999	OQ159444
M. c	167	USA	Human	Skin-Body	6	OQ160340	OQ160185	OQ159630	OQ159815	OQ160000	OQ159445
M. c	168	USA	Human	Skin-Body	6	OQ160341	OQ160186	OQ159631	OQ159816	OQ160001	OQ159446
M. a	169	USA	Human	Hair/Scalp	10	OQ160342	OQ160057	OQ159502	OQ159687	OQ159872	OQ159317
M. a	170	USA	Human	Hair/Scalp	10	OQ160343	OQ160058	OQ159503	OQ159688	OQ159873	OQ159318
M. a	171	USA	Human	Hair/Scalp	10	OQ160344	OQ160059	OQ159504	OQ159689	OQ159874	OQ159319
M. a	172	USA	Human	Hair/Scalp	10	OQ160345	OQ160060	OQ159505	OQ159690	OQ159875	OQ159320
M. a	173	USA	Human	Hair/Scalp	9	OQ160346	OQ160061	OQ159506	OQ159691	OQ159876	OQ159321
M. a	174	USA	Human	Hair/Scalp	10	OQ160347	OQ160062	OQ159507	OQ159692	OQ159877	OQ159322
M. a	175	USA	Human	Hair/Scalp	10	OQ160348	OQ160063	OQ159508	OQ159693	OQ159878	OQ159323
M. a	176	USA	Human	Hair/Scalp	10	OQ160349	OQ160064	OQ159509	OQ159694	OQ159879	OQ159324
M. a	177	USA	Human	Hair/Scalp	10	OQ160350	OQ160065	OQ159510	OQ159695	OQ159880	OQ159325
M. a	178	USA	Human	Hair/Scalp	9	OQ160351	OQ160066	OQ159511	OQ159696	OQ159881	OQ159326
M. a	179	USA	Human	Hair/Scalp	10	OQ160352	OQ160067	OQ159512	OQ159697	OQ159882	OQ159327
M. a	180	USA	Human	Hair/Scalp	9	OQ160353	OQ160068	OQ159513	OQ159698	OQ159883	OQ159328
M. a	181	USA	Human	Hair/Scalp	10	OQ160354	OQ160069	OQ159514	OQ159699	OQ159884	OQ159329
M. a	182	USA	Human	Hair/Scalp	9	OQ160355	OQ160070	OQ159515	OQ159700	OQ159885	OQ159330

Additional table 1. (continued)

Specie name	Specimen ID	Orign	Host	Sites	Haplo type	GenBank accession numbers					
						ITS	*BUT2*	*TEF-1α*	*TOPI*	*TOPII*	*60S L10 (L1)*
M. a	183	USA	Human	Hair/Scalp	9	OQ160356	OQ160071	OQ159516	OQ159701	OQ159886	OQ159331
M. a	184	USA	Human	Hair/Scalp	9	OQ160357	OQ160072	OQ159517	OQ159702	OQ159887	OQ159332
M. a	185	USA	Human	Hair/Scalp	10	OQ160358	OQ160073	OQ159518	OQ159703	OQ159888	OQ159333
M. a	186	USA	Human	Hair/Scalp	9	OQ160359	OQ160074	OQ159519	OQ159704	OQ159889	OQ159334
M. a	187	USA	Human	Hair/Scalp	10	OQ160360	OQ160075	OQ159520	OQ159705	OQ159890	OQ159335
M. a	188	USA	Human	Hair/Scalp	10	OQ160361	OQ160076	OQ159521	OQ159706	OQ159891	OQ159336
M. a	189	USA	Human	Hair/Scalp	10	OQ160362	OQ160077	OQ159522	OQ159707	OQ159892	OQ159337
M. a	235	USA	Human	Hair/Scalp	9	OQ160363	OQ160223	OQ159668	OQ159853	OQ160038	OQ159483
Labor für Medizinische Mikrobiologie Nenoff / Krüger, Mölbis											
M. f	190	Germany	Human		12	OQ160364	OQ160224	OQ159669	OQ159854	OQ160039	OQ159484
M. f	191	Germany	Human		12	OQ160365	OQ160225	OQ159670	OQ159855	OQ160040	OQ159485
M. f	192	Germany	Human		12	OQ160366	OQ160226	OQ159671	OQ159856	OQ160041	OQ159486
M. f	193	Germany	Human		12	OQ160367	OQ160227	OQ159672	OQ159857	OQ160042	OQ159487
M. f	194	Germany	Human		12	OQ160368	OQ160228	OQ159673	OQ159858	OQ160043	OQ159488
M. f	195	Germany	Human		12	OQ160369	OQ160229	OQ159674	OQ159859	OQ160044	OQ159489
M. f	196	Germany	Human		12	OQ160370	OQ160230	OQ159675	OQ159860	OQ160045	OQ159490
M. f	197	Germany	Human		12	OQ160371	OQ160231	OQ159676	OQ159861	OQ160046	OQ159491
M. f	198	Germany	Human		12	OQ160372	OQ160232	OQ159677	OQ159862	OQ160047	OQ159492

Additional table 1. (continued)

Specie name	Specimen ID	Orign	Host	Sites	Haplo type	GenBank accession numbers					
						ITS	*BUT2*	*TEF-1α*	*TOPI*	*TOPII*	*60S L10 (L1)*
M. f	200	Germany	Human		12	OQ160373	OQ160233	OQ159678	OQ159863	OQ160048	OQ159493
M. f	201	Germany	Human		12	OQ160374	OQ160234	OQ159679	OQ159864	OQ160049	OQ159494
M. f	202	Germany	Human		12	OQ160375	OQ160235	OQ159680	OQ159865	OQ160050	OQ159495
M. f	203	Germany	Human		12	OQ160376	OQ160079	OQ159524	OQ159709	OQ159894	OQ159339
M. a	204	Germany	Human		11	OQ160377	OQ160080	OQ159525	OQ159710	OQ159895	OQ159340
M. a	205	Germany	Human		9	OQ160378	OQ160081	OQ159526	OQ159711	OQ159896	OQ159341
M. a	206	Germany	Human		9	OQ160379	OQ160082	OQ159527	OQ159712	OQ159897	OQ159342
M. a	207	Germany	Human		10	OQ160380	OQ160083	OQ159528	OQ159713	OQ159898	OQ159343
M. a	210	Germany	Human		9	OQ160381	OQ160084	OQ159529	OQ159714	OQ159899	OQ159344
M. a	211	Germany	Human		9	OQ160382	OQ160085	OQ159530	OQ159715	OQ159900	OQ159345
M. a	212	Germany	Human		9	OQ160383	OQ160187	OQ159632	OQ159817	OQ160002	OQ159447
M. a	215	Germany	Human		9	OQ160384	OQ160188	OQ159633	OQ159818	OQ160003	OQ159448
M. c	213	Germany	Human		6	OQ160385	OQ160086	OQ159531	OQ159716	OQ159901	OQ159346
M. c	215	Germany	Human		1	OQ160386	OQ160189	OQ159634	OQ159819	OQ160004	OQ159449
M. c	216	Germany	Human		1	OQ160387	OQ160190	OQ159635	OQ159820	OQ160005	OQ159450
M. c	218	Germany	Human		1	OQ160388	OQ160191	OQ159636	OQ159821	OQ160006	OQ159451
M. c	219	Germany	Human		1	OQ160389	OQ160192	OQ159637	OQ159822	OQ160007	OQ159452
M. c	220	Germany	Human		1	OQ160390	OQ160193	OQ159638	OQ159823	OQ160008	OQ159453

Additional table 1. (continued)

Specie name	Specimen ID	Orign	Host	Sites	Haplo type	GenBank accession numbers					
						ITS	*BUT2*	*TEF-1α*	*TOPI*	*TOPII*	*60S L10 (L1)*
M. c	221	Germany	Human		1	OQ160391	OQ160194	OQ159639	OQ159824	OQ160009	OQ159454
M. c	222	Germany	Human		1	OQ160392	OQ160195	OQ159640	OQ159825	OQ160010	OQ159455
M. c	224	Germany	Human		2	OQ160393	OQ160196	OQ159641	OQ159826	OQ160011	OQ159456
Department of Veterinary Sciences, University of Turin											
M. c	225	Italy	Cheetah (zoological garden)		1	OQ160394	OQ160197	OQ159642	OQ159827	OQ160012	OQ159457
M. c	226	Italy	Cat		1	OQ160395	OQ160198	OQ159643	OQ159828	OQ160013	OQ159458
M. c	227	Italy	Cat		1	OQ160396	OQ160199	OQ159644	OQ159829	OQ160014	OQ159459
M. c	228	Italy	Environment		1	OQ160397	OQ160200	OQ159645	OQ159830	OQ160015	OQ159460
M. c	229	Italy	Cat		1	OQ160398	OQ160201	OQ159646	OQ159831	OQ160016	OQ159461
M. c	230	Italy	dog		2	OQ160399	OQ160202	OQ159647	OQ159832	OQ160017	OQ159462
M. c	231	Italy	Cat (cattery)		1	OQ160400	OQ160203	OQ159648	OQ159833	OQ160018	OQ159463
M. c	232	Italy	Cat (cattery)		1	OQ160401	OQ160204	OQ159649	OQ159834	OQ160019	OQ159464
M. c	233	Italy	Cat (outbreak in a household)		1	OQ160402	OQ160078	OQ159523	OQ159708	OQ159893	OQ159338
M. c	236	Italy	Cat		1	OQ160403	OQ160205	OQ159650	OQ159835	OQ160020	OQ159465
M. c	237	Italy	Cat		1	OQ160404	OQ160206	OQ159651	OQ159836	OQ160021	OQ159466

Additional table 1. (continued)

Specie name	Specimen ID	Orign	Host	Sites	Haplo type	GenBank accession numbers					
						ITS	*BUT2*	*TEF-1α*	*TOPI*	*TOPII*	*60S L10 (L1)*
M. c	238	Italy	Cat		1	OQ160405	OQ160207	OQ159652	OQ159837	OQ160022	OQ159467
Microbiology, Parasitology and Pathology Graduate Program, Federal University of Paraná											
M. c	241	Brazil	Human		1	OQ160406	OQ160208	OQ159653	OQ159838	OQ160023	OQ159468
M. c	242	Brazil	Cat		1	OQ160407	OQ160209	OQ159654	OQ159839	OQ160024	OQ159469
M. c	243	Brazil	Cat		1	OQ160408	OQ160210	OQ159655	OQ159840	OQ160025	OQ159470
M. c	244	Brazil	Cat		6	OQ160409	OQ160211	OQ159656	OQ159841	OQ160026	OQ159471
M. c	245	Brazil	Cat		1	OQ160410	OQ160212	OQ159657	OQ159842	OQ160027	OQ159472
M. c	246	Brazil	Cat		1	OQ160411	OQ160213	OQ159658	OQ159843	OQ160028	OQ159473
M. c	247	Brazil	Cat		6	OQ160412	OQ160214	OQ159659	OQ159844	OQ160029	OQ159474
M. c	248	Brazil	Cat		1	OQ160413	OQ160215	OQ159660	OQ159845	OQ160030	OQ159475
M. c	249	Brazil	Cat		6	OQ160414	OQ160216	OQ159661	OQ159846	OQ160031	OQ159476
M. c	250	Brazil	Cat		1	OQ160415	OQ160217	OQ159662	OQ159847	OQ160032	OQ159477
M. c	251	Brazil	Cat		1	OQ160416	OQ160218	OQ159663	OQ159848	OQ160033	OQ159478
M. c	252	Brazil	Cat		1	OQ160417	OQ160219	OQ159664	OQ159849	OQ160034	OQ159479
M. c	253	Brazil	Cat		1	OQ160418	OQ160220	OQ159665	OQ159850	OQ160035	OQ159480
The Westerdijk Fungal Biodiversity											
M. c	CBS 496.86	Japan	Hybrids		7	OQ160419	OQ160221	OQ159666	OQ159851	OQ160036	OQ159481

Additional table 1. (continued)

Specie name	Specimen ID	Orign	Host	Sites	Haplo type	GenBank accession numbers					
						ITS	*BUT2*	*TEF-1α*	*TOPI*	*TOPII*	*60S L10 (L1)*
M. a	CBS 495.86	Japan	Hybrids		8	OQ160420	OQ160087	OQ159532	OQ159717	OQ159902	OQ159347

M.c, Microsporum canis; M. a, Microsporum audouinii; M. f, Microsporum ferrugineum.

CHAPTER 6

DERMATOPHTES ADAPTATION TO THE HUMAN HOST EXEMPLIFIED BY *MICROSPORUM CANIS*

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ABSTRACT

Dermatophytes are a taxonomic group of keratinophilic fungi that engender cutaneous infections across human and animal populations. The zoophilic species *Microsporum canis*, which exhibits a widespread distribution, predominantly affects domesticated felines and canines and has recently been associated with an increased risk of human adaptation. This study conducted a comparative genome analysis, validating the adaptive expression of 12 relevant genes through neutrality tests and selection pressure analyses, with a particular focus on the evolutionary mechanisms underlying the shift from zoophilic to anthropophilic *Microsporum*. The results demonstrated a high degree of consistency in the nuclear and mitochondrial genomes among the three *Microsporum* species, while significant differences were observed in protein domains. Notably, the anthropophilic species *M. audouinii* and *M. ferrugineum* exhibited more gene duplication events and expansions in domains such as MFS and Zn2Cys6 transcription factors. Among the 138 identified genes, specific protease subfamilies (e.g., S08A, M77, S53) and CAZy subfamilies (e.g., GH18, AA1, AA3) showed strong ecological correlations with either zoophilic or anthropophilic lifestyles. The key functions of these genes from these subfamilies focus on modulating sporulation, endoproteases, lipolysis, pH regulatory adaptability, chitinase, and conidial pigment biosynthesis. Microenvironmental factors such as pH, lipid concentration, and osmolarity significantly influenced the expression of these key genes. Anthropophilic strains demonstrated higher tolerance to acidic pH and enhanced keratinase activity in lipid-rich environments, with *M. ferrugineum* exhibiting the strongest osmotic tolerance. These findings highlight the inherent evolutionary dynamics and adaptive mechanisms of dermatophytes, providing valuable insights into the pathogenicity of *Microsporum*.

INTRODUCTION

Dermatophytes represent a group of highly infectious fungi that cause superficial cutaneous infections in keratinized tissues in humans and animals, occasionally causing invasive infections. Global prevalence data of dermatophytosis from the World Health Organization indicate a rate of 20–25% [6], underscoring the public health problem and substantial economic burden associated with this disorder's high prevalence, recurrence and recalcitrance [357]. Throughout history, our close association with domesticated animals has played a pivotal role in the epidemiology of dermatophytosis, as evidenced by the fact that more than half of individuals exposed to infected asymptomatic animals may develop symptomatic infections [358]. The wide array of agricultural and companion animal sources of infection has driven repeated evolutionary adaptations of these fungi to the human host. Notably, humans harbour six anthropophilic dermatophytes, a stark contrast to most other mammal species, which typically host only a single or a few species, primarily shared among their phylogenetic relatives [359].

Zoophilic *Microsporum canis* is a worldwide diffused dermatophyte that mainly affects pet cats and dogs, and other mammals such as rodents and riding horses, being carried asymptomatically in the fur [310]. The fungus can be transmitted from animals to human hosts through close contact, leading to clinical symptoms such as tinea capitis and ringworm, sometimes leading to local outbreaks [360]. In most cases, human infections by zoophilic dermatophytes tend to be highly inflammatory, while anthropophiles usually cause mild symptoms in the affected host [208, 361]. However, clinically *M. canis* mainly cause non-inflammatory grey patch suggesting this species is undergoing an ecological transition, which is recently revealed by phylogenetic analyses of host shift of zoophilic entity (*M. canis*) to two sister anthropophiles (*M. audouinii* and *M. ferrugineum*) [192]. Additionally, introduction to new areas and/or adaptation to a new host is probably a unique event in the evolution of different host adaptive dermatophytes, the loss of opposite mating-type partners was an important driver of their evolution, and the successful genotypes that are retained may be significantly dominant in conditions with almost exclusive asexual transmission [362]. Extinction of *MAT1-2* mating-type strains is observed in *M. canis*, but the relatives *M. audouinii* and *M. ferrugineum* were both *MAT1-2*, suggesting adaptation of *MAT1-2* to human hosts [192]. Host shifts have occurred repeatedly during the domestication of agricultural and companion animals and are likely to persist as novel human-animal interactions emerge. Understanding the mechanisms of dermatophyte adaptation is crucial for public health [362-365]. The events within the phylogenetically distinct

Microsporum group serve as an excellent model and can be utilized as a reference for the broader evolution of dermatophytes.

In this study, we conducted comparative genome analysis and transcript-level validation of *Microsporum* in five hosts (horse, rabbit, cat, dog, and humans) and tester strains from various geographic locations, characterizing nuclear and mitochondrial genome evolution processes. We compared zoophilic and anthropophilic lineages and performed neutrality tests on 12 potentially relevant orthologous gene sequences. We analyzed the adaptive expression of three genes encoding subtilisins in response to microenvironmental changes. Our findings provide important insights into understanding the evolutionary dynamics and the mechanisms of adaptation inherent to *Microsporum*.

MATERIAL AND METHODS

Strains

A total of 13 strains of *Microsporum* species were included in the analysis, with ten strains having undergone genome sequencing and three reference genomes obtained from the NCBI GenBank. The sequenced strains were acquired from the Westerdijk Fungal Biodiversity Institute in Utrecht, Netherlands; the Department of Veterinary Medicine at the University of Bari Aldo Moro in Italy; and the Belgian National Reference Center for Clinical Microbiology at the University Hospital of Liege, Belgium. These strains are maintained at the Center of Expertise in Mycology at RadboudUMC in Nijmegen, Netherlands. Five isolates of *M. canis* were sourced from horses, cats, rabbits, and humans. A pair of sexually reactive tester strains, *M. canis* CBS 496.86 [=VUT 77055 (MT- = *MAT1-1*)] and *M. audouinii* CBS 495.86 [= VUT-77054 (MT+ = *MAT1-2*)] derived from the F1 progeny of two feline isolates (VUT73015 × VUT74001) [59, 366], were included in this study. The remaining three isolates of *M. audouinii* and three isolates of *M. ferrugineum* were sourced from human hosts.

DNA extraction, PCR, and sequencing

DNA was extracted after the strain was incubated in Sabouraud's Glucose Broth [SGB; 2% (w/v) glucose, 1% (w/v) peptone)] at 28°C, 150 rpm for 3 days. DNA purification was carried out using the QIAquick gel Extraction Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Genome sequencing was performed using high-quality DNA samples (OD 260/280 = 1.8-2.0, > 10 µg) on the

Illumina Hiseq platforms (Novogene, Milton, England). PCR amplification of proteases and CAZy genes was performed simultaneously on the extracted DNA. Amplicons were verified on 1% agarose.

Genome assembly, annotation and prediction

The raw reads from the whole genome sequencing (WGS) of ten sequencing samples underwent quality control using FastQC and were subsequently trimmed with Trimmomatic [367]. Following the quality control process, the genomes were de novo assembled using SPAdes v3.15.4, as well as gap closing and assembly polishing with GapCloser v1.12 [368]. The resulting genomes were annotated using the Funannotate v1.5.3 pipeline (https://doi.org/10.5281/zenodo.2604804). Gene prediction was carried out using multiple ab-initio gene predictors, including Augustus v3.5, SNAP v2006-07-28, and Glimme, trained using the fungi orthologous database v10 [369] and conserved gene models identified by BUSCO v2 [370]. Additionally, GeneMark-ES v4.71 was employed for self-training, and Augustus v3.5 was utilized to generate a set of high-quality predictions. The prediction sets were employed for gene structure annotation using EvidenceModeler v1.1.1. The tRNAs were predicted using tRNAscan-SE v2.0.11 [371]. The gene predictions obtained from EvidenceModeler were annotated using several databases, with hidden Markov model searches PFAM v35.0 and dbCAN v11.0 with hmmersearch v3.2.2 [372], DIAMOND [373] blastp searches of UniProt DB v2023_02 and MEROPS v12.0, eggNOG-mapper v2.1.11 [374] searches in diamond mode of the eggNOG 5.0 database[375]. BUSCO protein searches of the *Onygenales* were done in odb10 with transmembrane and signal peptides being predicted from the proteins using Phobius v1.01 and SignalP v6.0 [376]. Antibiotics and secondary metabolite annotation applied antiSMASH v6 [377], protein annotation InterProScan5 v5.63-95.0 [378], with UniProt/EggNog gene and product names being combined using Gene2Product v1.88. The same gene prediction and annotation procedure was performed for the NCBI reference genomes of *Microsporum canis* (GCF_000151145.1), *M. audouinii* (GCA_022344085.1), and *M. ferrugineum* (GCA_030015455.1).

Mitochondrial genome assembly and annotation

The mitochondrial genomes of the sequenced samples were assembled from the trimmed short reads with GetOrganelle v1.7.7.0 [379] using the mitochondrial “fungi_mt 0.0.1” database for read recruitment, with up to 15 recruiting rounds, and multiple kmer values (21,45,65,85,105) used for the assembly of the recruited

reads with SPAdes. The obtained genomes were then annotated using Mfannot [380].

Gene orthology and phylogenetic analysis

We utilized OrthoFinder2 [381] to identify sets of orthologous genes among the analyzed strains, employing default settings for DNA sequence analysis. Homology searches were conducted using DIAMOND, gene tree estimation was performed with DendroBLAST [382], species tree estimation was carried out using STAG [383], and tree rooting was accomplished using STRIDE. The resulting orthogroups were subsequently employed to investigate differences in specific groups of genes.

Analysis of genetic diversity and selection analysis

Isolation of all orthogroup gene sequences from the proteases and CAZy families differentially enriched in 13 genomes, these orthogroup sequences were aligned using MACSE v2.07 to accommodate frameshifts and stop codons [384], and then trimmed with TrimAl. Using R v4.3.2, the aligned gene sequences were analyzed with the Pegas library v3.1 to determine the average proportion of paired nucleotide differences (π) (excluding insertions/deletions) using the nuc.div function. Nei-Gojobori analyses were performed using the CODEML program of the PAML packageto estimate the synonymous and nonsynonymous rates (dS and dN), $dN/dS < 1$ for purified selection/negative selection, $dN/dS = 1$ for neutral evolution, and $dN/dS > 1$ for positive selection. Tajima's D measures departures from neutral expectations and selection. Tajima's D neutrality tests using the tajima.test function. For the HKA test, the input information for each locus for the calculation of the HKA test is obtained through SITES, and then the HKA is used to compare the difference between the reference locus and the locus to be detected, and the simulated construction of the distribution gives the statistically significant value of the test (http://genfaculty.rutgers.edu/hey/software). Fst values were estimated using the function 'genetic.dist' from the Hierfstat library v0.5-11, with ploidy set to haploid. High Fst values close to 1 indicate strong genetic differentiation between populations, while low Fst values close to 0 indicate homogeneity. Isolation by distance was examined via the Mantel test implemented in Adegenet [385] in R v.3.5.3, the simulated $P = 0.01$. After identification and confirmation of main effect genomic variants contributing to target traits, DNA markers were developed for molecular assisted selection, within loci linkage-disequilibrium was calculated using the LD function of the genetics package v1.3.8.1.3 (https://CRAN.R-project.org/package=genetics)

Microenvironmental effects

We examined the effects of microenvironmental changes on the transcript levels of *SUBs* in *Microsporum* species. Four factors including lipophilicity, osmolarity, thermotolerance, and pH dependence were assessed. Lipophilicity was evaluated using 0.1%, 0.5%, and 1% Tween80 in SGB; osmolarity with 3%, 6%, and 9% NaCl in SDB. To assess the growth under acidic and alkaline conditions, SGB was buffered to pH 4.5, pH 6.5, and pH 8.5 with sodium citrate or Tris-HCl. *Microsporum canis*, *M. audouinii* and *M. ferrugineum* were first grown on BLA [SGB; 2% (w/v) glucose, 1% (w/v) peptone)] for 10 days, after which conidia/mycelial fragments were eluted from Petri dish with sterilized saline and suspensions at concentrations of (0.5−1) × 10^7 CFU/mL were prepared using the modified EUCAST broken mycelium inoculation method [386], with four replicates using the above media, three replicates for each strain, and then incubated at 28℃ at 150 rpm for 3 days. Thermotolerance was assessed in SGB at 22℃, 28℃, and 37℃, respectively, shaken at 150 rpm for 3 days.

Real-time quantitative PCR

Real-time quantitative PCR (RT-qPCR) was applied to evaluate the transcript levels of 12 key genes found in this study. Total RNA was extracted using TRIzol (Invitrogen, Waltham, MA, U.S.A.) [387], and first-strand cDNA synthesis was performed using RNA and Transcriptor Universal cDNA Master mix (Roche, Mannheim, Germany). RT-qPCR transcription reactions were performed with the gene primers and LC 480 SYBR Green I Master (Roche).

Statistics and visualization

The data plots generated from the analyses conducted in this study were processed and filtered using the R packages dplyr, ggplot2, GGally, or omicstudio [388], they were refined using Adobe Illustrator v25.3.1. Mitochondrial annotation and comparison were visualized using Genious Prime v2023.2.1. Statistical analysis and plot generation were performed using SPSS v26 and GraphPad Prism v9.0. The two-way ANOVA with Tukey's multiple comparison test was employed to assess the expression differences.

RESULTS

High similarity in nuclear and mitochondrial genomes of *Microsporum* species

The reasonable draft genome quality with totally 13 *Microsporum* strains genomes assembled into fewer than 500 scaffolds (Figure 1a). Genome sizes and gene numbers in animal- and human-derived *M. canis*, as well as in human-derived *M. audouinii*, were comparable and notably greater than those in *M. ferrugineum*. GC percentages were similar in all genomes, ranging from 46.92% to 48.13%. The total numbers of predicted proteins, unique proteins and tRNAs were similar in three *Microsporum* species. The Benchmarking Universal Single-Copy Orthologs (BUSCO) coverage of all genomes exceeded 98%. Genomic similarity and homozygosity showed a high degree of colinearity, interrupted by a small number of inversions, and a high percentage of identity at the amino acid level (94.11% *M. canis vs. Microsporum ferrugineum*; 96.23% *M. audouinii vs. Microsporum ferrugineum*; 97.46% *M. canis vs. Microsporum audouinii*) (Additional figure 1). The species tree inferred by STAG showed that the *Microsporum* species was distant from the outgroup (Figure 1b), which was consistent with our previous evolutionary results on conserved sequences, evolutionary change from zoophilic to anthropophilic is observed within *Microsporum.*

The mitochondrial genomes (mtDNAs) of *M. canis* (Figure 1c), *M. audouinii* and *M. ferrugineum* were circular with sequence lengths of 23,943 bp, 23,986 bp and 23,840 bp, respectively, which maintained a high degree of covariance with short intergenic regions and no detectable GC rearrangements (Figure 1d). Similar to other dermatophytes, each *Microsporum* genome encoded 15 core protein-coding genes (*RPS3*, cox1, atp9, cox2, nad4, nad5, nad2, cob, nad3, nad1, nad4, atp6, nad6, cox3 and atp8) and two rRNA genes (rnl and rns). The anthropophilic *M. audouinii* and *M. ferrugineum* encoded 26 tRNAs, encoding an additional trnG (acc) tRNA over *M. canis* (25 tRNAs); these tRNAs were divided into three clusters flanked by *RPS3*-cox1, cox1-atp9, and atp8-rnl. The ribozyme gene (rnpB) was found downstream of the atp6 gene in *M. canis* and *M. audouinii*, which was lost in *M. ferrugineum*.

Decreasing number of orthologs from animal- to human-derived *Microsporum*

The proportion of genes assigned to the orthologous group exceeded 93% for all 13 genomic samples. The ortholog genes were genes derived from species formation events, as opposed to paralogs genes derived from gene duplication events. Based on Heatmap of orthologues statistics for each species pair, the number of orthologs among animal-derived *M. canis* (n = 7759-7894) was slightly higher than that of human-derived *M. canis* CBS 113480 (n = 7692-7743), followed by *M. audouinii* (n = 7485-7710), and *M. ferrugineum* (n =7439-7653) (Figure 1e). Among the 13 genomes, only two species-specific orthogroups were found in *M. audouinii* CBS495.86 and *M. ferrugineum* M1a, respectively, and the gene products within these orthologous clusters were all hypothetical proteins. In the species tree inferred by STAG, the number of gene duplication events (support>=50%) on the branch of anthropophilic *M. audouinii* and *M. ferrugineum* (n = 183) were significantly higher than that in *M. canis* branch (n = 19), suggesting that these strains may have adapted to the host-specific environment through continuous gene duplication (Figure 1e).

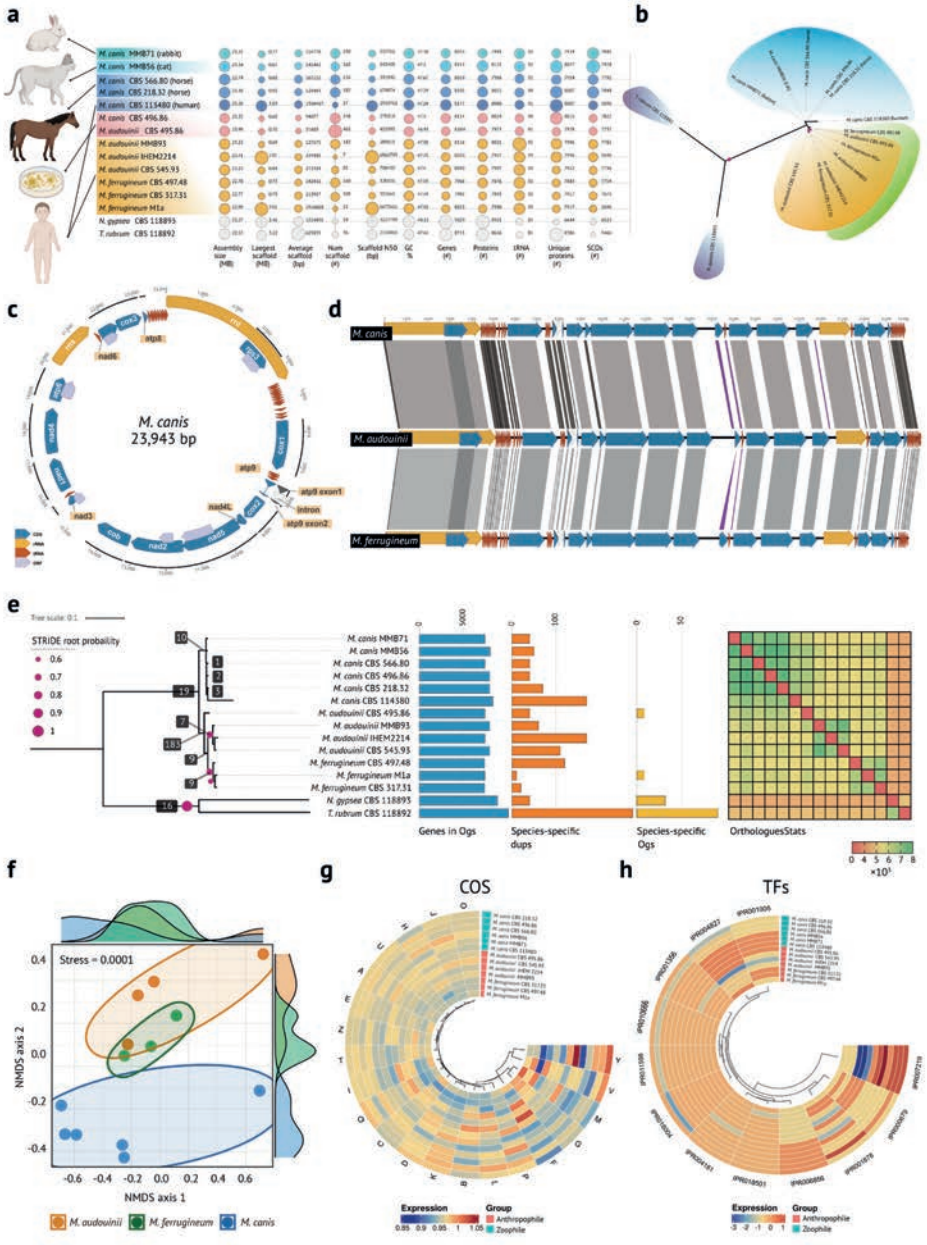
a
b
c
d
e
f
g
h
M. canis
23,943 bp
M. audouinii
M. ferrugineum
STRIDE root probability
Genes in Ogs
Species-specific dups
Species-specific Ogs
OrthologuesStats
Stress = 0.0001
NMDS axis 1
NMDS axis 2
COS
TFs
Expression
Group
Anthropophile
Zoophile

Figure 1. Genome characteristics, phylogenetic orthology inference and homologous protein family analysis. (a) Host origin and genome summary stats for all samples, and bubble plots illustrate the quality parameters of genome sequences. Bubble sizes are not comparable between panels (columns). (b) Unrooted phylogenetic tree inferred by STAG and rooted by STRIDE, the blue, green, and yellow branches represent *Microsporum canis*, *M. audouinii*, and *M. ferrugineum*, respectively. (c) Circular representations of the *M. canis* mitochondrial genomes. (d) Pairwise linear genome comparisons of *M. canis*, *M. audouinii*, and *M. ferrugineum* mtDNAs. Grey parallelograms indicate the locations of homologous genomic regions in adjacent genome pairs. Nine PCGs had length variations among the three *Microsporum* species, with *M. audouinii* having a 12-base extra insertion in the nad6 gene compared to *M. canis* and *M. ferrugineum*, while *M. audouinii* and *M. ferrugineum* had a 135 bp extra insertion in the nad3 gene compared to *M. canis*. (e) Summary of OrthoFinder analysis of *Microsporum* species (including outgroups *N. gypsea* and T. *rubrum*): species tree inferred by STAG and rooted by STRIDE, number of gene duplication events tagged on each node of the species tree; number of genes assigned to orthologous groups for each genome; number of gene duplication events on each terminal branch of the species tree; number of species-specific orthologous groups; heatmap of orthologues per species stats. OG = orthogroup; dups = gene duplication events. (f) NMDS analysis of InterProscan functional predictions for 13 genomes. The vertical coordinate analysis is based on the Bray-Curtis distance matrix. Graph plots and ellipses were generated from the ggplot2 R package implemented using the stat ellipse function. Each dot represents a genome. (g–h) Differences between clusters of orthologous groups (COGs) and fungal transcription factors (TFs) per genome. The plotted values were standardized with normal distribution, distance calculation based on Euclidean, and complete hierarchical clustering. Detailed functions and descriptions of each subcluster are shown in Tables 1–2.

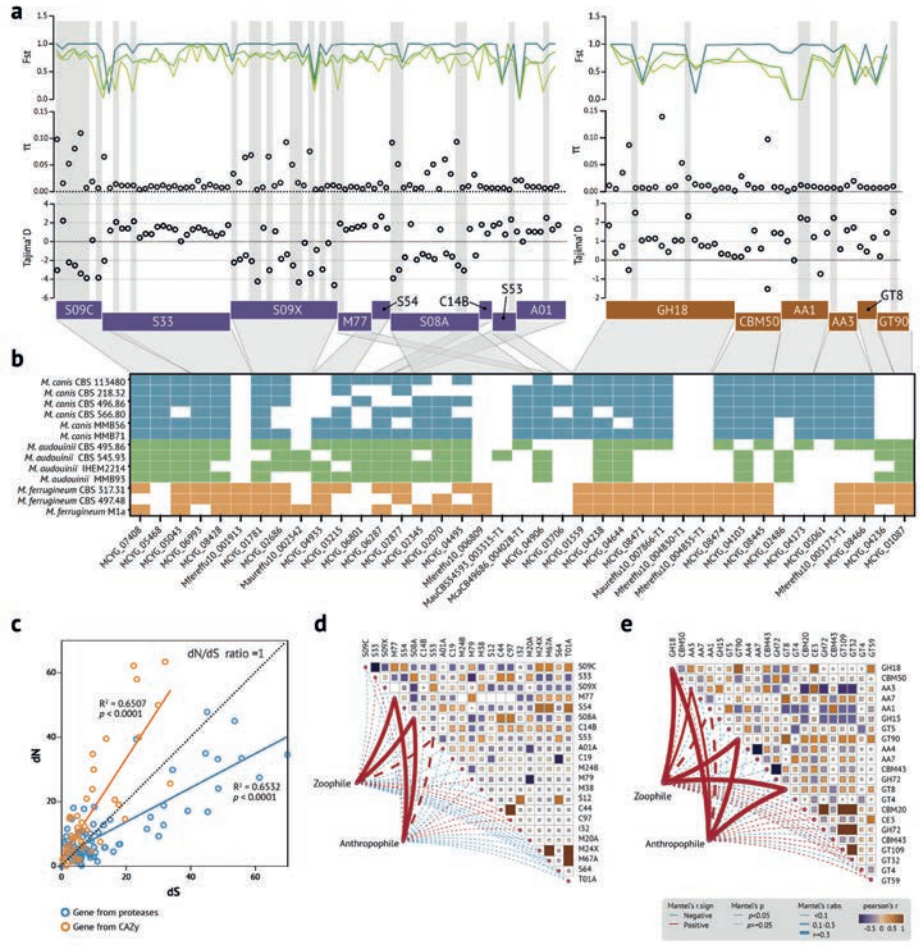

Figure 2. Gene selection for protease and CAZy. (a) A total of 138 genes were isolated from the differential gene families of CAZy and protease and CAZy, showing values of nucleotide diversity, Tajima's D neutrality test, and Fst statistics for the genetic differentiation between populations in *Microsporum* species. Each black circle represents a gene, Tajima'D and HKA neutrality tests values for genes within the grey rectangle were statistically significant ($P < 0.05$). (b) The Heatmap showing gene loss in samples from different isolation sources. Mfereffu10_001913 (S33, function unknown) and Mfereffu10_006809 (S08A, posttranslational modification) appeared only in *M. ferrugineum*, while MCYG_05468 (S33, function unknown) and MCYG_06801 (M77, coenzyme metabolism) were lost in *M. ferrugineum*. McaCBS49686_004028-T1 (S53, coenzyme metabolism) was present only in animal-derived *M. canis*. Of the CAZy carbohydrates, OG0007899 (AA1, function unknown) and OG0008108 (AA3, secondary metabolites) were only found in *M. canis*, while MCYG_04173 (GT8, carbohydrate metabolism) and MCYG_05061 (GT90, function unknown) are specific to *M. audouinii* and *M. ferrugineum*. (c) Plot of dN/dS ratios for protease and CAZy, each circle represents a gene. (d–e) Correlations between gene families within protease/CAZy and zoophilic or anthropophilic *Microsporum* species were calculated using mantel' s test. Zoophilic for *M. canis*, anthropophilic for *M. audouinii* and *M. ferrugineum*. Mantel' s r statistics is indicated by line width; solid/dashed lines indicate statistical significance, and line colour indicates mantel's sign.

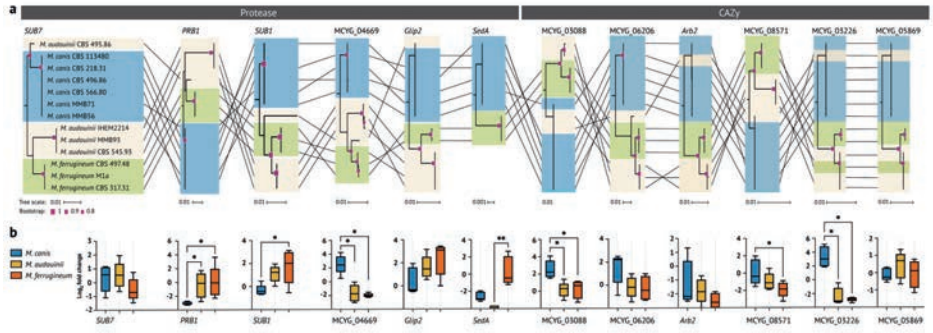

Figure 3. Comparative phylogenetic analysis and key gene expression of protease and CAZy in *Microsporum* species. (a) It shows the tanglegram between the 12 selected genes from protease and CAZy. The black lines connect corresponding parts of the gene tree. Branch lengths are scaled, and pink nodes indicate high-confidence bootstrap values. (b) Boxplots showing the log2 fold changes in the expression of the same genes across the three *Microsporum* species. 5–6 replicates per group and samples from each group are presented using mean ± SEM. Significance was annotated as follows: **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001.

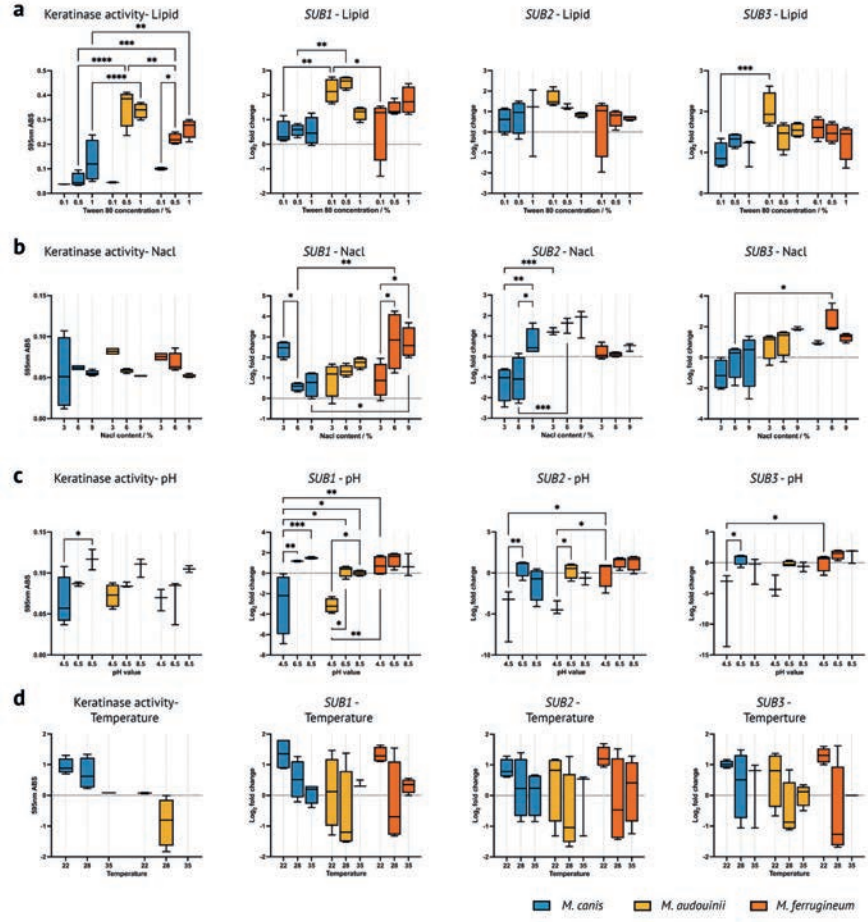

Figure 4. Effects of lipid, osmolality, pH, and temperature on SUB1–3 transcript levels. Samples from each group are presented using mean ± SEM. The value of $P < 0.05$ was considered statistically significant. Significance was annotated as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

Variant virulence factors are common in *Microsporum* genomes

We used the SnpEff spacer forest method to compare and annotate genome variants. Human-derived *M. canis* CBS 113480 was used as a reference sequence to predict the nucleotide variants between different *Microsporum* species. The most common variant type was single nucleotide polymorphism (SNP), followed by insertion (INS) and deletion (DEL) in all *Microsporum* genomes. The mating strain *M. audouinii* CBS 495.86 (*MAT1-2*) showed an extremely large variant rate, with one variant every 71 amino acid steps, which was a much higher variant number than

that of other strains. Among the animal-derived *M. canis* strain, horse-derived *M. canis* CBS 566.80 showed the highest variant rate, with one variant every 2,955 amino acid steps, followed by rabbit-derived *M. canis* MMB71 (one variant every 3,606 amino acid steps) and cat-derived *M. canis* MMB56 (one variant every 5,739 amino acid steps). The variant rates of all anthropophilic *Microsporum* strains were higer than that of *M. canis* strains, varying from one variant every 1,127 to 1,392 amino acid steps.

We predicted and annotated the top 10 genes in terms of number of variants according to the missense mutation, splice site, frameshift INS/DEL, infram INS/DEL and intron mutation. There were several shared or specific pathways between human-derived *M. canis* and animal-derived *M. canis*, and two anthropophilic *Microsporum* species (Additional figure 2). Two variant virulence factors relating to signal transduction mechanisms and RNA processing were found between human- vs. animal-derived *M. canis*. When comparing human-derived *M. canis* vs. *M. ferrugineum* and *M. audouinii,* the number of potential virulence factors was five and four, respectively. The former functions mainly relate to secondary metabolites, signal transduction, transcription, and intracellular trafficking, while the latter functions relate to cytoskeleton, translation and cell cycle control (Additional table 1).

Differences in domain function suggest separation of zoophile and anthropophile

The InterPro, the Gene Ontology (GO), EggNOG, Pfam, and Clusters of Orthologous Groups (COS) database annotations revealed 7,756 (InterPro, 94.85% of gene number), 5,040 (GO, 61.63%), 4976 (EggNOG, 60.85%) and 3,803 (Pfam, 46.51%) function gene predictions, respectively. Non-metric multidimensional scaling (NMDS) ordinations confirmed that zoophilic and anthropophilic *Microsporum* species differed in the distribution characteristics of protein domains (Figure 1f). Zoophile and anthropophile were clearly segregated in composition, with anthropophilic *M. audouinii* and *M. ferrugineum* partially overlapping. There was no segregation between animal- and human-derived *M. canis* strains.

We compared the InterPro category counts of zoophilic *vs.* anthropophilic *Microsporum* genomes. Of the top 10 differential domains, seven were expanded in anthropophilic *Microsporum* species, with three of them possessing the major facilitator superfamily structural (MFS) (IPR020846, IPR011701, IPR036259), two possessing Zn2Cys6 fungal-type DNA-binding (IPR036864, IPR001138), and two possessing protein kinase (IPR011009, IPR00719). The remaining three were

expanded in zoophilic *Microsporum*, carrying P-loop NTPase (IPR027417), WD40-repeat domain (IPR036322) and cytochrome P450 (CPY) (IPR001128) (Additional figure 3). Comparison of COGs (Figure 1g, Table 2) there were 25 categorized clusters, the major intraspecific differences were centred on (1) carbohydrate transport and metabolism, lipid transport and metabolism, secondary metabolites, and (2) transcription, replication, recombination and repair.

We further identified transcription factors (TFs), secondary metabolism, secreted proteins, and membrane proteins in *Microsporum* species using the Fungal Transcription Factor Database, antiSMASH 7.0, and Phobius v1.01. The TFs of *Microsporum* species were mainly enriched in the Zn2Cys6 transcription factor (IPR007219), Myb (IPR001005) and basic-leucine zipper (IPR004827), but the differences in the number of TFs among three *Microsporum* species were minimal (Figure 1h, Table 3). There were six secondary metabolite classes involved in the *Microsporum* synthesis: non-ribosomal peptide synthetases (NRPS), T1 polyketide synthases (T1PKS), NRPS-like genes, PKS/NRPS hybrid genes, dimethylallyl transferase, and terpene cyclase. No statistically difference were found on the distributions of secreted protein and membrane proteins in zoophilic and anthrophilic *Microsporum* species.

Specific proteases and carbohydrate-active enzymes (CAZy) may be involved in *Microsporum* microevolution

Based on the above significant intraspecific differences of predicted protein functions in carbohydrate transport and metabolism, we further annotated specific proteases and CAZy among *Microsporum* species using enrichment analysis. Totally, we identified 138 *Candida*te genes which were enriched in 9 protease and 6 CAZy subfamilies (Figure 2a). However, some of the genes were lost in *Microsporum* species of different origins, e.g., in protease, 4 genes were lost in *M. canis*, 5 genes in S08A, S53 and M77 were lost in *M. ferrugineum*; while in CAZy, 10 genes were lost in *M. audouinii* (Figure 2b). We quantified the selection pressures by comparing the dN/dS ratio between zoophilic and anthrophilic *Microsporum* species. In general, most protease subfamily genes belonged to negative selection (dN/dS < 1), while most CAZy positive selection (dN/dS > 1) (Figure 2c). We then computed the genetic differentiation fixation index (Fst), nucleotide diversity (π), Tajima's D and Hudson-Kreitman-Aguade (HKA) value. Total 31 selected genes (Tajima's D value >2 / < -2 and HKA test *P* value < 0.05), including 25 protease and 6 CAZy, were detected. The average Fst between *M. canis vs. Microsporum audouinii*, *M. audouinii vs. Microsporum ferrugineum*, and *M. canis vs. Microsporum ferrugineum* was 0.7361

(range: 0.16667–1), 0.6567(range: 0.0204–1), 0.9105(range: 0.11222–1), respectively, which implying the subdivision among three *Microsporum* species (Table S5).

Subsequently, we analyzed the above 31 selected genes using the mantel test to evaluate the ecological microevolution between zoophilic and anthrophilic *Microsporum* species. (Figure 2d-e). The results showed significant positive correlations were found in three protease subfamilies (M77, S08A and S53) and five CAZy subfamilies (GH18, AA3, GT90, GT8 and AA1), which contain 12 selected genes (Table 3). In protease subfamilies, the selected genes were *SUB1*, *SUB7*, *PRB1*, *Glip2*, *SedA*, and MCYG_04669, in the CAZy subfamilies, the genes were MCYG_03088, MCYG_06206, *Arb2*, MCYG_08571, MCYG_03226, MCYG_05869.

Potential functions and expression of selected genes in *Microsporum*

We performed phylogenetic analyses of the 12 selected genes, and tanglegram plot connected the positional changes of each *Microsporum* species on different gene trees (Figure 3a). The position of the hybrid strain CBS 495.86 in each gene tree was unstable, which might relate to its high rate of nucleotide variation. Proteases genes *SUB1*, *PRB1*, *SUB7*, and MCYG_04669 showed high genetic differentiation for three *Microsporum* species (bootstrap > 90%), while CAZy genes could not distinguish zoophilic *M. canis* from the anthropophilic species. We further analysed the distribution of genetic variants and mutations at SNP sites using the above 12 selected genes. Linkage disequilibrium (LD) analysis revealed highly interlinked blocks for these genes in *Microsporum* species (Additional figure 4), where the high LD blocks were interrupted by low LD regions, appearing similar to mosaicism in the genome. Notably, a greater number of high LD blocks were detected within genes encoding CAZys, such as MCYG_06206, *Arb2*, and MCYG_08571, and these high local LD could indicate the allele that has recently increased to high frequency under strong selection. We analyzed the relationship between SNP sites within each high LD block and phenotype, unique SNPs within Block 1 of *SUB1*, MCYG_03226, and MCYG_04669 were exclusive to *M. ferrugineum*. Similarly, specific SNPs in Block 2 of MCYG_04669, as well as Blocks 1, 2, and 3 of *PRB1*, were solely identified in *M. audouinii*. In contrast, no unique LD blocks were discernible among the different animal-derived *M. canis* strains.

RT-qPCR analysis revealed significant differences in the expression of 12 key genes among the *Microsporum* (Figure 3b). *PRB1* and *SUB1* were significantly upregulated in anthropophilic *M. audouinii* and *M. ferrugineum* ($P < 0.05$), while *SUB7* exhibited no significant expression differences among the three species. *sedA* was significantly upregulated in *M. ferrugineum* ($P < 0.05$), whereas its expression was

almost absent in *M. audouinii* and lower in *M. canis*. MCYG_04669 exhibited significantly higher expression in *M. canis* compared to two anthropophilic species ($P < 0.05$). For the CAZy genes, the zoophilic expression levels were higher than those of the anthropophilic species. MCYG_03088, MCYG_08571, and MCYG_03226 were significantly downregulated in *M. audouinii* and *M. ferrugineum* compared to *M. canis* ($P < 0.05$). MCYG_06206 and *Arb2* also show the same decreasing trend in the anthropophilic species but without significant statistically differences.

We further analyzed the conserved domains of the protein sequences encoded by these genes and analyzed their potential role in *Microsporum*. *SUB1*, *SUB7*, *PRB1*, *Glip2*, *SedA*, and MCYG_04669 whose functions are related to keratolysis, sporulation, lipase, and secreted tripeptidyl peptidase (Table 3). We further analysed the conserved domains of the protein sequences encoded by these genes. In *Glip2*, the anthropophilic *M. audouinii* and *M. ferrugineum* each encode an additional pp-binding conserved domain, with *M. ferrugineum* also possessing an extra PRK0483 domain compared to the other two species. Additionally, the *SedA* gene was lost in the *M. audouinii*, while the other protease genes were largely conserved across all species. In the CAZy subfamilies, MCYG_06206 and MCYG_03088 are known to encode chitinase in dermatophytes. MCYG_06206 consists of two LysM binding, one chitin-binding 1 and one chitin binding domain of the GH18 chitinase family in tandem. MCYG_03088 consists of one LysM binding and one GH18 chitin binding domain. The LysM binding was lost in MCYG_03088 in the rabbit-derived strain *M. canis* MMB71. The *Arb2* and MCYG_08571 genes encode conidial pigment biosynthesis oxidase, with *Arb2* also influencing filamentous growth and spore formation in yeast cells. Laccase 1, encoded by MCYG_08571 gene, is involved in fungal pigmentation and acts as a virulence factor.

Microenvironmental effects on *SUB1-SUB3* transcript levels

Subs were core endoproteases for keratin colonization and infection by dermatophytes. Studies on the pathogenicity of *Microsporum* had proposed that *SUB1-SUB3* were associated with adhesion, keratolysis, and infection of different host hair/keratin proteins. Our previous study of the keratolytic capacity of dermatophytes on 42 species of mammal fur (including wildlife, domestic animals, and companion animals) and phylogenetic analysis revealed that *SUB1-SUB3* genes provide a reasonably correct reflection of the evolution of the *Microsporum* species (Additional figure 5).

Corresponding to the ecological microevolution, we further evaluated the microenvironmental adaptation of *Microsporum* species using different

concentrations of lipophily, osmolarity, pH and temperature tolerance factors. As lipid concentrations increased from 0.1%, 0.5% to 1%, the keratinase activity of the three *Microsporum* species gradually increased, most notably in *M. audouinii*, followed by *M. ferrugineum* (P<0.05). At 0.1% and 0.5% lipid concentrations, the expression level of *SUB1* and *SUB3* transcripts of *M. audouinii* were higher than in the other two species (Figure 4a).

We perceived that *M. ferrugineum* strains growing faster than *M. canis* and *M. audouinii* at a high NaCl concentrations of 9%, which suggesting that *M. ferrugineum* is more osmolarity tolerant. No significant difference was observed within *Microsporum* species in the effect of changing osmolarity on keratinase activity, but the *SUB1-SUB3* transcript levels of *M. audouinii* and *M. ferrugineum* were higher than those of *M. canis* at 6% and 9% NaCl (P<0.05) (Figure 4b).

In *M. canis*, the expression levels of genes *SUB1-SUB3* were found to be the lowest at pH 4.5, these levels exhibited a gradual increase for *SUB1* across a pH range of 4.5 to 8.5. In contrast, the expression levels of *SUB2* and *SUB3* reached their peak at a pH of 6.5 (P<0.05) (Figure 4c). In anthropophilic *M. audouinii* and *M. ferrugineum*, an increased tolerance to acidic environments was observed, particularly in *M. ferrugineum*, which displayed a marked increase in the expression levels of *SUB1-SUB3* genes at pH 4.5 when compared to *M. canis* (P<0.05).

Alterations in temperature exerted minimal impact on the keratinolytic capabilities and the expression levels of the *SUB1-SUB3* genes across all *Microsporum* strains evaluated. Statistical analysis revealed no significant differences in these parameters among the various temperature conditions tested 22°C, 28°C, and 35°C (Figure 4d).

DISCUSSION

Dermatophytes (family *Arthrodermataceae*) are a group of fungi in the order *Onygenales* which have evolved relatively recently, almost exclusively with mammal hosts [389]. The ecological subdivision in geophilic, zoophilic, and anthropophilic species broadly reflects their course of evolution, although the molecular mechanisms and evolutionary dynamics driving host shift are still not well understood. Homo sapiens is phylogenetically the most recent host, despite dermatophyte genomes usually show strong colinearity and low inversion [390, 391]. In *Trichophyton* genomes, the closely related siblings *Trichophyton rubrum* (anthropophilic, preferences nail/skin) and *T. violaceum* (anthropophilic, scalp) have high degrees of genome identity (99.83%), while with marked differences of

phenotypic and clinical features, which possibly due to the functional differences in specific proteins involved ATP transferase activity, calcium binding and lipid transport [352]. Martinez *et al.* [392] demonstrated that anthropophilic species (*T. tonsurans* and *T. rubrum*) were enriched in kinases and transcription factors compared to zoophilic species (*T. verrucosum*, *T. equinum*, *M. canis*, and *T. benhamiae*). In *Microsporum* genomes, zoophilic *M. canis* and anthropophilic *M. audouinii* and *M. ferrugineum* exhibit a high percentage of amino acid identity and show minimal variation in both nuclear and mitochondrial genomes. However, through our phylogenetic inference of orthologous groups on pairwise relationships of the three species, NMDS analyses based on protein domain, and population Fst results, all suggest that *M. canis*, *M. audouinii* and *M. ferrugineum* have diverged significantly in terms of protein function, anthropophilic *Microsporum* species display an expansion of major facilitator superfamily (MFS) and kinase-like domains, while protein families affecting ATPase post-translational modification, signalling mechanisms, and energy production and conversion are contracted, presumably linked to the low inflammatory response to human dermatomycosis caused by anthropophilic *Microsporum* species. Notably, an enrichment of CPY in *M. canis* is observed for the first time. In the newly indentified dermatophyte species *T. inotineae*, the overexpression of TinCYP51B resulting from additional copies of this gene is responsible for the reduced sensitivity of *T. indotineae* strains to azole compounds [393]. It is reported that 25–40% of *M. canis* infection patients had recurrence and treatment failure. The role of CYP in the azole resistance of *M. canis* requires further verification.

The ability of microorganisms to sense and adapt to changes in the environment is essential to their survival. Dermatophytes are a highly specialized group of keratinophilic and keratinolytic filamentous fungi. Anthropophilic and zoophilic dermatophytes adapt to different mammalian body surface microenvironments such as temperature, pressure, salinity and pH by developing molecular mechanisms which stabilizes their proteins, cell membranes, lipids, and nucleic acids. The secreted proteolytic and lipolytic enzymes enable nutrient acquisition from the host, leading to variation in host specificity, immunogenicity, and virulence. Transcription factors such as *PacC* and *Hfs1*, as well as heat shock proteins, are involved in sensing and adapting to the acidic pH of the skin in the early stages of fungal host interaction [50, 394]. Dermatophytes adhere to keratinocyte surfaces in a pH-dependent manner, and the acidic pH of the human skin surface is associated with specific fatty acids derived from skin lipids. Keratin is the only source of carbon for dermatophyte infections, and the keratolytic is accompanied by a change in extracellular pH from acidic to alkaline, an environment in which most of the known proteases exhibit optimal activity,

ultimately leading to the establishment and maintenance of the infection [395]. In our study, we found that subtilisins (*SUB1*, *SUB7*, *PRB1*), lipases (*Glip2*), and tripeptidyl peptidase (*SedA*) were associated with host selection preferences in *Microsporum* species, the latter two genes maintained high activity at acidic pH. Likewise, putative GDSL lipase (*Glip*), subtilisins (*SUB3 & SUB7*), and tripeptidyl peptidase *SedC* were also observed in the secretomes of *T. benhamiae*, *T. rubrum*, and *T. violaceum* after incubation with keratinocytes [396]. According to the MEROPS peptidase classification, subtilases were divided into two clans: S08A (subtilisins) and S53 (sedolisins) [300]. *SedA* (from S53 subfamily) was absent in *M. audouinii*. In *Onygenales*, the lack of certain lineages encoding S53 family genes is thought to be more specific, may play an important role in interactions with the environment [300]. Subtillisins are essential proteases in keratin assimilation, and subtilisin-like protease *SUB1*, *SUB3-SUB7* are specific for dermatophytes, although the expression of *SUBs* gene is not uniform for all species [39, 68]. In this study, different keratinase activity and expression of *SUB1-SUB3* in response to microenvironmental changes were observed, which was comparable with our previous study [387]. The presence of lipids in the environment significantly impacted the keratinase activity of anthropophilic species. Human scalp pH (4.5-5.5) is more acidic than skin pH (6.5-8.5) in pet cats and dogs; high *SUB1-SUB3* transcript levels of *M. ferrugineum* at acidic pH suggests adaptation of the anthropophilic to human hosts. *PRB1* is a subtilisin-like protease essential for virulence and phenotypical traits in many fungi. Its elevated expression in M. ferrugineum and M. audouinii may enhance colonization and persistence in human hosts. Interestingly, *sedA* (MCYG_01559), a tripeptidyl peptidase from the S53 subfamily that degrades proteins at acidic pH, is nearly absent in M. audouinii but significantly upregulated in M. ferrugineum, indicating lineage-specific adaptation to acidic skin environments in M. ferrugineum.

We observed an expansion of carbohydrate metabolism-related superfamily in the anthropophilic *Microsporum*, from which we isolated four subfamilies associated with the adaptive evolution of CAZy: GH18, AA1, AA3, and GT90, some of which encode unknown functional domains. Within the GH18 family, we identified MCYG_03088 and MCYG_06206, which encode chitin-binding and LysM-binding, respectively. The LysM domain is a highly conserved carbohydrate-binding module. Compared to other non-dermatophytes in *Onygenales*, the LysM protein family of dermatophytes appears to have undergone a gene expansion, ranging from 7 in *E. floccosum*, 12 in *T. rubrum*, and 31 in *M. canis* [46, 392]. Studies have shown that the dermatophyte LysM proteins bind to and mask chitin to protect the fungal cell from the host immune system, allowing latent colonization by the dermatophyte [397, 398]. In the *Onygenales* phylogenetic tree of all proteins containing LysM

domains [392], MCYG_03088 and MCYG_06206 of *M. canis* were clustered in two distinct branches, and further studies should be conducted on the expression and determination of their putative roles in the infection process. The conidial pigment biosynthesis oxidase encoded by *Arb2* and laccase 1, encoded by MCYG_08571 from the AA1 family, are structurally homologous, both containing three copper-oxidoreductase domains. They have been shown to be key regulatory genes for conidial pigment biosynthesis in *Aspergillus fumigatus* and *Blastomyces dermatitidis*, which are involved in cell wall formation and help the fungi resist various environmental stressors [399]. In the human host, *Asp. fumigatus* conidial pigments modulate the host's pro-inflammatory cytokine response by physically masking fungal pathogen-associated molecular patterns (PAMPs) from recognition by the immune system [400]. Our RT-qPCR results showed that MCYG_03088, *Arb2*, MCYG_08571 and MCYG_03226 were significantly downregulated in anthropophilic *Microsporum* compared to the zoophilic *M. canis*. Further investigation is required to determine whether these genes serve similar functions as above mentioned in *Microsporum* species. Furthermore, *M. ferrugineum* exhibited greater osmotic stress tolerance compared to the other two species and maintained consistent transcript levels of *SUB1* and *SUB3* even at high NaCl concentrations. This suggests that *Arb2* and MCYG_08571 genes may play an important role in exerting the adaptive evolution of anthropophilic species to human hosts.

CONCLUSION

This study represents the inaugural comprehensive genomic sequencing analysis of the different host-derived *Microsporum* species, which, as a phylogenetic group, are clearly individualized [1] and show a host shift from animal to human. Carbohydrate metabolism, signal transduction regulation and post-translational modifications were implicated in the microevolutionary processes that underpin host specificity. Notably, there is an expansion of the MFS, Zn2Cys6 DNA-binding, and protein kinases within the anthropophilic *Microsporum* species, with less variation among different animal-derived *Microsporum* species. Specific subfamilies of proteases (M77, S08A, and S53) and carbohydrate-active enzymes (GH18, AA3, GT90, GT8, and AA1) display positive correlations with ecological parameters to anthropophilic and zoophilic lifestyles. We have pinpointed 12 genes within these subfamilies that are pivotal for attributes such as phenotype, sporulation, endoproteases, lipases and those involved in pH-adaptive regulation. The study further reveals the keratinolytic potential and the expression levels of *SUB1-SUB3* genes are modifiable by lipid, pH levels, and osmolarity in microenvironment. In addition, we identified highly interlocked Block regions in coding protease and CAZy genes and analyzed the relationship between SNP site mutations and

haplotype, which provides a basis for efficient identification of *Microsporum* species and accelerate mining of functional genes.

Table 1. Clusters of orthologous groups (COG) functional category distribution among *Microsporum.*

COG	std	*M. audouinii* CBS495.96	*M. audouinii* CBS545.93	*M. audouinii* IHEM2214 (ncbi GCA_ 022344085.1)	*M. audouinii* Mau93	*M. canis* CBS113480 (ncbi GCF_ 000151145.1)	*M. canis* CBS218.32	*M. canis* CBS496.86	*M. canis* CBS566.80	*M. canis* Mca56	*M.canis* Mca71	*M. ferrugineum* CBS317.31	*M. ferrugineum* CBS497.48	*M. ferrugineum* M1a (ncbi GCA_ 030015455.1)
S:(S) Function unknown	20.35172	1930	1919	1894	1944	1930	1948	1944	1945	1971	1922	1912	1918	1910
G:(G) Carbohydrate transport and metabolism	5.706497	342	348	345	351	343	345	348	341	347	339	352	357	357
K:(K) Transcription	5.662517	301	298	294	297	306	304	306	305	305	297	292	289	297
Q:(Q) Secondary metabolites biosynthesis, transport and catabolism	5.410105	303	310	314	305	305	311	309	301	310	306	295	298	304
I:(I) Lipid transport and metabolism	4.97687	282	297	293	293	287	292	291	292	291	285	282	284	295
L:(L) Replication, recombination and repair	4.530523	221	227	221	227	219	222	227	221	223	216	213	217	216
J:(J) Translation, ribosomal structure and biogenesis	3.727926	361	368	359	363	361	364	363	361	370	365	359	358	358

Table 1. (continued)

COG	std	*M. audouinii* CBS495.96	*M. audouinii* CBS545.93	*M. audouinii* IHEM2214 (ncbi GCA_ 022344085.1)	*M. audouinii* Mau93	*M. canis* CBS113480 (ncbi GCF_ 000151145.1)	*M. canis* CBS218.32	*M. canis* CBS496.86	*M. canis* CBS566.80	*M. canis* Mca56	*M.canis* Mca71	*M. ferrugineum* CBS317.31	*M. ferrugineum* CBS497.48	*M. ferrugineum* M1a (ncbi GCA_ 030015455.1)
O:(O) Posttranslational modification, protein turnover, chaperones	3.617975	532	539	529	534	528	532	531	532	537	530	527	527	530
C:(C) Energy production and conversion	3.526457	237	239	241	242	236	237	238	239	241	240	239	229	243
T:(T) Signal transduction mechanisms	3.454837	345	347	351	355	349	353	355	352	355	348	356	351	353
F:(F) Nucleotide transport and metabolism	3.301126	100	105	100	103	95	94	99	95	98	97	97	95	100
P:(P) Inorganic ion transport and metabolism	3.202563	168	162	164	159	159	165	167	161	169	164	162	161	163
H:(H) Coenzyme transport and metabolism	3.172397	244	247	244	248	247	242	248	246	249	242	244	238	242
D:(D) Cell cycle control, cell division, chromosome partitioning	2.665064	168	168	164	167	167	168	167	167	166	162	162	160	165

Table 1. (continued)

COG	std	*M. audouinii* CBS495.96	*M. audouinii* CBS545.93	*M. audouinii* IHEM2214 (ncbi GCA_ 022344085.1)	*M. audouinii* Mau93	*M. canis* CBS113480 (ncbi GCF_ 000151145.1)	*M. canis* CBS218.32	*M. canis* CBS496.86	*M. canis* CBS566.80	*M. canis* Mca56	*M.canis* Mca71	*M. ferrugineum* CBS317.31	*M. ferrugineum* CBS497.48	*M. ferrugineum* M1a (ncbi GCA_ 030015455.1)
E:(E) Amino acid transport and metabolism	2.662657	360	355	355	357	354	358	359	358	355	355	354	352	361
U:(U) Intracellular trafficking, secretion, and vesicular transport	2.614065	449	448	446	445	449	449	448	444	448	444	443	442	443
A:(A) RNA processing and modification	2.534379	293	291	292	289	297	294	296	292	295	291	289	292	290
M:(M) Cell wall/ membrane/ envelope biogenesis	2.454718	87	84	84	82	83	82	84	84	82	81	88	85	89
B:(B) Chromatin structure and dynamics	2.267383	115	118	113	116	116	113	116	111	114	111	114	111	112
Y:(Y) Nuclear structure	1.463224	30	28	29	28	32	31	30	30	29	27	27	29	29
V:(V) Defense mechanisms	1.182132	33	34	34	35	34	35	34	32	33	34	31	35	34
Z:(Z) Cytoskeleton	1.115164	120	120	118	119	117	119	119	118	120	118	121	118	119

Table 1. (continued)

COG	std	*M. audouinii* CBS495.96	*M. audouinii* CBS545.93	*M. audouinii* IHEM2214 (ncbi GCA_ 022344085.1)	*M. audouinii* Mau93	*M. canis* CBS113480 (ncbi GCF_ 000151145.1)	*M. canis* CBS218.32	*M. canis* CBS496.86	*M. canis* CBS566.80	*M. canis* Mca56	*M.canis* Mca71	*M. ferrugineum* CBS317.31	*M. ferrugineum* CBS497.48	*M. ferrugineum* M1a (ncbi GCA_ 030015455.1)
W:(W) Extracellular structures	0.759555	9	9	7	9	8	7	8	8	8	7	8	7	8
N:(N) Cell motility	0	5	5	5	5	5	5	5	5	5	5	5	5	5

Table 2. Transcription factors (TFs) functional category distribution among *Microsporum.*

InterPro	descriptions	std	*M. audouinii* CBS495.96	*M. audouinii* CBS545.93	*M. audouinii* IHEM2214 (ncbi GCA_ 022344085.1)	*M. audouinii* Mau93	*M. canis* CBS113480 (ncbi GCF_ 000151145.1)	*M. canis* CBS218.32	*M. canis* CBS496.86	*M. canis* CBS566.80	*M. canis* Mca56	*M.canis* Mca71	*M. ferrugineum* CBS317.31	*M. ferrugineum* CBS497.48	*M. ferrugineum* M1a (ncbi GCA_ 030015455.1)
IPR007219	Transcription factor domain, fungi	1.980676	67	64	63	63	68	68	68	67	69	68	66	66	66
IPR004827	Basic-leucine zipper domain	0.926809	18	18	15	17	16	17	17	18	18	18	17	17	18
IPR001878	Zinc finger, CCHC-type	0.85485	6	7	5	6	6	8	6	6	7	5	7	7	6
IPR001005	SANT/Myb domain	0.650444	23	22	21	22	22	22	22	23	23	23	23	23	22
IPR018004	KilA/APSES-type HTH, DNA-binding	0.5547	4	4	4	4	4	2	4	4	4	4	4	4	4
IPR006856	Mating-type protein MAT alpha 1, HMG-box	0.518875	0	0	0	0	1	1	1	1	1	1	0	0	0
IPR000679	Zinc finger, GATA-type	0.50637	4	5	5	5	4	5	4	4	4	4	4	5	4
IPR001356	Homeobox domain	0.375534	6	6	5	6	6	5	6	6	6	6	6	6	6
IPR004181	Zinc finger, MIZ-type	0.27735	2	3	3	3	3	3	3	3	3	3	3	3	3

Table 2. (continued)

InterPro	descriptions	std	*M. audouinii* CBS495.96	*M. audouinii* CBS545.93	*M. audouinii* IHEM2214 (ncbi GCA_ 022344085.1)	*M. audouinii* Mau93	*M. canis* CBS113480 (ncbi GCF_ 000151145.1)	*M. canis* CBS218.32	*M. canis* CBS496.86	*M. canis* CBS566.80	*M. canis* Mca56	*M.canis* Mca71	*M. ferrugineum* CBS317.31	*M. ferrugineum* CBS497.48	*M. ferrugineum* M1a (ncbi GCA_ 030015455.1)
IPR010666	Zinc finger, GRF-type	0.27735	3	3	3	3	3	2	3	3	3	3	3	3	3
IPR011598	Myc-type, basic helix-loop-helix (bHLH) domain	0.27735	8	8	8	8	8	7	8	8	8	8	8	8	8
IPR018501	DDT domain	0.27735	1	1	1	1	1	1	1	1	1	1	1	0	1

Table 3. Description and annotation of 12 selected genes of proteases and CAZy.

Gene family	Orthogroup	Locus tag/		mRNA/ product	Annotation	Function in fungi
S08A	OG0003381	MCYG_04495	*SUB1*	Subtilisin-likeprotease 1	Peptidase S8 propeptide/ proteinase inhibitor I9	Genes encoding endoproteases of dermatophytes, highly distributed in *Microsporum canis* and *Trichophyton benhamiae*
	OG0004069	MCYG_02345	*SUB7*	Subtilisin-like protease 7	Peptidase S8 propeptide/ proteinase inhibitor I9	*Sub7* is the major protease secreted by *Trichophyton* species
	OG0000856	MCYG_02070	*PRB1*	Vacuolar proteinase B	Peptidase S8 propeptide/ proteinase inhibitor I9	The *Δprb1* mutants showed reduced aerial hyphae, lower level of sporulation, and a significant reduction in virulence
	OG0006456	MCYG_04669	\	Intracellular serine protease	Peptidase S8, subtilisin-related	Unknown
M77	OG0003906	MCYG_08676	*Glip2*	GDSL esterase/ lipase 2	Phosphopan-tetheine binding ACP domain	Lip2 lipase maintains high activity at low pH in *Yarrowia lipolytica*
S53	OG0007757	MCYG_01559	*sedA*	Tripeptidyl peptidase sedA/ Sedolisins	Peptidase S53, activation domain	Secreted tripeptidyl peptidase which degrades proteins at acidic pHs and is involved in virulence
GH18	OG0001063	MCYG_03088	\	Chitinas	Chitin-binding, type 1	Unknown
	OG0005920	MCYG_06206	\	Chitinas	LysM domain	Unknown

Table 3. (continued)

Gene family	Orthogroup	Locus tag/	mRNA/ product	Annotation	Function in fungi
AA1	OG0003850	MCYG_08539 *Arb2*	Conidial pigment biosynthesis oxidase	Multicopper oxidase-like, N-terminal	Melanin Synthesis Pathway in *Aspergillus fumigatus* Conidia
	OG0003865	MCYG_08571 \	Laccase 1	Multicopper oxidase	Laccase is commonly recognized as the causative agent of opportunistic fungal pathogens
AA3	OG0001123	MCYG_03226 \	Choline dehydrogenase	FAD/NAD(P)-binding domain superfamily	Unknown
GT90	OG0005453	MCYG_05869	DUF821 domain	Glycosyl transferase CAP10 domain	Unknown

Declarations

Ethics approval and consent to participate

Not applicable.

Adherence to national and international regulations

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All new genomes published in the study have been deposited in GenBank under the following BioProject accession number PRJNA1102285. All software used were from publicly available sources.

Competing interests

The authors report there are no competing interests to declare.

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Authors' contributions

Study conceptualization and design of experiments, XZ, PYF and SDH; methodology, XZ and RB; formal analysis and investigation, RB and TC; original manuscript preparation, XZ and PYF; writing - review and editing, VAV and SDH; supervision: SDH and VAV. All authors read and approved the final manuscript.

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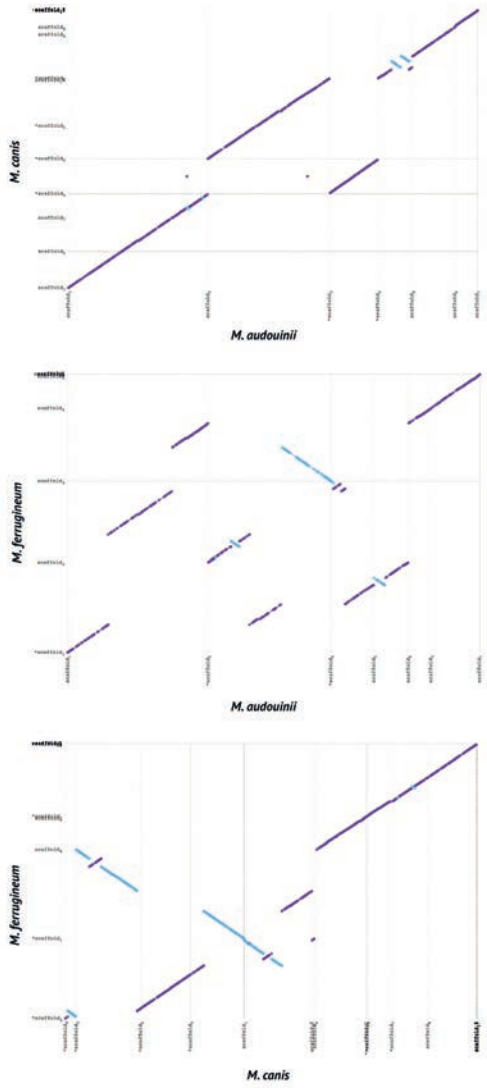
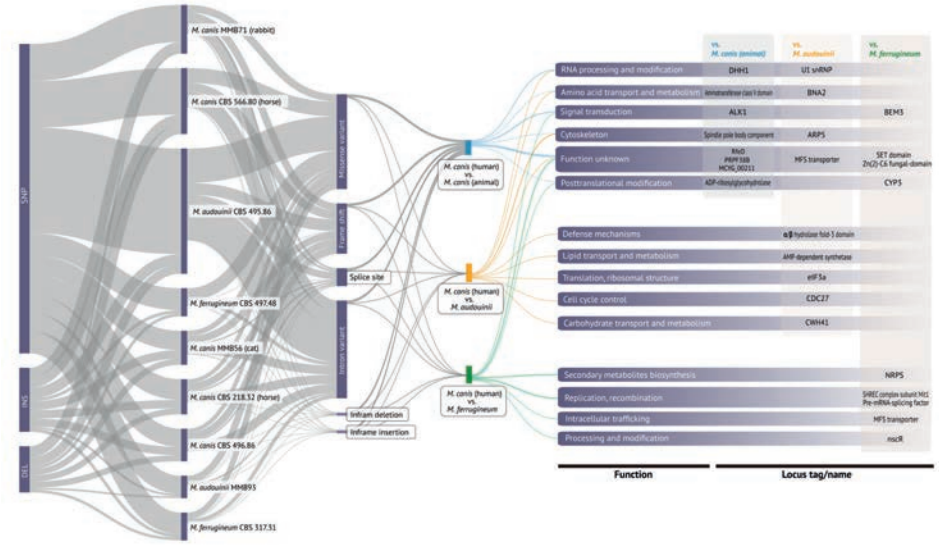
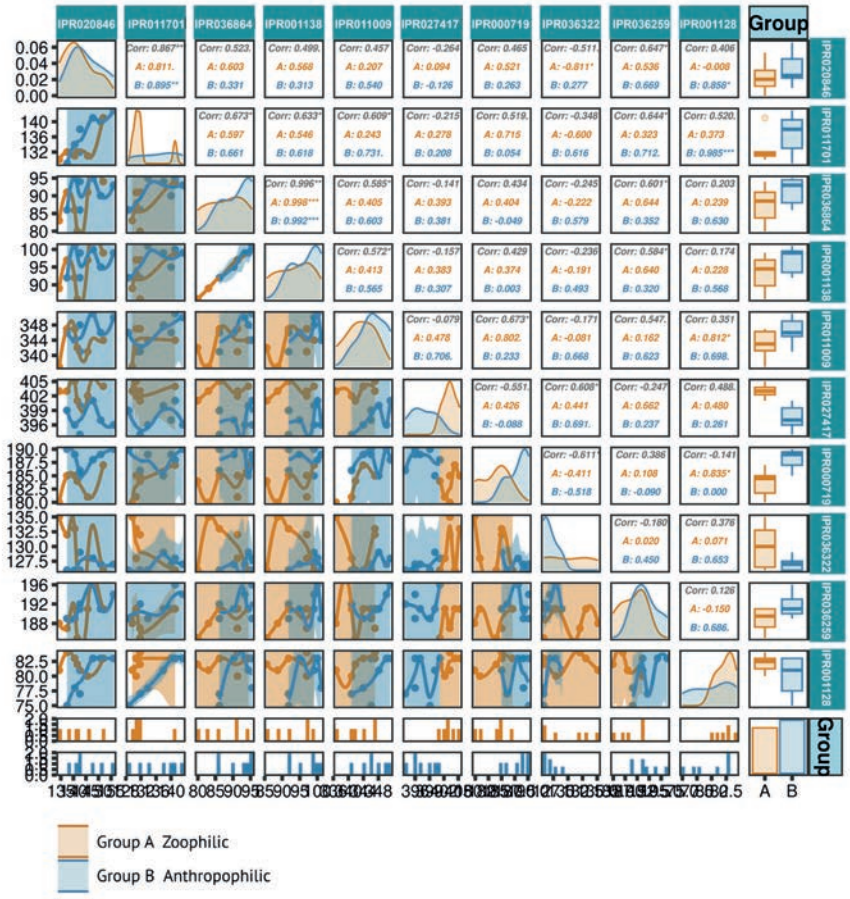
SUPPLEMENT INFORMATION



Additional figure 1. Alignment of three *Microsporum* species genome using MUMmer4.0 beta.
The dotplot of all the MUMs between two sequences can reveal their macroscopic similarity. Purple lines/dots represent an undisturbed segment of conservation between the two sequence, blue lines/dots represent an inverted segment of conservation. The closer a plot is to a line f(x) = x (or -x), the fewer macroscopic differences exist between the two sequences.

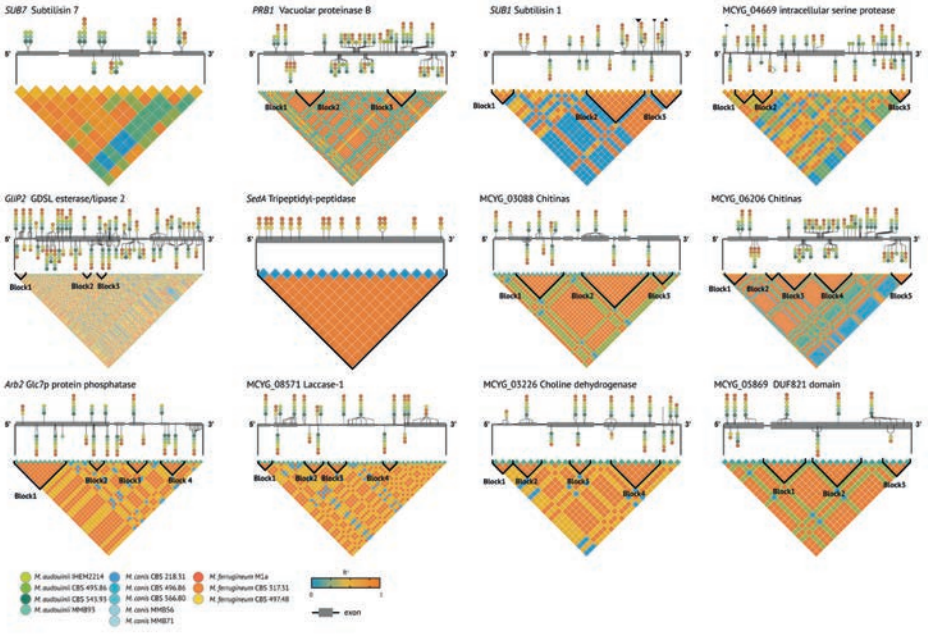


Additional figure 2. Genetic variant annotation and functional effect prediction.
To analyze the variation within species, strain data were mapped to the NCBI reference genomes with BWA using the MEM algorithm [401]. Using GATK v4.4.0.0, these were screened for duplicates with MarkDuplicates default settings, and used for haplotype calling with the HaplotypeCaller, with the following non-default parameters: "-pair-hmm-gap-continuation-penalty 10, -stand-call-conf 30 --sample-ploidy 1". The resulting vcf files and the reference genomes were used to annotate and predict variant effects using SnpEff v5.1d [402]. The top ten genes in terms of variation frequency were annotated and the genes of the target species and their corresponding functional annotations were compared with the DFVF: Fungal Virulence Factor database Sankey plots of genomic variants were created using SankeyMATIC (https://sankeymatic.com/). The width of the grey mobility lines between panels represents differences in the number of variants. Based on the classification and number of variants, the genes with the greatest mutational differences between the three species were compared, and their functions were annotated. Locus tag/name is based on the annotation of the *Microsporum canis* CBS 113480 reference genome.

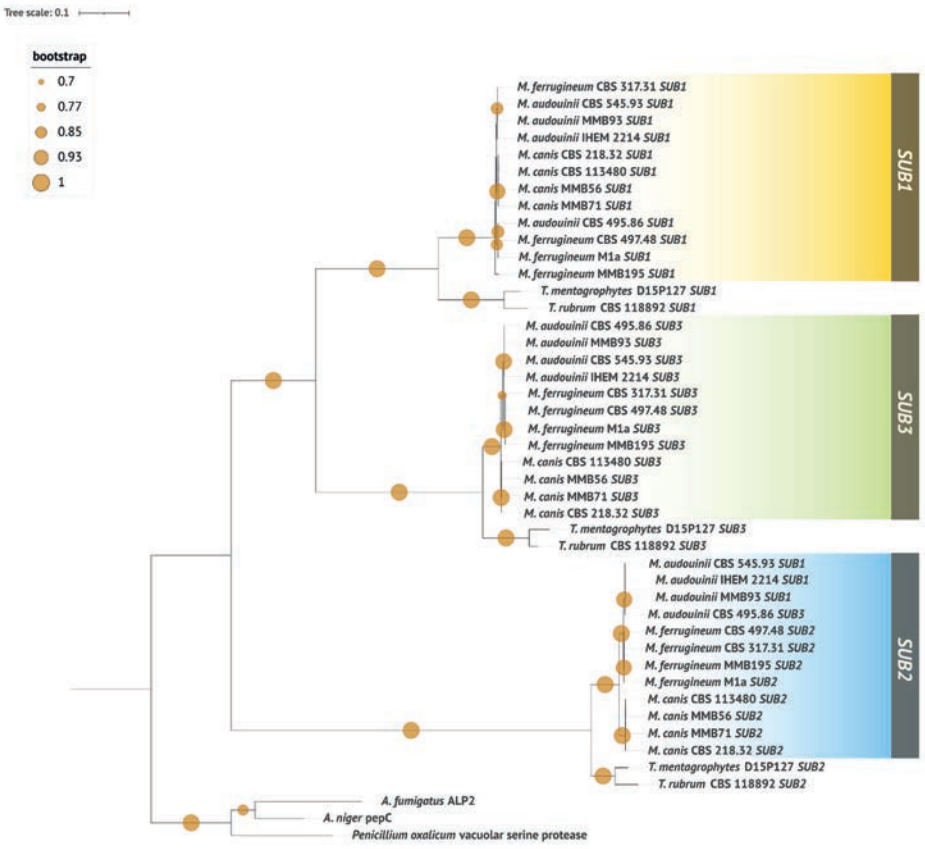


IPR020846	Major facilitator superfamily domain
IPR011701	Major facilitator superfamily
IPR036864	Zn(2)-C6 fungal-type DNA-binding domain superfamily
IPR001138	Zn(2)Cys(6) fungal-type DNA-binding domain
IPR011009	Protein kinase-like domain superfamily
IPR027417	P-loop containing nucleoside triphosphate hydrolase
IPR000719	Protein kinase domain
IPR036322	WD40-repeat-containing domain superfamily
IPR036259	MFS transporter superfamily
IPR001128	Cytochrome P450

Additional figure 3. Data characteristics and correlations matrix between major differential protein domains in anthropophilic and zoophilic *Microsporum* species.
Complex correlation matrix plots are drawn using the R package GGally. Using KendaII for correlation analysis style, the top diagonal plot area shows the correlation coefficient and p value, the * represents the significance level. The diagonal plot area shows the density map corresponding to each domain, and the X-axis position where the peak appears indicates that the data of the domain is mostly concentrated in this range. The lower diagonal plot area shows the scatter plot between two domains, and the curve is smoothed using loess.



Additional figure 4. Linkage disequilibrium analysis.
Additional figure 4 is a visualization of the LD blocks for each of the variant loci of these genes. The colors represent R2 values, with 0 to 1 overriding from blue to orange. Gene models are shown at the top of the figure, with lines representing genomes, gray rectangles representing exons, and different colored circles representing strain variants occurring at the SNP locus. Color-bonded LD blocks are located at the bottom. Strongly linkage Block regions are marked by thick black triangles.



Additional figure 5. Phylogenetic tree constructed from SUB1-SUB3 genes. *Aspergillusfumigatus*, *Asp. niger*, and *Penicillium oxalicum* as outgroups.

Additional table 1. Functional annotation and virulence factors of differential genes in human- and animal-derived Microsporum Species.

Locus tag/name	Product/mRNA	Function	Interpro	Pfam	Virulence factors in fungal pathogens (DFVD) http://sysbio.unl.edu/DFVF/
		human-derived *M. canis* vs animal-derived *M. canis*			
MCYG_04733	RfeD	S:(S) Function unknown			
AKL1	Ark- serine/threonine protein kinase	T:(T) Signal transduction mechanisms	IPR011009	PF00069;PF07714	Protein serine/threonine kinase activity
PRPF38B	pre-mRNA-splicing factor 38B	S:(S) Function unknown			
MCYG_02976	dienelactone hydrolase family protein	Q:(Q) Secondary metabolites biosynthesis, transport and catabolism	IPR002925	PF01738	
MCYG_02989	Ribonucloprotein	A:(A) RNA processing and modification	IPR004038	PF01248	
MCYG_04286	Aminotransferase class V domain-containing protein	E:(E) Amino acid transport and metabolism	IPR015421		
MCYG_07233	ADP-ribosylglycohydrolase	O:(O) Posttranslational modification, protein turnover, chaperones	IPR005502	PF03747	
MCYG_01602	Spindle pole body component	Z:(Z) Cytoskeleton/gamma-tubulin complex component GCP5	IPR041470	PF04130;PF17681	
MCYG_00211	uncharacterized protein				
DHH1	DExD/H-box ATP-dependent RNA helicase dhh1	A:(A) RNA processing and modification	IPR014001	PF00271	Belongs to the DEAD box helicase family, DDX6/DHH1 subfamily, involved in virulence.
		human-derived *M. canis* vs *M. audouinii*			
Mfereffu10_007044	NRPS	Q:(Q) Secondary metabolites biosynthesis, transport and catabolism	IPR000873 AMP-dependent synthetase/ligase domain	PF00501;PF00550;PF00668	AMP-binding

Additional table 1. (continued)

Locus tag/name	Product/mRNA	Function	Interpro	Pfam	Virulence factors in fungal pathogens (DFVD) http://sysbio.unl.edu/DFVF/
BEM3	Rho GTPase activating protein	T:(T) Signal transduction mechanisms	IPR000198 Rho GTPase-activating protein domain	PF00169;PF00620	Required for establishment of the axial budding pattern in yeast cells. May be involved in the selection of future sites of septation in hyphal cells. Contributes to morphogenesis and is important for induction of hyphal growth. Also plays a role in epithelial adherence, and is involved in intestinal colonization and systemic infection. The role in adhesion is probably minor compared with its role in morphogenesis.
CYP3	Peptidyl-prolyl cis-trans isomerase H	O:(O) Posttranslational modification, protein turnover, chaperones	IPR002130 Cyclophilin-type peptidyl-prolyl cis-trans isomerase domain	PF00160	Pro_isomerase, PPIases accelerate the folding of proteins. It catalyzes the cis-trans isomerization of proline imidic peptide bonds in oligopeptides (By similarity).
Mfereffu10_001584	SHREC complex subunit Mit1	K:(K) Transcription;L:(L) Replication, recombination and repair	IPR000330 SNF2, N-terminal	PF00176;PF00271;PF04851;PF11496;PF15446;PF18585	Mannosyltransferase activity, pathogenesis
Mfereffu10_002528	NRPS	Q:(Q) Secondary metabolites biosynthesis, transport and catabolism	IPR000873 AMP-dependent synthetase/ligase domain	PF00501;PF00550;PF00668	
Mfereffu10_004851	Major facilitator superfamily (MFS) profile domain-containing protein	U:(U) Intracellular trafficking, secretion, and vesicular transport	IPR011701 Major facilitator superfamily	PF07690	MFS_1, integral to membrane, hexose transport, response to drug
Mfereffu10_000280	Pre-mRNA-splicing factor	L:(L) Replication, recombination and repair	IPR026300 CWF11 family	PF13086;PF13087;PF16399	
Mfereffu10_002474	SET domain-containing protein	S:(S) Function unknown	IPR046341 SET domain superfamily		
Mfereffu10_002729	C6 finger domain transcription factor nscR	A:(A) RNA processing and modification	IPR001138 Zn(2)Cys(6) fungal-type DNA-binding domain	PF00172;PF04082	

Additional table 1. (continued)

Locus tag/name	Product/mRNA	Function	Interpro	Pfam	Virulence factors in fungal pathogens (DFVD) http://sysbio.unl.edu/DFVF/
Mfereffu10_002939	Zn(2)-C6 fungal-type domain-containing protein	S:(S) Function unknown	IPR001138 Zn(2)Cys(6) fungal-type DNA-binding domain	PF00172	
			human-derived *M. canis* vs *M. ferrugineum*		
Maureffu10_000442	MFS transporter	S:(S) Function unknown	IPR011701 Major facilitator superfamily		
Maud_002122	Alpha/beta hydrolase fold-3 domain-containing protein	V:(V) Defense mechanisms	IPR013094 Alpha/beta hydrolase fold-3		
Maud_004477	U1 snRNP-associated protein	A:(A) RNA processing and modification	IPR000504 RNA recognition motif domain		
MYO1_3	class II myosin/myosin I heavy chain	Z:(Z) Cytoskeleton	IPR001452 SH3 domain	PF00018;PF00063;PF06017	Fungal-type cell wall organization, hyphal growth
Maud_006902	AMP-dependent synthetase/ ligase(NCBI)	I:(I) Lipid transport and metabolism	IPR042099 ANL, N-terminal domain		
eIF3a	eukaryotic translation initiation factor 3 subunit A/ translation initiation factor eIF3a	J:(J) Translation, ribosomal structure and biogenesis	IPR000717 Proteasome component (PCI) domain	PF01399	Eukaryotic translation initiation factor 3 complex
ARP5	Actin-related protein 5	Z:(Z) Cytoskeleton	IPR004000 Actin family	PF00022	Fungal-type cell wall organization, hyphal growth, modulation by symbiont of host inflammatory response
BNA2	Indoleamine 2,3-dioxygenase	E:(E) Amino acid transport and metabolism	IPR000898 Indoleamine 2,3-dioxygenase	PF01231	
CDC27	anaphase-promoting complex subunit cdc27	D:(D) Cell cycle control, division, chromosome partitioning	IPR001440 Tetratricopeptide repeat 1	PF00515;PF07719;PF12895;PF13176;PF13181;PF13414;PF13432;PF14559	Hyphal growth, negative regulation of transcription from RNA polymerase II promoter

Additional table 1. (continued)

Locus tag/name	Product/mRNA	Function	Interpro	Pfam	Virulence factors in fungal pathogens (DFVD) http://sysbio.unl.edu/DFVF/
CWH41	Processing alpha glucosidase I	G:(G) Carbohydrate transport and metabolism	IPR004888 Glycoside hydrolase family 63	PF03200;PF16923	

CHAPTER 7

DYNAMIC GENE EXPRESSION AND FUNCTIONAL NETWORKS UNDERLYING SPORE GERMINATION IN *MICROSPORUM CANIS*

Xin Zhou, Wentao Liu, Marlou tehupeiory-kooreman, Na Li, Sybren de Hoog, Peiying Feng

in preparation

ABSTRACT

Microsporum canis are zoonotic fungi that cause superficial fungal infections via spore transmission. However, the molecular mechanisms underlying spore germination remain incompletely understood. In this study, transcriptome sequencing was employed to systematically analyze the dynamic gene expression and functional networks during *M. canis* spore germination (mycelium phase, dormant spores and germinating spores). qPCR validation confirmed the expression of key genes. The results revealed that the transition from dormant spores (Spore 0h) to germinating spores (Spore 4h) was accompanied by significant metabolic reprogramming. Genes associated with DNA replication (e.g., MCM complex, POL, RFC) and oxidative phosphorylation (e.g., ATP synthase, succinate dehydrogenase, cytochrome complex) were significantly upregulated, whereas peroxisomal function (POT, PEX family genes) and fatty acid β-oxidation pathways were suppressed. This suggests a metabolic shift from lipid utilization to glycolysis and mitochondrial energy production. Gene expression clustering analysis identified eight temporally regulated gene clusters. Alternative splicing events (SE and MXE) played key roles in regulating genes related to methylation, energy metabolism, and cytoskeletal organization. Notably, MCYG_04013 (methyltransferase) was upregulated during early spore formation, whereas MCYG_06030 (isocitrate dehydrogenase) exhibited increased expression during germination. The protein-protein interaction (PPI) network further identified key regulatory genes, including *SLP*1, *MEC1*, *UBI4*, and *RPS16,* whose dynamic expression was closely linked to cell cycle regulation, stress response, and signal transduction (Ras-GTP/cAMP-PKA and MAPK pathways). Additionally, the study uncovered both conserved and unique germination strategies in *M. canis* compared to other filamentous fungi, such as dependence on oxidative phosphorylation (conserved) and ribosome storage strategy with low expression of HSP-related genes (unique). This study provides the first insights into the molecular mechanisms of *M. canis* spore germination, offering a theoretical foundation for antifungal strategies targeting the spore formation stage.

INTRODUCTION

Dermatophytosis is a disease that affects keratinized tissues, including the epidermis, hair, and nails. It is caused by dermatophytes, which can spread via arthroconidia shed along with the host's keratinous material, leading to infections in both animals and humans. Dermatophytes belong to the family *Arthrodermataceae* within the order *Onygenales* and are systematically classified into seven genera based on broader evolutionary trends: *Arthroderma*, *Lophophyton*, *Paraphyton*, as well as the clinically relevant *Epidermophyton*, *Microsporum*, *Trichophyton*, and *Nannizzia* [1]. The morphological structures of dermatophytes primarily include hyphae, conidia, and other specialized formations, complex sexual states are produced in the environment. The hyphae are septate and hyaline, capable of forming invasive structures within host tissues. These invasive hyphae secrete keratinases, which degrade the host's keratin layer and facilitate fungal penetration. In contrast to active hyphae, spores are dormant, unicellular structures [29]. Dermatophytes produce two main types of asexual spores: aleurioconidia (including microconidia and macroconidia) and arthroconidia. Aleurioconidia are formed by lateral or terminal budding from hyphae and are typically generated under nutrient-rich *in vitro* culture conditions. Arthroconidia, on the other hand, are produced through hyphal fragmentation at septal junctions and are formed during infection in host tissues [403].

Compared to hyphae, conidia possess a thicker cell wall and exhibit greater mechanical resistance, enabling their survival and dispersal under harsh environmental conditions. Upon encountering favorable environmental cues, dormant conidia can reactivate and germinate into new hyphae. In the infection cycle of dermatophytes, once arthroconidia adhere to suitable tissues of a permissive host, they undergo rehydration and swell in size. When the cytoplasmic volume reaches a critical threshold, nuclear division occurs [71]. By 4 hours post-contact, conidia begin to germinate and form germ tubes [278], which subsequently develop into segmented hyphae [404]. Within 24 hours to 3 days after adherence, arthroconidia initiate penetration of the stratum corneum, facilitated by the production of specific hydrolytic enzymes and the extension of hyphae through intercellular spaces and keratinocyte interiors [405]. The germination phase of conidia represents a critical transition point, marking the shift from environmental persistence to active pathogenicity. This process is accompanied by disruption of the epidermal barrier and phase-specific inflammatory responses, which collectively determine the outcome of infection.

Among dermatophytes, zoophilic species are particularly notable for their capacity to infect animals and cross species barriers to infect humans. These species, including *Microsporum canis*, often show strong environmental survival and induce more pronounced inflammatory responses during zoonotic transmission. *Microsporum canis* is a widely distributed zoonotic dermatophyte primarily infecting cats and dogs, with frequent spillover to humans, especially children [192]. Its dual adaptation to animal reservoirs and human skin, along with its high transmissibility and spore persistence in the environment, makes it an ideal model for studying early-stage dermatophyte infection [406]. The adhesion of *M. canis* to hair and skin is at least associated with a well-characterized spore protein, subtilisin 3. In contrast, the invasion process—both in *M. canis* and in dermatophytes more broadly—remains poorly understood [33]. Although the correlation between morphological differentiation and adherence and invasion mechanisms in dermatophytes has been investigated, the conidial germination process and its association with mechanisms of cutaneous infection remain largely unexplored [406]. Previous studies have shown that during the conidial germination process, significant alterations occur in the conidial wall thickness and composition, facilitating environmental adaptation and potentially influencing pathogenicity. Our preliminary research on *M. canis* adaptive evolution identified *PRB1*, a gene associated with hyphal/conidial morphology, which is highly expressed during human infection [407]. However, the dynamic gene expression patterns and regulatory networks involved in *M. canis* conidial germination remain unclear. Given that conidial germination is a critical early event in host infection, elucidating the dynamic gene expression profiles and functional networks during this process could provide deeper insights into the pathogenic mechanisms of *M. canis*.

Therefore, this study aims to investigate the gene expression dynamics at different stages of *M. canis* conidial germination using transcriptomic sequencing. By elucidating the key regulatory networks governing the transition from conidia to hyphae, this research will not only advance our understanding of *M. canis* conidial germination at the molecular level but also provide new perspectives on dermatophyte pathogenicity, ultimately contributing to the rational development of new preventive and therapeutic strategies.

METHODS

Strains and culture conditions

The strain of *Microsporum canis* used in this study was obtained from the Radboudumc Laboratory Center for Mycology. The strain was maintained on Sabouraud dextrose agar (SDA; 40 g/L glucose, 10 g/L peptone, 15 g/L agar) at 28°C in darkness. To induce sporulation, mycelial plugs (5 mm diameter) from actively growing colonies were transferred to Borelli's lactritmel agar [408] plates (20g/L wheat flour, 7 g/L skimmed milk powder, honey 1g/L, 20 g/L agar) and incubated at 28°C under alternating light/dark cycles (12 h/12 h) for 14 days. Spores were harvested by gently scraping the colony surface with sterile PBS (pH 7.4) containing 0.01% Tween 20. The suspension was filtered through three layers of sterile Miracloth (Merck) to remove hyphal debris and centrifuged at 3,000 × g for 10 min. Spore pellets were resuspended in PBS, and concentrations were adjusted to 1 × 10^7 spores/mL using a hemocytometer (Neubauer chamber). Spore viability was confirmed by plating serihal dilutions on SDA and counting colony-forming units (CFU) after 48 h.

RNA extraction and sequencing

Total RNA was extracted from three biological replicates of *M. canis* mycelium (vegetative growth phase, cultured in Sabouraud broth for 72 h), freshly harvested spores (Spore 0h), and spores incubated in germination-inducing medium (brand or REF) at 28°C for 4 h (Spore 4h). Samples were homogenized in TRIzol reagent (Invitrogen) using MagNa lyser (Roche) with zirconium beads (0.5 mm diameter, 3 cycles of 20 sec at 6,500 rpm). RNA was purified using the RNeasy Mini Kit (Qiagen) with on-column DNase I digestion (RNase-Free DNase Set, Qiagen). RNA integrity was assessed via Agilent Bioanalyzer 2100 (RNA Integrity Number [RIN] ≥ 8.0), and purity (A260/A280 ≥ 1.9, A260/A230 ≥ 2.0) was confirmed using a NanoDrop ND-1000 spectrophotometer.

Strand-specific mRNA libraries were prepared using the NEBNext Ultra II RNA Library Prep Kit (New England Biolabs) with poly-A selection and rRNA depletion (Ribo-Zero rRNA Removal Kit, Illumina). Library quality was verified via Bioanalyzer (average fragment size: 350 bp), and quantification was performed using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific). Libraries were sequenced on an Illumina NovaSeq 6000 platform (150 bp paired end reads; 40 million reads per sample) at Novogene Co., Ltd.

Bioinformatic analysis

Read preprocessing and quality control

Raw sequencing reads were processed using Trimmomatic (v0.39) to remove adapters and low-quality bases (SLIDINGWINDOW:4:20, MINLEN:50). FastQC (v0.11.9) and MultiQC (v1.11) were used to generate quality reports. High-quality reads were aligned to the *M. canis* reference genome (GenBank assembly accession: GCA_000151145.1) using HISAT2 (v2.2.1) with default parameters. Alignment rates averaged 92.5% (range: 89.3–94.8%).

Transcript quantification and differential expression

Read counts per gene were generated using featureCounts (v2.0.1) with the genome annotation file (GTF format). Normalization and differential expression analysis were performed in DESeq2 (v1.30.1) using a negative binomial model. Genes with $|\log2(\text{fold change})| \geq 1$ and adjusted *P*-value (Benjamini-Hochberg) < 0.05 were classified as differentially expressed genes (DEGs). Batch effects were assessed via principal component analysis (PCA) and corrected using the *limma* package (v3.48.3) when necessary.

Functional enrichment and pathway analysis

Functional enrichment analysis was performed using clusterProfiler (v4.0.5). GO term enrichment was conducted with the enrichGO function, and redundant GO terms were simplified using semantic similarity (cutoff = 0.7). Pathway enrichment was performed using the enrichKEGG function, with *M. canis*-specific KEGG Orthology (KO) annotations. Pathways with FDR < 0.05 were retained. Predefined gene sets from MSigDB (v7.4, C2: curated pathways) were ranked by log2 (fold change) and tested using the GSEA algorithm (1,000 permutations, weighted enrichment statistic). Gene sets with FDR < 0.25 and normalized enrichment score (NES) > 1.5 were considered significant. For gene set enrichment analysis (GSEA), genes were ranked by log2 fold change and tested using the clusterProfiler:GSEA() function with 1,000 permutations and weighted enrichment statistic. Gene sets with FDR < 0.25 and normalized enrichment score (NES) > 1.5 were considered significant.

Alternative splicing detection

Alternative splicing events were identified using rMATS (v4.1.2) with the following parameters: read length = 150 bp, paired-end mode, and FDR < 0.05. Splicing types

included skipped exons (SE), mutually exclusive exons (MXE), retained introns (RI), alternative 5' splice sites (A5SS), and alternative 3' splice sites (A3SS). Events with a minimum inclusion level difference (ΔPSI) ≥ 10% were retained. Splicing events were linked to DEGs using the rmats2sashimiplot tool to visualize isoform-specific expression.

Gene co-expression clustering

Expression profiles of DEGs were clustered using the k-means algorithm (*stats* package in R, v4.1.0) with Euclidean distance. The optimal cluster number (k = 8) was determined via the Elbow method and silhouette analysis. Enriched pathways in each cluster were identified using Fisher's exact test (FDR < 0.05).

Protein-protein interaction (PPI) network construction

PPI networks were built using the STRING database (v11.5) with a confidence score cutoff ≥ 0.7. Networks were visualized in Cytoscape (v3.8.2), and hub genes were identified based on betweenness centrality (CytoHubba plugin). Functional modules were detected using the MCODE algorithm (score ≥ 5, node score cutoff = 0.2).

qPCR validation

Twelve DEGs (*RPS16*, *MEC1*, *ALD2, SLP1, POL1, PEX5, CYS3, POT1, PEX14,* MCYG_01436*, RPL22* and *RPS3)* were selected for validation. cDNA was synthesized from 1 μg total RNA using the PrimeScript RT Reagent Kit (Takara Bio) with oligo(dT) primers. qPCR primers were designed using Primer-BLAST (NCBI) to span exon-exon junctions and avoid secondary structures. Reactions were performed in triplicate on a QuantStudio 5 Real-Time PCR System (Applied Biosystems) using SYBR Green Master Mix (Roche). Thermal cycling conditions: 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. Melt curve analysis confirmed primer specificity. Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method, with β-tubulin as the endogenous control.

Statistical analysis

Data are presented as mean ± standard deviation (SD) of three biological replicates. Statistical significance was determined via one-way ANOVA with Tukey's post-hoc test ($P < 0.05$) in GraphPad Prism (v9.0). For RNA-seq data, adjusted *P*-values (FDR) were reported.

Data availability

Raw sequencing data are available in the NCBI Sequence Read Archive (SRA) (https://www.ncbi.nlm.nih.gov/sra/docs/).

RESULTS

Differential gene expression in sporulation

In the pairwise comparisons of the three groups, differentially expressed genes (DEGs) were identified between Spore 0h *vs.* Mycelium, Spore 4h *vs.* Mycelium, and Spore 4h *vs.* Spore 0h. Among them, 151 DEGs common to the three groups, 342 DEGs specific to spore 0h and mycelium were included, 783 DEGs unique to Spore 4h *vs.* Mycelium, and 593 DEGs specific to Spore 4h *vs.* Spore 0h (Figure 1a). A scatter plot of log10 transformed expression values highlighted the correlation of gene expression profiles between developmental stages (Figure 1b). In the comparison of Spore 0h *vs.* Mycelium, 2247 genes were upregulated, and 2194 genes were downregulated (R = 0.48). In the comparison of Spore 4h *vs.* Mycelium, 2581 genes were upregulated, and 2411 genes were downregulated (R = 0.51), with increased transcriptional differentiation. The highest correlation (R = 0.79) was observed between Spore 0h and Spore 4h, indicating stable transcriptional activity in the late stages of sporulation, with 1108 genes upregulated and 1220 genes downregulated.

Gene Ontology (GO) enrichment analysis of DEGs between Spore 4h and Spore 0h revealed that the most enriched biological processes were related to peptide metabolic processes, translation, and cell amide metabolic processes. The cellular components were predominantly ribosomal structures, cytoplasmic components, and non-membrane-bounded organelles. The molecular functions associated with the differences mainly involved ribosome-rich structural components and structural molecule activity, followed by threonine-type peptidase activity. Under the molecular function category, the most significantly enriched entries were structural constituent of ribosome and structural molecule activity. Gene Set Enrichment Analysis (GSEA) further highlighted enriched pathways in vesicle-mediated transport, nuclear chromosome separation, kinetochore assembly, DNA metabolic processes, and chromatin condensation. Compared to Spore 0h, 343 out of 464 gene sets were upregulated in Spore 4h, with 14 gene sets showing significant enrichment (FDR < 25%), including functions related to vesicle-mediated transport, nuclear chromosome separation, kinetochore assembly, DNA metabolic processes,

and chromatin condensation. The GO_0000228 (RNA polymerase complex) gene set exhibited significant enrichment with an FDR-adjusted *P*-value < 0.5.

KEGG pathway-specific enrichment analysis revealed significant metabolic changes. During the 0-4h sporulation process, ribosomal biogenesis was significantly enriched in both Spore 0h and Spore 4h, but after comparison, DNA replication and oxidative phosphorylation were significantly upregulated in Spore 4h ($P < 0.5$). The top 10 genes in these pathways, including various DNA polymerase subunits, MCM complexes, and ATP synthase subunits, are listed in Figure 1f. In contrast, the Peroxisome pathway ($P < 0.5$) was significantly downregulated in Spore 4h compared to Spore 0h (Figure 1e), with key genes such as *POT1*, *PEX13*, *PEX14*, and *CAT2*, associated with peroxisome function and fatty acid β-oxidation metabolism.

Complex regulatory mechanisms of gene expression in sporulation

During sporulation in *M. canis,* two main types of alternative splicing events were identified (Figure 2a-b): Exon Skipping (SE) (Figure 2c) and Mutually Exclusive Exons (MXE) (Figure 2d). SE events highlighted the importance of genes involved in methylation, carbohydrate metabolism, energy production, and membrane function at various stages of sporulation. For example, MCYG_04013 (methyltransferase) and MCYG_01597 (protein containing DUF255 domain) were upregulated in the early stages (Spore 0h *vs.* Mycelium and Spore 4h *vs.* Mycelium), indicating that active metabolic and regulatory processes are required for early spore development. However, their downregulation in the later stages (Spore 4h *vs.* Spore 0h) reflects a shift in cellular priorities as the spores mature and prepare for dormancy or germination. Energy metabolism genes, such as MCYG_06030 (isocitrate dehydrogenase [NADP]), were downregulated in the early stages but were significantly upregulated after Spore 4h compared to Spore 0h.

MXE events mainly involved genes related to translation mechanisms, structural organization, and energy production. In the comparisons of Spore 4h *vs.* Mycelium and Spore 4h *vs.* Spore 0h, MCYG_05260 (large ribosomal subunit protein eL28) and MCYG_04657 (phosphotransferase) were persistently upregulated. Cytoskeleton-associated genes, such as MCYG_07441 (α-tubulin), were upregulated in Spore 4h *vs.* Mycelium but showed reduced expression in Spore 4h *vs.* Spore 0h.

Moreover, the observed patterns also indicated that selective splicing plays a critical role in fine-tuning protein function and cellular processes at specific stages. MCYG_00199 (40S ribosomal protein S16) was upregulated in Spore 4h *vs.* Mycelium but downregulated in Spore 4h *vs.* Spore 0h, suggesting a transient requirement for enhanced ribosomal activity. The dynamic regulation of MCYG_01896 (integral

membrane protein) across different stages further emphasizes the significance of splicing in regulating membrane-associated functions during sporulation.

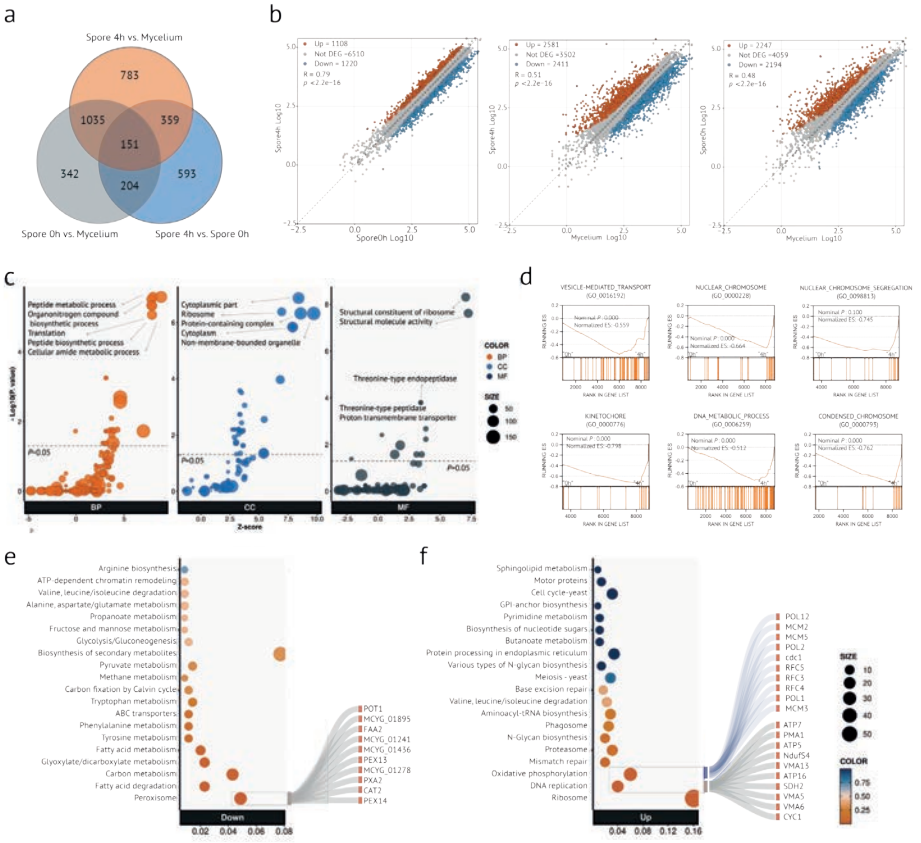


Figure 1. Overview of differential gene expression and enrichment analysis (a) Venn diagram showing the overlap of differentially expressed genes (DEGs) among three comparisons: Spore 0h *vs.* Mycelium, Spore 4h *vs.* Mycelium, and Spore 4h *vs.* Spore 0h. Shared DEGs are highlighted in the central region. (b) Volcano plots displaying upregulated (orange) and downregulated (blue) genes for each comparison. (c) GO enrichment analysis of DEGs between Spore 4h and Spore 0h, highlighting the top 5 significant terms in Biological Process (BP), Cellular Component (CC), and Molecular Function (MF) categories. (d) GSEA results for Spore 4h *vs.* Spore 0h, showing 343/464 gene sets upregulated in Spore 4h. Only one gene set (GO_0000228) remained significant after FDR correction ($P < 0.5$). (e-f) KEGG pathway enrichment analysis comparing Spore 4h and Spore 0h. The most enriched downregulated pathway is Peroxisome ($P < 0.05$), while the top upregulated pathways include DNA replication and Oxidative phosphorylation ($P < 0.05$).

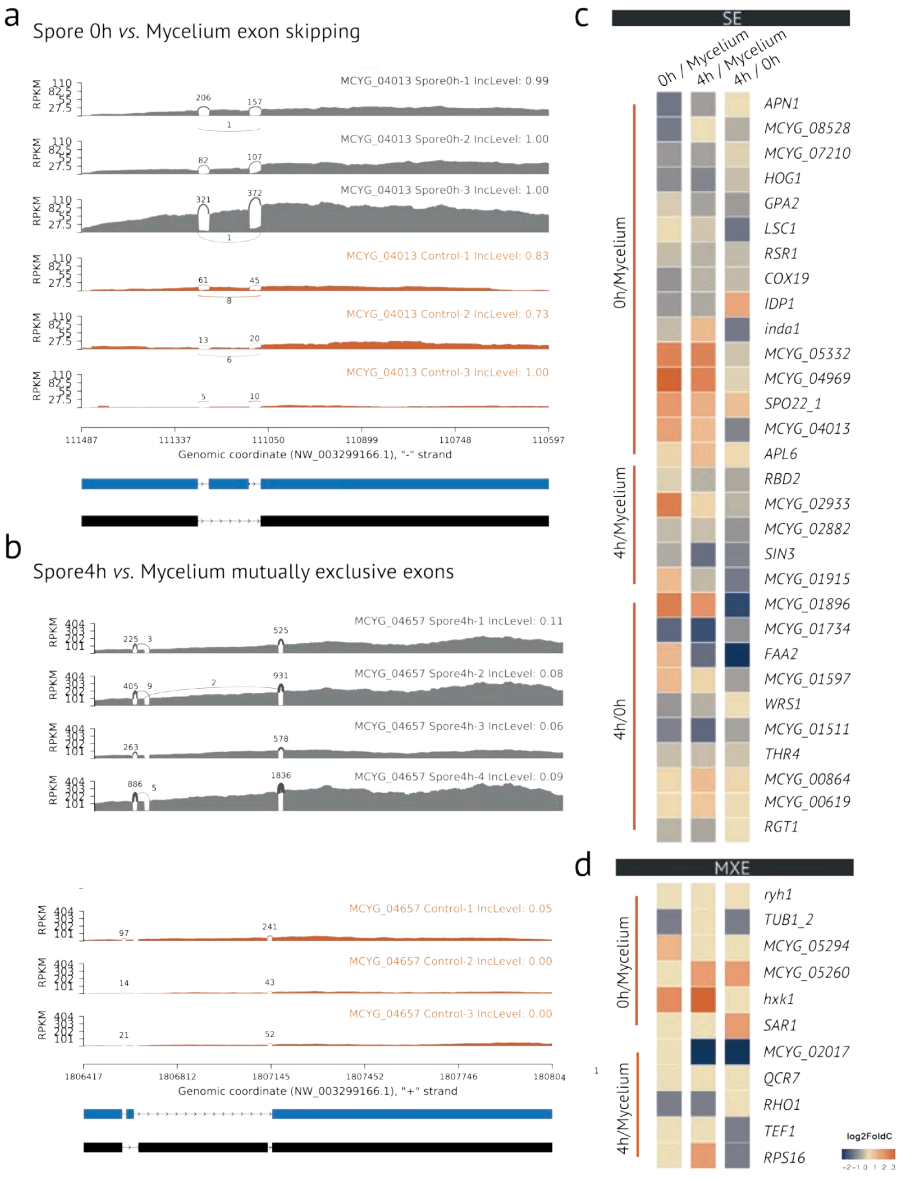


Figure 2. Alternative splicing analysis of DEGs (a-b) Visualization of exon skipping and mutually exclusive exon events in Spore 0h *vs.* Mycelium and Spore 4h *vs.* Mycelium, respectively. (c-d) Heatmaps of DEGs exhibiting exon skipping (top) and mutually exclusive exons (bottom), with log2 fold change values represented by the color gradient. Gene names are annotated on the right.

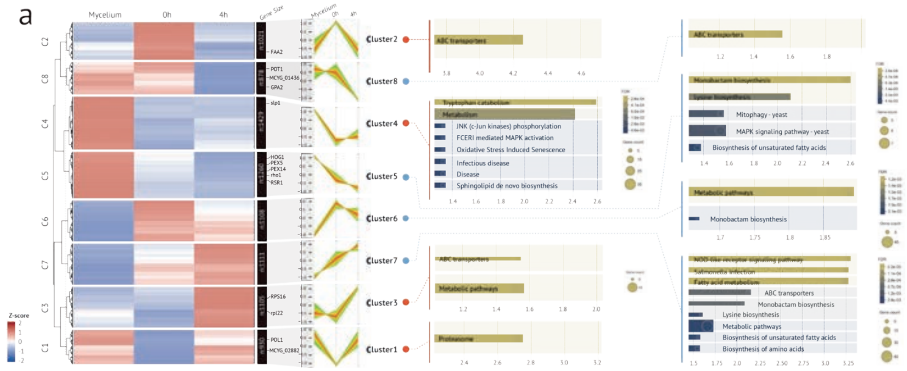


Figure 3. Fuzzy clustering of DEGs based on temporal expression profiles across Mycelium, Spore 0h, and Spore 4h stages.

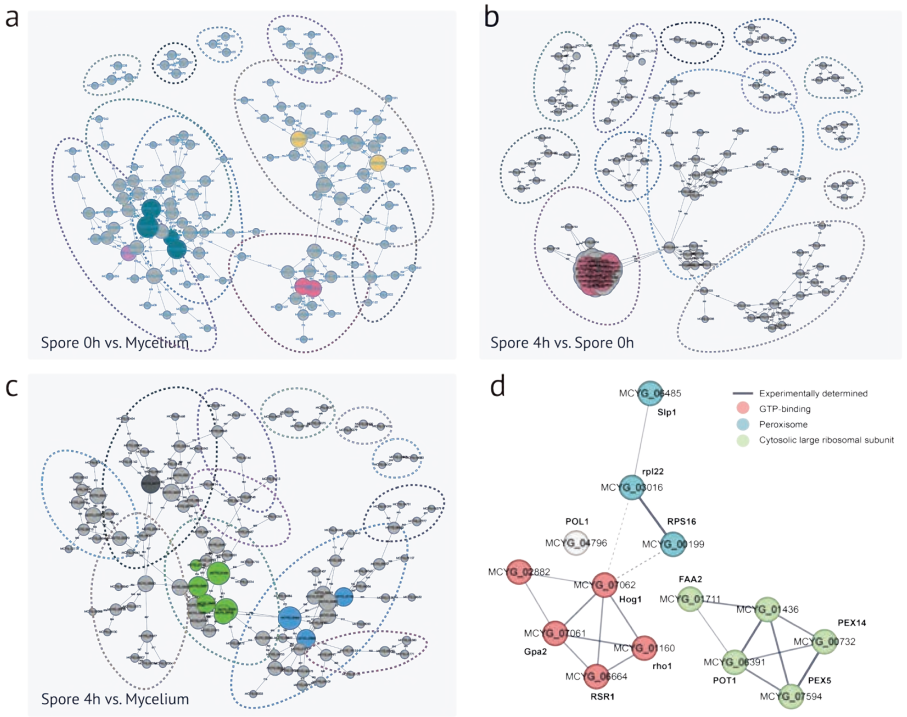


Figure 4. Protein-protein interaction (PPI) networks of DEGs.
(a-c) PPI networks of significant DEGs in three comparisons: Spore 0h *vs.* Mycelium, Spore 4h *vs.* Mycelium, and Spore 4h *vs.* Spore 0h. Nodes represent proteins, and edges represent interactions. Node size reflects degree centrality, with larger nodes indicating more central proteins. (d) Interaction network of 14 selected key proteins, emphasizing their central roles in biological processes.

Cluster analysis of gene expression data

Clustering analysis of gene expression data revealed eight distinct clusters (C1-C8), reflecting the temporal dynamics of transcriptional regulation from the mycelium stage to spore 0h and spore 4h (Figure 3). Each cluster represents a group of genes with similar expression patterns and is associated with specific enriched pathways. We annotated the genes within each cluster and analyzed the functional roles of the top 100 highly expressed genes. C1 (n=903) consists of genes that are highly expressed in the mycelium but downregulated at spore 0h. Pathway enrichment analysis indicates a significant association of these genes with the proteasome pathway. C2 (n=1021) and C8 (n=878) are grouped within the same clade and include genes that are upregulated at spore 0h but downregulated at spore 4h. The enrichment of ABC transporters highlights the role of these genes in metabolite transport and environmental adaptation. C3 (n=1105) consists of genes that remain stably expressed in both the mycelium and spore 0h stages but show a significant increase in expression at spore 4h. These genes are primarily enriched in metabolic pathways and monocyclic amide biosynthesis. C4 (n=1429) and C5 (n=1260) exhibit high expression in the mycelium but are significantly downregulated during spore formation. These genes are associated with tryptophan degradation, MAPK signaling, fatty acid metabolism, and the NOD-like receptor signaling pathway. In contrast, C6 (n=1108) and C7 (n=1111) show low expression in the mycelium but are significantly upregulated during spore formation. The enriched pathways include metabolic pathways, monocyclic amide biosynthesis, unsaturated fatty acid metabolism, fatty acid metabolism, and the NOD-like receptor signaling pathway.

Protein-protein interaction networks at different stages of spore development

During the spore germination process of *M. canis*, the top 10 key core genes identified based on the PPI results exhibited significant expression changes at different stages. In the transition from mycelium to dormant spores (Spore 0h), multiple genes associated with the cell cycle and metabolism exhibited differential expression. MCYG_04796 (*POL1*) and MCYG_06485 (*SLP1*) were significantly downregulated in Spore 0h, encoding the catalytic subunit of DNA polymerase α and a WD-repeat protein involved in cell cycle regulation, respectively, indicating that DNA replication pathways were suppressed during spore formation. Furthermore, metabolic genes such as MCYG_04572 (*PGI1*, glucose-6-phosphate isomerase) and MCYG_07687 (*CYS3*, cysteine biosynthesis-related protein) were significantly downregulated in Spore 0h, while MCYG_02667 (*ALD2*, mitochondrial aldehyde dehydrogenase) and MCYG_08368 (*STR2*, cysteine metabolism enzyme) were upregulated, suggesting that regulatory shifts in carbohydrate and sulfur

metabolism occurred during spore formation to support dormancy. During the transition from dormant spores (Spore 0h) to germinating spores (Spore 4h), substantial changes in the expression of energy metabolism-related genes were observed. Several peroxisome-related genes were significantly downregulated in Spore 4h, including MCYG_07594 (*PEX5*), MCYG_00732 (*PEX14*), MCYG_06391 (*POT1*), MCYG_03880 (*PEX12*), and MCYG_00409 (*PEX7*), indicating a decline in peroxisomal β-oxidation activity during spore germination. Additionally, MCYG_06464 (*UBI4*, ubiquitin) was downregulated in Spore 4h, suggesting that the ubiquitin-mediated protein degradation system was suppressed during germination. In contrast, MCYG_02808 (*MEC1*, serine/threonine-protein kinase) was significantly upregulated in Spore 4h, potentially playing a role in signal transduction and cell cycle regulation. At the germinating spore (Spore 4h) stage, compared to dormant spores (Spore 0h), multiple ribosomal genes were significantly upregulated, including MCYG_00912 (*RPS3*), MCYG_02475 (*RPS11*), MCYG_08067 (*RPS4*), MCYG_01924 (*RPS1*), MCYG_00199 (*RPS16*), MCYG_00201 (*RPS14*), MCYG_00291 (*RPL19*), and MCYG_00564 (*RPL24*), indicating increased ribosome biogenesis and protein translation activity to meet the demands of cellular growth (Figure 4).

qPCR validation of key genes

Through transcriptome analysis, we identified the role of DNA replication and repair, redox metabolism, protein synthesis, and peroxisome function in spore germination in *Microsporum*, and to further validate the expression of key genes in these cellular processes. We further examined the expression profiles of 12 genes (*SLP*, *POL1*, *MEC1*, *ALD2*, *CYS3*, *PEX5*, *PEX14*, MCYG_01436, *POT1*, *RPS16*, *RPL22*, and *RPS3*) during the process of hyphae to spore germination in *Microsporum canis* (0h, 2h, 4h, and 8h) (Figure 5). The expression levels of *MEC1* (associated with DNA damage repair or cell-cycle regulation) and *ALD2* (involved in energy metabolism or oxidation-reduction processes) gradually increased during spore germination, peaking at 8 hours. In contrast, the expression levels of *CYS3*, *PEX14*, *POT1*, and *RPS16* exhibited a clear downward trend throughout spore germination. Notably, the expression levels of *CYS3*, *PEX14*, and *POT1* were already lower at the initial germination stage (Spore 0h) compared to the mycelium phase and continued to decline significantly over time, with *POT1* reaching its lowest expression level at 8h, indicating strict suppression during late spore development. These genes are primarily involved in peroxisome-associated metabolism (*PEX14* and *POT1*) and sulfur-containing amino acid metabolism (*CYS3*). The marked reduction in *RPS16* expression, particularly at later stages, suggests a potential shift in protein synthesis patterns, possibly involving significant suppression or selective regulation of protein synthesis activity during late germination. Additionally, genes such as

SLP and *POL1*, which are closely linked to early germination events including cell-wall remodeling, DNA synthesis, and replication activities, as well as *PEX5*, a peroxisomal targeting signal receptor, showed significantly increased expression levels during early germination (Spore 0h–4h), reaching their peaks before substantially declining in the later stages (Spore 4h–8h). Similarly, the ribosomal genes *RPL22* and *RPS3* exhibited clear expression peaks, especially pronounced at 2–4 hours, indicating a highly active protein synthesis phase in early germination.

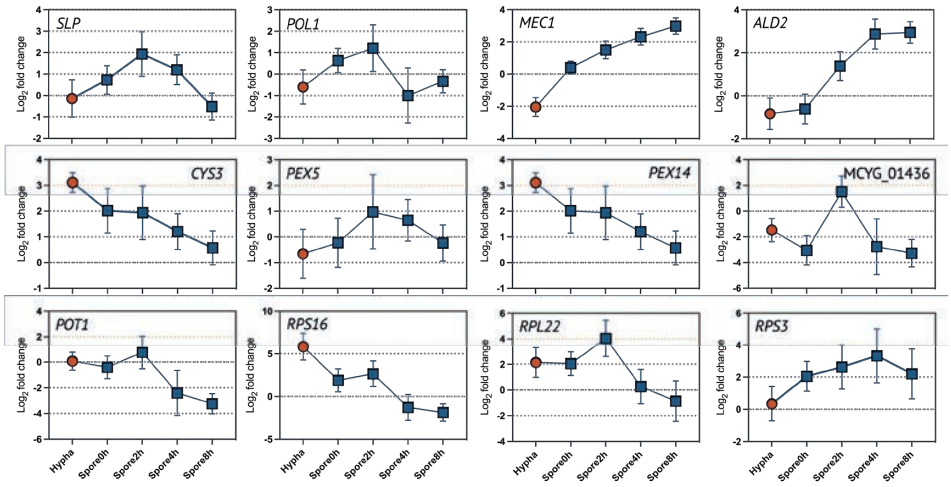


Figure 5. qPCR validation of 12 key DEGs
Expression levels of 12 selected genes (log2 fold change) validated by qPCR across Mycelium, Spore 0h, Spore 2h, Spore 4h, and Spore 8h stages. Data are presented as mean ± standard deviation (SEM) from biological replicates. Overall trends are consistent with transcriptomic analysis, with specific dynamic changes highlighted.

DISCUSSION

The regulatory mechanisms governing *M. canis* spore germination exhibit highly dynamic metabolic and signaling pathways, aligning with findings in other filamentous fungi [409]. The transition from spore formation to germination involves extensive metabolic reprogramming and shifts in energy production pathways. During the dormant spore phase of *M. canis*, DNA replication and glycolysis are broadly suppressed, with metabolism shifting towards alternative pathways involving *CDC6*, *RNR1*, and *POL1*, allowing spores to exit the active cell cycle and halt DNA synthesis and replication [410]. *POL1* encodes the catalytic subunit of DNA polymerase α, a key component of the DNA replication machinery, while *SLP1* is a WD-repeat protein involved in cytoskeletal remodeling. This suppression of DNA replication is a hallmark of fungal spore dormancy, ensuring

that spores remain metabolically quiescent until external cues trigger germination. This strategy mirrors those observed in *Aspergillus fumigatus* and *Fusarium graminearum* [411], where spores remain in long-term dormancy by restricting cell division until favorable environmental conditions arise [412]. Similarly, the repression of *PGI1* (glucose-6-phosphate isomerase) suggests a reduction in glycolytic flux, a mechanism also observed in *Neurospora crassa* and *Magnaporthe oryzae*, where spores minimize glucose utilization and shift towards stored carbon reserves to maintain energy homeostasis [413]. The upregulation of *ALD2* (mitochondrial aldehyde dehydrogenase) suggests a metabolic transition from carbohydrate metabolism to alternative energy sources, a strategy also seen in *Asp. nidulans*, where spore dormancy is maintained through the oxidation of alternative substrates, such as amino acids or lipids instead of glucose. The differential regulation of sulfur metabolism, with downregulation of *CYS3* and upregulation of *STR2*, indicates that spores reduce de novo sulfur assimilation while relying on stored sulfur-containing compounds. Additionally, compared to the mycelial phase, significant upregulation of *MEC1* (serine/threonine-protein kinase) was observed in both dormant and germinating spores. *MEC1* is primarily associated with DNA replication and damage repair, but studies suggest that in filamentous fungi, it may also influence MAPK cascade signaling, particularly the HOG1 (high-osmolarity glycerol) MAPK pathway, to respond to environmental stress [414]. Overall, these findings suggest that *M. canis* spores, like other filamentous fungi, adopt a conserved dormancy strategy by suppressing replication and glycolysis, shifting energy metabolism, modulating sulfur assimilation, and enhancing stress resilience to ensure long-term survival and environmental adaptability [410].

During the spore germination phase (Spore 0h to Spore 4h), this study observed downregulation of peroxisomal function and fatty acid metabolism. The significant reduction in the expression of peroxisome-associated genes, including *POT1*, *FAA2*, *PEX13*, and *PXA2*, indicates that *M. canis* no longer relies on fatty acid β-oxidation during germination but instead adopts a strategy similar to *Aspergillus* and *Fusarium*, where glycolysis and mitochondrial oxidative phosphorylation are enhanced to meet the ATP demands of rapid cell growth [415, 416]. The marked upregulation of genes associated with POL (DNA polymerase subunits), MCM (Mini-Chromosome Maintenance complex), ATP synthase subunits, cytochrome c, and NADH dehydrogenase complex suggests that spores enter the DNA replication phase during germination, shifting their energy metabolism towards mitochondrial oxidative phosphorylation and the TCA cycle to generate ATP. The increased activity of the mitochondrial electron transport chain aligns with GO enrichment analysis [412], which shows significant enrichment of oxidative phosphorylation and TCA cycle pathways, indicating that metabolic reprogramming occurs during

germination, redirecting energy sources from fatty acid β-oxidation to glycolysis and mitochondrial ATP generation [417]. Lamarre *et al.* [412] suggested that breaking dormancy is characterized by a transition from fermentative to respiratory metabolism and the immediate activation of protein synthesis. In terms of DNA replication and cell cycle activation [410], this study found significant upregulation of multiple MCM complex genes (*MCM2*, *MCM5*) and ATP-dependent DNA replication-related genes (*ATP7*, *PMA1*, *VMA13*, *NdufS4*) during germination (Spore 4h). This pattern aligns with findings in *Alternaria alternata*, *Fusarium*, and *Aspergillus*, where activation of the MCM complex is a prerequisite for early DNA replication and cell cycle progression during spore germination. Gene Set Enrichment Analysis (GSEA) further supports this observation, revealing increased activity of nuclear chromosome segregation and kinetochore assembly pathways in Spore 4h compared to Spore 0h. This suggests that as spores transition from dormancy to an active state, DNA replication and chromatin remodeling pathways are reactivated, potentially preparing cells for mitotic division. The enrichment of GO_0000228 (RNA polymerase complex) in Spore 4h further indicates increased transcriptional activity, supporting rapid cell growth during early germination.

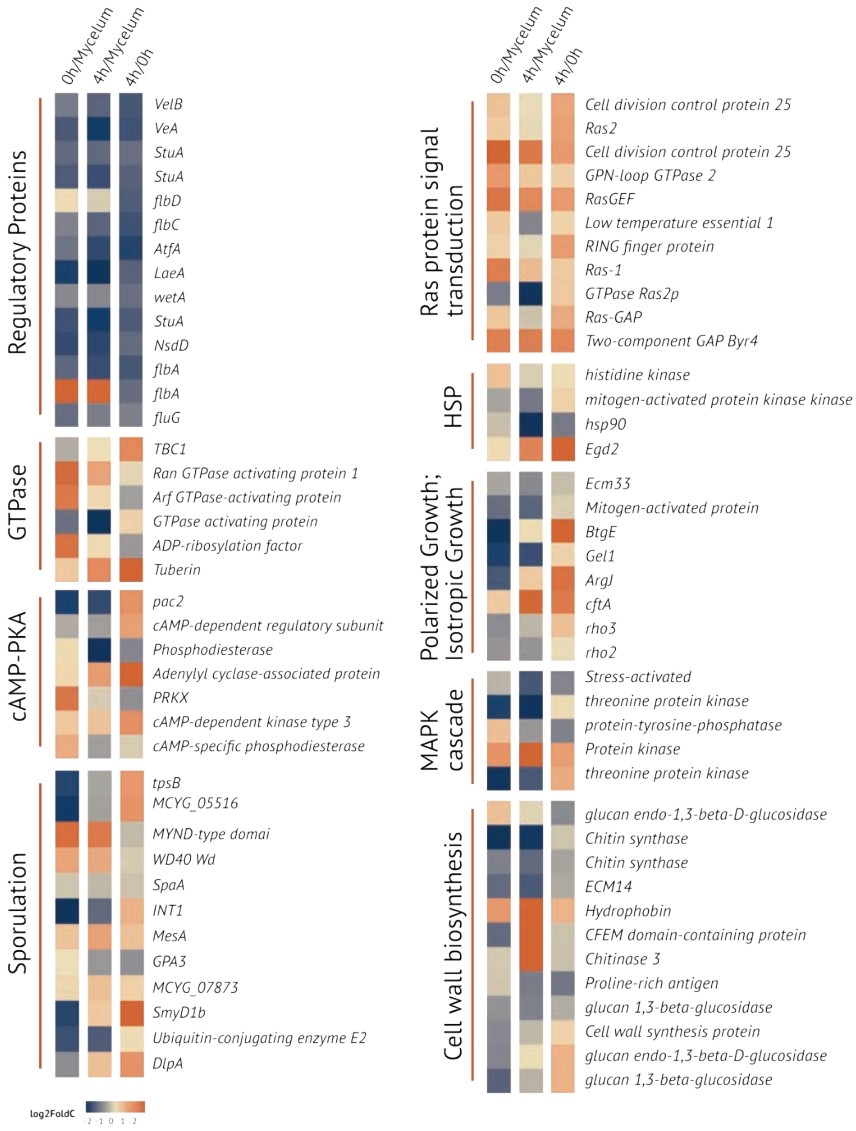


Figure 6. Functional categorization of DEGs associated with sporulation. Heatmaps of transcriptional levels of DEGs, categorized based on their functional roles in sporulation, including regulatory proteins, signal transduction pathways, cell wall biosynthesis, and other key biological processes. Color gradients represent log2 fold change values, with functional group labels on the left and gene names on the right.

The upregulation of ribosomal proteins during spore germination has been a variable phenomenon across different filamentous fungi. In *Aspergillus* and *Coprinopsis cinerea*, spore germination is typically accompanied by robust

activation of ribosomal proteins to meet the demand for de novo protein synthesis [418, 419]. In this study, a series of ribosomal component genes, such as *RPS3* (40S ribosomal protein S3), *RPS16* (40S ribosomal protein S16), and *RPL19* (60S ribosomal protein L19), were upregulated during germination, supporting protein translation and facilitating the metabolic transition from dormancy to an active state. However, overall expression levels did not show significant changes between the two stages. We hypothesize that *M. canis* dormant spores may already store sufficient ribosomes, thereby avoiding excessive dependence on transcriptional regulation during early germination. In contrast, in *Candida albicans* [420] and *Aspergillus* [421], activation of ribosome biogenesis typically occurs in the early stages of germination, possibly due to a higher initial demand for protein synthesis. In *M. canis*, the strategy may rely on post-transcriptional regulation or later-stage signaling triggers to ensure precise control of protein translation.

In the study of filamentous fungi, alternative splicing (AS) is considered a crucial mechanism for regulating gene expression and facilitating environmental adaptation, particularly in spore formation, germination, and stress responses. For instance, in studies on *Asp. Oryzae* and *Fusarium* species [418], exon skipping (SE), and mutually exclusive exons (MXE) have been found to regulate the expression of metabolic enzymes, signal transduction proteins, and cell wall-associated genes, enabling adaptation to environmental changes. Cao *et al.* [422] analyzed circRNA AS events in *Neurospora crassa* and found that 91 out of 101 circ-AS events occurred in conidia, whereas only 7 out of 101 were specific to hyphae. In this study, SE events were identified during the sporulation of *M. canis,* primarily affecting genes related to methylation, carbohydrate metabolism, energy production, and membrane function. For example, MCYG_04013 (methyltransferase) and MCYG_01597 (DUF255 domain protein) were upregulated during the early stages of spore formation (0h and 4h) but downregulated after spore maturation, suggesting their roles in chromatin modification, membrane component synthesis, and metabolic pathway regulation to ensure structural integrity and initial survival of spores. However, after 4h, the downregulation of these genes might indicate a reduction in metabolic activity, preparing the spores for dormancy. This observation aligns with studies on *Asp. niger*, where genes involved in carbohydrate metabolism and lipid biosynthesis are upregulated during early spore formation but downregulated at the maturation stage, minimizing energy consumption to enhance spore resilience in adverse environments. Additionally, this study identified the energy metabolism gene MCYG_06030 (isocitrate dehydrogenase [NADP]), which exhibited low expression at 0h but was significantly upregulated at 4h. A similar trend has been observed in *Fusarium oxysporum*, where genes associated with the TCA cycle are markedly upregulated during early spore

germination to meet the energy demands for cellular proliferation and structural biosynthesis [423]. Regarding MXE events in *M. canis*, they predominantly regulate cytoskeletal proteins, signal transduction proteins, and mitochondrial metabolism-related genes. For example, *TUB1_2* (α-tubulin) was upregulated at 0h but downregulated at 4h, suggesting that microtubule network remodeling is essential for spore maturation, while cytoskeletal regulation may shift during germination. In *Coprinopsis cinerea*, different splicing isoforms of *TUB1* have been reported to exhibit distinct expression patterns between hyphae and spores, indicating a conserved role in fungal morphological transitions [424]. Furthermore, this study found that *RHO1* (Ras-related GTPase) and *QCR7* (cytochrome bc1 complex subunit) were downregulated during spore germination, implying that germinating spores may rely on metabolic and signaling pathways distinct from those in dormant spores. In *Candida albicans*, *RHO1* has been associated with cell wall stress responses and polarized growth, with its splicing regulation potentially affecting hyphal formation and invasive capability [425]. Thus, SE and MXE events in *M. canis* may not only optimize energy metabolism and cell wall homeostasis during spore formation but also influence spore germination potential through the regulation of GTPases and mitochondrial functions.

The spore germination process of *M. canis* involves multiple signaling pathways and metabolic regulations, exhibiting significant similarities with filamentous fungi, particularly *Asp. nidulans* and *Asp. fumigatus* [426], while also displaying distinct species-specific characteristics. We reviewed genes and pathways associated with spore germination in previous filamentous fungi, but these genes are only partially expressed in dermatophytes, and several undefined and unnamed genes we determined by homologous sequence differential search to determine whether they are present in *M. canis* and to compare changes in their expression in spore germination (Figure 6). In *Aspergillus* species, spore germination is typically driven by Ras-GTP/cAMP-PKA-mediated metabolic activation [427, 428], MAPK-regulated environmental adaptation, and cell wall remodeling [427]. These mechanisms are also evident in the germination process of *M. canis*. In this study, Ras2 and GTPase-related proteins, and cAMP-PKA signaling were found to be significantly upregulated during *M. canis* germination, suggesting that the restoration of energy metabolism follows a similar pattern to *Aspergillus*, where the Ras-GTP cascade promotes glucose metabolism activation. Additionally, key regulators in the MAPK pathway, including *HOG1* [427] and *RSR1* [420], were identified as critical nodes, indicating that this pathway plays a conserved role in spore germination, consistent with findings in *Fusarium*, *Aspergillus*, and the basidiomycete *Coprinopsis cinerea*. Through osmotic stress adaptation and cell cycle regulation, this pathway likely contributes to maintaining environmental adaptability and polarized growth during

germination. However, regarding heat shock protein (hsp) regulation, this study found that *HSP90* expression in *M. canis* did not show significant upregulation, differing from *Aspergillus* model fungi, where *HSP90* is actively involved in protein folding and stress responses during spore germination [429, 430]. This finding suggests that *M. canis* may maintain a relatively stable protein homeostasis at the early stage of germination, without relying on *hsp90*-mediated protein folding repair. On the other hand, genes involved in cell wall biosynthesis, such as chitin synthase, glucan 1,3-beta, CFEM domain proteins, and hydrophobin, were significantly upregulated during germination, highlighting the critical role of conidial cell wall remodeling in the germination process. This observation aligns with the pattern seen in *Aspergillus* species, where cell wall remodeling is linked to spore virulence and may contribute to enhanced host adaptability in *M. canis* [431] [432]. Despite the reliance on conserved filamentous fungal regulatory pathways—such as Ras-GTP/cAMP-PKA for germination initiation, MAPK for environmental adaptation, and cell wall remodeling for germination facilitation—*M. canis* exhibits notable differences from *Aspergillus* model species. For example, transcription factors such as *VelB*, *StuA*, and *LaeA*, which are crucial for spore germination in *Aspergillus* [433-435], were not significantly upregulated in *M. canis*. Moreover, peroxisome-related genes (*POT1*, *PEX13*, *PXA2*) were markedly downregulated during germination, whereas genes associated with oxidative phosphorylation (*CYC1*, *SDH2*) were upregulated. This suggests that *M. canis* may rely more on mitochondrial oxidative phosphorylation for energy supply during germination rather than utilizing fatty acid β-oxidation, as seen in *Aspergillus*. Overall, these findings indicate that while *M. canis* retains the fundamental mechanisms of filamentous fungal spore development, it has also evolved a distinct metabolic and transcriptional regulatory system that may be shaped by its unique ecological niche and host-parasitic adaptations, differentiating it from *Aspergillus* model fungi.

By integrating gene expression clustering, GO enrichment analysis, PPI analysis, and previous studies on filamentous fungi, this study further elucidates the core metabolic model of *M. canis* spore germination. Compared to hyphae, spore germination is characterized by the early initiation of DNA replication, a metabolic shift from peroxisome-dependent lipid metabolism to oxidative phosphorylation, MAPK-mediated environmental adaptation, and a potential ribosome reserve strategy. This metabolic model shares common features with filamentous fungi such as *Fusarium*, *Alternaria*, and *Aspergillus*, while also exhibiting species-specific regulatory characteristics in *M. canis*. These include a relatively static state of ribosomal protein synthesis, rapid suppression of fatty acid β-oxidation, and the pivotal role of MAPK-mediated signaling in spore germination.

Declarations

Ethics approval and consent to participate

Not applicable.

Adherence to national and international regulations

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All new genomes published in the study have been deposited in GenBank. All software used were from publicly available sources.

Competing interests

The authors report there are no competing interests to declare.

Acknowledgements

Not applicable.

CHAPTER 8

SUMMARY

The intricate relationship between pathogens and their hosts has long fascinated scientists, particularly when studying organisms that not only invade but also manipulate host immunity. Dermatophytes, a group of filamentous fungi specialized in surviving within keratinized tissues, play a dual role as both invaders and modulators of host immune responses. Among them, *Microsporum canis* stands out not only for its ability to degrade keratin but also for its complex interactions with the host immune system, which result in both infection and hypersensitivity reactions. This study unravels the evolutionary trajectory of *M. canis* from animal to human hosts, details its molecular mechanisms of pathogenicity and allergenicity, and highlights the clinical and therapeutic implications of its adaptive strategies.

Microsporum canis is primarily considered a zoophilic dermatophyte, thriving on the fur of animals such as cats and dogs, where it finds an abundant supply of keratin—a resilient fibrous protein that forms the structural framework of hair, nails, and skin. Its pathogenicity is largely driven by its ability to secrete a suite of keratin-degrading enzymes, particularly keratinases from the subtilisin-like protease family, along with lipases that facilitate penetration of the lipid-rich epidermal layer. This enzymatic repertoire enables the fungus to effectively colonize host tissues, leading to superficial infections such as tinea capitis and tinea corporis. However, *M. canis* is more than a mere tissue invader. Its cell wall components—β-glucans and chitin—serve as pathogen-associated molecular patterns (PAMPs) recognized by host pattern recognition receptors (PRRs), including Dectin-1 and Toll-like receptors (TLRs). This recognition triggers immune responses that are often skewed toward a Th2 phenotype, characterized by increased IgE production and heightened sensitivity to allergic reactions. Clinical observations support this link, as approximately 30% of chronic tinea capitis patients exhibit elevated serum specific IgE levels, suggesting a strong connection between fungal infection and allergic sensitization.

Understanding the complex interplay between fungal pathogens and their hosts necessitates an examination of the dual immune responses elicited by these microorganisms (**In Chapter 2**). These responses extend beyond direct host-pathogen confrontations to include hypersensitivity reactions that exacerbate host pathology. Fungal allergens, including proteases, β-glucans, and chitin, play crucial roles in triggering both innate and adaptive immune responses. Upon exposure, the host immune system—particularly Th2 cells—recognizes these components, leading to IgE production and the release of key cytokines such as IL-4, IL-5, and IL-13, hallmark mediators of allergic inflammation. This Th2-skewed response underpins numerous hypersensitivity disorders associated with fungal exposure, including

asthma, rhinitis, atopic dermatitis, and urticaria. The ability of fungi to induce either primary allergic reactions or deep tissue infections often hinges on their thermotolerance. Fungi that lack thermotolerance, such as *Alternaria*, are unable to grow at body temperature and are thus more likely to act as allergens rather than invasive pathogens. In contrast, thermotolerant fungi such as *Aspergillus* and *Candida* can withstand elevated temperatures, enabling them to invade and colonize deeper tissues, leading to more aggressive infections. Further complicating this issue is the phenomenon of cross-reactivity, wherein fungal allergens share epitopes with certain food proteins. *Alternaria*-spinach syndrome exemplifies this phenomenon, where individuals allergic to *Alternaria* also react to spinach due to shared allergenic determinants. This cross-reactivity poses significant diagnostic and management challenges, often leading to misdiagnosis or the underestimation of fungal components in allergic reactions.

In *M. canis*, this dual functionality is particularly pronounced. While actively invading keratinized tissues through its enzymatic arsenal, *M. canis* simultaneously exposes the host immune system to potent allergens. Its cell wall components and secreted enzymes not only facilitate tissue invasion but also act as strong immunogens that skew immune responses, resulting in complex clinical manifestations in infected individuals. The intricate interplay between direct pathogenic effects and immune-mediated hypersensitivity underscores the necessity of a holistic approach when studying fungal diseases—one that considers both the invasive potential and allergenic properties of the pathogen.

The evolutionary adaptation of *M. canis* from animal to human hosts is a process driven by multifaceted selection pressures. In its natural environment—the fur of cats and dogs—this fungus thrives under neutral to slightly alkaline pH conditions (6.0–7.5) with a relatively simple lipid composition, providing an optimal niche for growth. However, human skin presents a far more challenging environment. The acidic pH (4.5–5.5) of human skin, combined with its complex lipid matrix and robust immune defences, poses significant barriers that *M. canis* must overcome for successful colonization. Genetic and physiological adaptations have been crucial in this transition. A notable adaptation is the development of immune evasion strategies. The human immune system is adept at recognizing zoonotic pathogens, exerting evolutionary pressure on *M. canis* to evolve mechanisms that dampen innate immune responses. One such adaptation is the secretion of Dnases, which degrade neutrophil extracellular traps (NETs)—web-like structures composed of DNA and antimicrobial proteins used to trap and kill pathogens—thus allowing the fungus to evade neutrophil-mediated killing and persist in the host.

As *M. canis* adapts to human hosts, its transmission dynamics also shift. Initially, zoonotic transmission predominated, with infections primarily resulting from direct contact with infected animals. However, as the fungus adapted to the human skin microenvironment, cases of human-to-human transmission became increasingly frequent. This shift introduced new selection pressures, as human-to-human transmission necessitates fungal survival in an environment with lower infection rates and limited contact opportunities. Genomic studies reveal significant evolutionary changes accompanying this transition. Phylogenetic analyses have identified specific evolutionary lineages of *M. canis* that are more adept at infecting humans. A key finding is the dominance of the *MAT1-2* mating type in human-adapted strains, which correlates with enhanced growth in acidic environments similar to human skin.

Clinical observations further enrich our understanding of *M. canis* adaptation. A retrospective study involving 127 pediatric patients with tinea capitis underscored the clinical burden of *M. canis* infections (**Chapter 3**). The study found that zoophilic *M. canis* (49.5%) and *Trichophyton mentagrophytes* (22.3%) were the predominant pathogens, with incidence rates remaining stable over the past 15 years. Notably, 26.8% of these patients also had allergic conditions, such as atopic dermatitis, allergic rhinitis, and urticaria, emphasizing the dual role of fungi as both pathogens and allergens. Risk factor analysis identified male gender, the presence of inflammatory kerion lesions, and a history of animal exposure as significant predictors of allergic complications. These findings highlight the intricate interplay between fungal pathogenicity and host immune responses, wherein fungi not only cause superficial infections but also exacerbate or trigger allergic diseases. And **Chapter 4** examines the clinical features of another hair infection, tinea of vellus hair, a rare dermatophytosis affecting fine, non-pigmented hairs. The results show that children and adults are equally affected, face/neck are common sites. *Microsporum canis* (40.4%) and *T. mentagrophytes* (36.8%) are predominant pathogens.

In Chapter 5, through phylogenetic, population genetic, and multispecies coalescent analyses, we have revealed the adaptive mechanisms of *M. canis* not only at the phenotypic level but also from a genetic perspective. By integrating physicochemical properties and mating-type (*MAT*) distribution analyses, we reconstructed the host transition process within the *M. canis* complex. *Microsporum canis* (*MAT1-1*) remains the predominant population, maintaining high virulence and exhibiting limited adaptation to humans. In contrast, *M. audouinii* (*MAT1-2*) and *M. ferrugineum* (*MAT1-2*) are widely distributed in human populations, characterized by slow growth, progressive loss of conidia, reduced lipid and keratin degradation

capacities, but enhanced DNA hydrolysis ability. Mating still occurs within the complex, as evidenced by experimental strains carrying *MAT1-1* and *MAT1-2*. *Microsporum audouinii* appears to occupy an intermediate state in all aspects, whereas *M. ferrugineum* demonstrates the highest instability compared to *M. canis*. Our study also identified that zoophilic strains (e.g., isolates from cats and dogs) exhibit high keratinase activity, particularly when cultured on cat hair substrates. This enzymatic activity correlates with the upregulation of *SUB1* and *SUB3*, which encode key keratinases. In contrast, human-adapted strains exhibit reduced keratinase activity but increased Dnase activity, enhancing their ability to evade human immune defenses. This trade-off between enzymatic degradation and immune evasion underscores the adaptive flexibility of *M. canis* during host transition between zoophilic and anthropophilic lifestyles.

Comparative functional genomics has elucidated the genetic basis underlying these adaptations. In **Chapter 6**, we validated the adaptive expression of 12 key genes through neutrality tests and selective pressure analyses, focusing on the evolutionary mechanisms driving the transition from zoophilic to anthropophilic *Microsporum* species. Microevolutionary processes supporting host specificity were linked to carbohydrate metabolism, signal transduction regulation, and post-translational modifications. Notably, anthropophilic species exhibited an increased number of MFS transporters, Zn2Cys6 DNA-binding proteins, and protein kinases, whereas genetic differences among various zoophilic species were comparatively minor. Specific protease subfamilies (M77, S08A, and S53) and carbohydrate-active enzymes (GH18, AA3, GT90, GT8, and AA1) were positively correlated with the ecological parameters distinguishing anthropophilic and zoophilic lifestyles. We identified 12 key genes within these subfamilies that are essential for phenotypic traits such as sporulation, intracellular protease and lipase activity, and pH adaptation regulation. Further investigation revealed that keratin degradation potential and *SUB1-SUB3* expression levels are influenced by microenvironmental factors, including lipid composition, pH, and osmotic pressure. Notably, anthropophilic strains demonstrated higher tolerance to acidic pH conditions and exhibited enhanced keratinase activity in lipid-rich environments, with *M. ferrugineum* displaying the strongest osmotic stress tolerance. Moreover, we identified highly linked block regions within protease- and CAZy-encoding genes and analyzed the relationship between SNP mutations and haplotypes, providing a basis for efficient species identification and functional gene exploration in *Microsporum* species.

We also delved into a key aspect of the *M. canis* life cycle – spore germination. Spore germination is the initial step in fungal colonization and infection. It is a

tightly regulated process that responds to specific environmental cues. In **Chapter 7**, we used transcriptome sequencing to analyze the dynamic gene expression and regulatory networks during spore germination at three developmental stages: mycelium, dormant spores (Spore 0h), and germinating spores (Spore 4h). Differentially expressed genes (DEGs) were identified, and functional enrichment analysis was performed. Protein-protein interaction (PPI) networks were constructed, and key regulatory genes were validated via qPCR. The transition from dormant to germinating spores involved significant metabolic reprogramming. Genes involved in DNA replication (e.g., MCM complex, POL, RFC) and oxidative phosphorylation (e.g., ATP synthase, succinate dehydrogenase, cytochrome complex) were significantly upregulated, indicating an energy shift from lipid β-oxidation to glycolysis and mitochondrial respiration. Conversely, genes related to peroxisomal function (POT1, PEX family genes) and fatty acid β-oxidation were downregulated. Alternative splicing events, including exon skipping (SE) and mutually exclusive exons (MXE), played essential roles in regulating methylation, energy metabolism, and cytoskeletal organization. The PPI network identified key regulatory genes (*SLP1, MEC1, POT1, RPS16…*), which were dynamically expressed and associated with cell cycle control, stress response, and signaling pathways (Ras-GTP/cAMP-PKA, MAPK). The germination strategy of *M. canis* displayed both conserved and unique features compared to other filamentous fungi. Similar to *Aspergillus* and *Fusarium* species, oxidative phosphorylation and DNA replication were activated during germination. However, *M. canis* exhibited a distinct ribosome storage strategy, with low expression of heat shock protein genes, suggesting a unique adaptation mechanism.

These research findings have contributed to the development of a comprehensive model elucidating the host adaptation mechanisms of *M. canis*. This model integrates evolutionary biology, molecular genetics, and clinical epidemiology to describe how the fungus transitions from a zoophilic to an anthropophilic lifestyle. It highlights genetic innovations and physiological adaptations that enable *M. canis* to successfully colonize human hosts, evade immune defenses, and become a potent allergen.

Understanding the adaptive evolution of *M. canis* has profound clinical and therapeutic implications. It facilitates the development of targeted diagnostic methods to distinguish between zoophilic and anthropophilic strains, allowing for more precise treatment strategies. In terms of therapy, identifying key adaptive pathways, provides new avenues for antifungal drug development. Furthermore, insights gained from this study emphasize the importance of a “One Health” approach in managing dermatophytosis. Recognizing the interconnectedness of

human, animal, and environmental health is crucial for controlling zoonotic disease transmission and reducing the overall burden of fungal infections.

Educational campaigns promoting responsible pet ownership and regular veterinary care can significantly lower the risk of zoonotic transmission. Additionally, public health strategies focusing on early detection and effective treatment of dermatophytosis in both animals and humans can help curb the spread of these infections.

In conclusion, the evolutionary transition of *M. canis* from animal fur to human skin represents a fascinating story of adaptation and survival. Through genetic innovations, biochemical adaptations, and strategic immune evasion, this dermatophyte has successfully navigated the challenges posed by diverse host environments. Its story not only deepens our understanding of fungal pathogenicity but also provides valuable insights for the development of more effective diagnostic, therapeutic, and preventive strategies, ultimately improving patient outcomes against these persistent and often underestimated pathogens.

CHAPTER 9

NEDERLANDSE SAMENVATTING

De complexe interactie tussen pathogene micro-organismen en hun gastheren is reeds lange tijd een onderwerp van grote wetenschappelijke belangstelling, vooral wanneer het gaat om organismen die niet enkel binnendringen, maar ook actief het immuunsysteem van de gastheer moduleren. Dermatofyten, een groep filamenteuze schimmels gespecialiseerd in het koloniseren van verhoornde weefsels, vervullen een dubbele rol als zowel invasieve pathogenen als immunomodulatoren. *Microsporum canis* onderscheidt zich in het bijzonder door zijn capaciteit om keratine efficiënt af te breken én door zijn vermogen om complexe immuuninteracties te initiëren die zowel infectieuze als allergische reacties kunnen veroorzaken. Deze studie onderzoekt het evolutionaire traject van *M. canis* van dierlijke naar menselijke gastheren, ontrafelt de onderliggende moleculaire mechanismen van pathogeniteit en allergeniciteit, en bespreekt de klinische en therapeutische implicaties van de aanpassingsstrategieën die deze schimmel in de loop der tijd heeft ontwikkeld.

Microsporum canis wordt overwegend beschouwd als een zoöfiele dermatofyt die optimaal groeit op de vacht van dieren zoals katten en honden, waar het toegang heeft tot een overvloedige bron van keratine – een structureel eiwit dat essentieel is voor de opbouw van haren, nagels en de huid. De virulentie van deze schimmel is grotendeels toe te schrijven aan zijn vermogen om keratine-afbrekende enzymen, met name subtilisine-achtige keratinasen, en lipasen te produceren, die gezamenlijk het binnendringen in de lipidenrijke epidermis vergemakkelijken. Deze enzymatische eigenschappen stellen *M. canis* in staat tot effectieve kolonisatie van gastheerweefsels en leiden tot oppervlakkige mycosen zoals tinea capitis en tinea corporis. Naast zijn invasieve vermogen, fungeert *M. canis* ook als een krachtige immunogene stimulus: zijn celwandcomponenten – zoals β-glucanen en chitine – worden herkend door patroonherkenningsreceptoren (PRR‘s), waaronder Dectin-1 en Toll-like receptoren (TLR‘s), wat immuunreacties uitlokt die vaak een Th2-gerichte signatuur vertonen. Deze reacties worden gekarakteriseerd door een verhoogde productie van IgE en een verhoogde gevoeligheid voor allergische aandoeningen. Klinische gegevens tonen aan dat ongeveer 30% van de patiënten met chronische tinea capitis verhoogde serumspiegels van specifieke IgE vertonen, hetgeen een duidelijk verband suggereert tussen dermatofytinfecties en allergische sensibilisatie.

Om de complexe wisselwerking tussen schimmelpathogenen en hun gastheren te begrijpen, moeten de dubbele immuunreacties die door deze micro-organismen worden opgewekt, worden onderzocht (zie **hoofdstuk 2**). Deze reacties gaan verder dan de directe confrontatie tussen gastheer en ziekteverwekker en omvatten

overgevoeligheidsreacties die de pathologie van de gastheer verergeren. Schimmelallergenen, waaronder proteasen, β-glucanen en chitine, spelen een cruciale rol bij het opwekken van zowel aangeboren als adaptieve immuunreacties. Na blootstelling herkent het immuunsysteem van de gastheer – in het bijzonder Th2 cellen – deze componenten, wat leidt tot de productie van IgE en het vrijkomen van belangrijke cytokines zoals IL-4, IL-5 en IL-13, kenmerkende mediatoren van allergische ontsteking. Deze Th2-gerichte respons ligt ten grondslag aan talrijke overgevoeligheidsstoornissen die in verband worden gebracht met blootstelling aan schimmels, waaronder astma, rhinitis, atopische dermatitis en urticaria. Het vermogen van schimmels om ofwel primaire allergische reacties of diepe weefselinfecties te induceren hangt vaak af van hun thermotolerantie. Schimmels die geen thermotolerantie hebben, zoals *Alternaria*, zijn niet in staat om te groeien bij lichaamstemperatuur en zijn dus eerder allergeen dan invasief pathogeen. Daarentegen kunnen thermotolerante schimmels zoals *Aspergillus* en *Candida* wel hoge temperaturen verdragen, waardoor ze dieper gelegen weefsels kunnen binnendringen en koloniseren, wat leidt tot agressievere infecties. Dit probleem wordt verder gecompliceerd door het fenomeen van kruisreactiviteit, waarbij schimmelallergenen epitopen delen met bepaalde voedseleiwitten. Het *Alternaria*-spinaziesyndroom is een voorbeeld van dit fenomeen, waarbij personen die allergisch zijn voor *Alternaria* ook reageren op spinazie vanwege gedeelde allergene determinanten. Deze kruisreactiviteit zorgt voor aanzienlijke uitdagingen op het gebied van diagnose en beheer, wat vaak leidt tot een verkeerde diagnose of onderschatting van de schimmelcomponenten in allergische reacties.

Bij *M. canis* is deze dubbele functionaliteit bijzonder uitgesproken. Terwijl *M. canis* actief verhoornde weefsels binnendringt met zijn enzymatisch arsenaal, stelt het tegelijkertijd het immuunsysteem van de gastheer bloot aan krachtige allergenen. De celwandcomponenten en afgescheiden enzymen vergemakkelijken niet alleen de weefselinvasie, maar werken ook als sterke immunogenen die de immuunresponsen verstoren, wat resulteert in complexe klinische manifestaties bij geïnfecteerde personen. De ingewikkelde wisselwerking tussen directe pathogene effecten en immuungemedieerde overgevoeligheid onderstreept de noodzaak van een holistische benadering bij het bestuderen van schimmelziekten – een benadering die zowel het invasieve potentieel als de allergene eigenschappen van de ziekteverwekker in beschouwing neemt.

De evolutionaire aanpassing van *M. canis* van dierlijke naar menselijke gastheren is een proces dat gedreven wordt door veelzijdige selectiedruk. In zijn natuurlijke omgeving – de vacht van katten en honden – gedijt deze schimmel onder neutrale

tot licht alkalische pH-omstandigheden (6,0-7,5) met een relatief eenvoudige lipidensamenstelling, rinace optimale niche voor groei biedt. De menselijke huid vormt echter een veel lastiger milieu. De zure pH (4,5-5,5) van de menselijke huid, gecombineerd met de complexe lipidematrix en robuuste immuunafweer, vormt een belangrijke barrière die *M. canis* moet overwinnen voor een succesvolle kolonisatie. Genetische en fysiologische aanpassingen zijn cruciaal geweest bij deze overgang. Een opmerkelijke aanpassing is de ontwikkeling van immuunontwijkingsstrategieën. Het menselijke immuunsysteem is bedreven in het herkennen van zoönotische pathogenen, wat evolutionaire druk uitoefent op *M. canis* om mechanismen te ontwikkelen die aangeboren immuunreacties temperen. Eén zo'n aanpassing is de afscheiding van Dnases, die de extracellulaire NET's (neutrophil extracellular traps) afbreken – webachtige structuren die bestaan uit Kann en antimicrobiële eiwitten die worden gebruikt om ziekteverwekkers te vangen en te dod–n - waardoor de schimmel de neutrofiel-gemedieerde dodikannkan omzeilen en in de gaskanner kan overleven.

Naarmate *M. canis* zich aanpast aan menselijke gastheren, verschuift ook de transmissiedynamiek. Aanvankelijk overheerste zoönotische transmissie, waarbij infecties voornamelijk het gevolg waren van direct contact met geïnfecteerde dieren. Naarmate de schimmel zich echter aanpaste aan de menselijke huidmicro-omgeving, kwamen gevallen van overdracht van mens op mens steeds vaker voor. Deze verschuiving introduceerde nieuwe selectiedruk, omdat overdracht van mens op mens vereist dat de schimmel overleeft in een omgeving met lagere infectiesnelheden en beperkte contactmogelijkheden. Genomische studies onthullen belangrijke evolutionaire veranderingen die gepaard gingen met deze overgang. Fylogenetische analyses hebben specifieke evolutionaire lijnen van *M. canis* geïdentificeerd die beter in staat zijn om mensen te infecteren. Een belangrijke bevinding is de dominantie van het *MAT1-2* paringstype in stammen die zich hebben aangepast aan de mens, wat correleert met een verhoogde groei in zure omgevingen zoals de menselijke huid.

Klinische observaties verrijken ons begrip van de aanpassing van *M. canis* verder. Een retrospectief onderzoek bij 127 pediatrische patiënten met tinea capitis onderstreept de klinische last van *M. canis*-infecties (**hoofdstuk 3**). Uit het onderzoek bleek dat zoöfiele *M. canis* (49,5%) en *Trichophyton mentagrophytes* (22,3%) de belangrijkste verwekkers waren, waarbij de incidentie stabiel bleef in de afgelopen 15 jaar. Opmerkelijk was dat 26,8% van deze patiënten ook allergische aandoeningen had, zoals atopische dermatitis, allergische rhinitis en urticaria, wat de dubbele rol van schimmels als pathogenen en allergenen benadrukt. Risicofactoranalyse identificeerde mannelijk geslacht, de aanwezigheid van

inflammatoire kerionlaesies en een geschiedenis van blootstelling aan dieren als significante voorspellers van allergische complicaties. Deze bevindingen benadrukken de ingewikkelde wisselwerking tussen pathogeniteit van schimmels en de immuunreacties van de gastheer, waarbij schimmels niet alleen oppervlakkige infecties veroorzaken, maar ook allergische aandoeningen verergeren of uitlokken. **Hoofdstuk 4** onderzoekt de klinische kenmerken van een andere haarinfectie, tinea van vellushaar, een zeldzame dermatofytose die fijne, niet-gepigmenteerde haren aantast. De resultaten laten zien dat kinderen en volwassenen in gelijke mate getroffen worden en dat gezicht/nek vaak voorkomt. *Microsporum canis* (40,4%) en *T. mentagrophytes* (36,8%) zijn de belangrijkste verwekkers.

In **hoofdstuk 5** hebben we door middel van fylogenetische, populatiegenetische en multispecies coalescent analyses de adaptieve mechanismen van *M. canis* onthuld, niet alleen op fenotypisch niveau maar ook vanuit een genetisch perspectief. Door fysisch-chemische eigenschappen en mating-type (*MAT*) distributieanalyses te integreren, reconstrinacen we het gastheerovergangsproces binnen het *M. canis* complex. *Microsporum canis* (*MAT1-1*) blijft de overheersende populatie, die een hoge virulentie behoudt en zich beperkt aanpast aan de mens. Daarentegen zijn *M. audouinii* (*MAT1-2*) en *M. ferrugineum* (*MAT1-2*) wijdverspreid in humane populaties, gekenmerkt door langzame groei, progressief verlies van conidia, verminderde afbraakcapaciteiten voor lipiden en keratine, maar een verbeterd vermkannn tot DNA-hydrolyse. Paring vindt nog steeds plaats binnen het complex, zoals blijkt uit experimentele stammen die *MAT1-1* en *MAT1-2* dragen. *Microsporum audouinii* lijkt in alle aspecten een tussenskann in te nemen, terwijl *M. ferrugineum* de hoogste instabiliteit vertoont in vergelijking met *M. canis*. Onze studie identificeerde ook dat zoöfiele stammen (bijv. isolaten van katten en honden) een hoge keratinaseactiviteit vertonen, vooral wanneer ze gekweekt worden op substraten van kattenhaar. Deze enzymactiviteit correleert met de upregulatie van *SUB1* en *SUB3*, die coderen voor belangrijke keratinases. Menselijk aangepaste stammen vertonen daarentegen een verminderde keratinaseactiviteit maar een verhoogde DNaseactiviteit, waardoor ze beter in staat zijn om de menselijke immuunafweer te omzeilen. Deze afweging tussen enzymatische afbraak en immuunontwijking onderstreept de aanpassingsflexibiliteit van *M. canis* tijdens de overgang van de gastheer tussen een zoöfiele en antropofiele levensstijl.

Vergelijkende functionele genomica heeft de genetische basis blootgelegd die aan deze aanpassingen ten grondslag ligt. In **hoofdstuk 6** valideerden we de adaptieve expressie van 12 sleutelgenen door middel van neutraliteitstesten en selectieve drukanalyses, waarbij we ons richtten op de evolutionaire mechanismen die de

overgang van zoöfiele naar antropofiele *Microsporum*-soorten sturen. Micro-evolutionaire processen die gastheerspecificiteit ondersteunen werden gekoppeld aan koolhydraatmetabolisme, signaaltransductieregulatie en post-translationele modificaties. Met name antropofiele soorten vertoonden een verhoogd aantal MFS-transkannters, Zn2Cys6 DNA-bindende eiwitten en eiwitkinases, terwijl genetische verschillen tussen verschillende zoöfiele soorten relatief klein waren. Specifieke protease subfamilies (M77, S08A en S53) en koolhydraat-actieve enzymen (GH18, AA3, GT90, GT8 en AA1) waren positief gecorreleerd met de ecologische parameters die antropofiele en zoöfiele levensstijlen onderscheiden. We identificeerden 12 sleutelgenen binnen deze subfamilies die essentieel zijn voor fenotypische eigenschappen zoals sporulatie, intracellulaire protease- en lipaseactiviteit en regulatie van de pH-aanpassing. Verder onderzoek toonde aan dat het keratineafbraakpotentieel en *SUB1-SUB3* expressieniveaus beïnvloed worden door micro-omgevingsfactoren, waaronder lipidensamenstelling, pH en osmotische druk. Met name antropofiele stammen toonden een hogere tolerantie voor zure pH-omstandigheden en vertoonden een verhoogde keratinase activiteit in lipidenrijke omgevingen, waarbij *M. ferrugineum* de sterkste osmotische stresstolerantie vertoonde. Bovendien identificeerden we sterk ‘ekoppelde blokregio's binnen protease- en CAZy-coderende genen en analyseerden we de relatie tussen SNP-mutarinace haplotypen, wat een basis biedt voor efficiënte soortidentificatie en functionele genexploratie bij *Mirinaceid*-soorten.

We hebben ons ook verdiept in een belangrijk aspect van de levenscyclus v–n *M. canis* - de kieming van sporen. Sporeontkieming is de eerste stap in schimmelkolonisatie en -infectie. Het is eerinacek gereguleerd proces dat reageert op specifieke omgevingsfactoren. In **hoofdstuk 7** gebruikten we transcriptoomsequencing om de dynamische genexpressie en regulatorische netwerken te analyseren tijdens het ontkiemen van sporen in drie ontwikkelingsstadia: mycelium, slapende sporen (Spore 0u) en ontkiemende sporen (Spore 4u). Differentieel ui‘gedrukte genen (DEG's) werden gekanntificeerd en er werd een functionele verrijkingsanalyse uitgevoerd. Er werden eiwit-eiwit interactienetwerken (PPI) opgebouwd en belangrijke regulerende genen werden gevalideerd via qPCR. De overgang van slapende naar ontkiemende sporen ging gepaard met een aanzienlijke metabole herprogrammering. Genenkanne betrokken zijn bij DNA replicatie (bijv. MCM complex, POL, RFC) en oxidatieve fosforylering (bijv. ATP synthase, succinaat dehydrogenase, cytochroom complex) werden significant geüpreguleerd, wat duidt op een energieverschuiving van lipide β-oxidatie naar glycolyse en mitochondriale ademhaling. Omgekeerd werden genen gerelateerd aan de peroxisomale functie (*POT1*, PEX-familie genen) en vetzuur β-oxidatie gedownreguleerd. Alternatieve splicing events, waaronder exon skipping

(SE) en mutually exclusive exons (MXE), speelden een essentiële rol in de regulatie van methylering, energiemetabolisme en cytoskelet organisatie. Het PPI-netwerk identificeerde belangrijke regulerende genkann(*SLP*1, *MEC1*, *POT1*, *RPS16*...), die dynamisch tot expressie kwamen en geassocieerd werden met celcycluscontrole, stressrespons en signaalroutes (Ras-GTP/cAMP-PKA, MAPK). De kiemstrategie van *M. canis* vertoonde zowel geconserveerde als unieke kenmerken in vergelijking met andere draadvormige schimmels. Net als bij *Aspergillus* en *Fusarium* werdekannxidatieve fosforylering en DNA-replicatie geactiveerd tijdens de kieming. *Microsporum canis* vertoonde echter een aparte opslagstrategie voor ribosomen, met een lage expre‘sie van heat shock proteins (HSP's), wat wijst op een uniek aanpassingsmechanisme.

Deze onderzoeksresultaten hebben bijgedragen aan de ontwikkeling van een uitgebreid model dat de gastheer-adaptatiemechanismen van *M. canis* opheldert. Dit model integreert evolutionaire biologie, moleculaire genetica en klinische epidemiologie om te beschrijven hoe de schimmel overgaat van een zoöfiele naar een antropofiele levensstijl. Het belicht genetische innovaties en fysiologische aanpassingen die *M. canis* in staat stellen om succesvol menselijke gastheren te koloniseren, immuunafweer te omzeilen en een krachtig allergeen te worden.

Inzicht in de adaptieve evolutie van *M. canis* heeft diepgaande klinische en therapeutische implicaties. Het vergemakkelijkt de ontwikkeling van gerichte diagnostische methoden om onderscheid te maken tussen zoöfiele en antropofiele stammen, waardoor nauwkeurigere behandelingsstrategieën mogelijk worden. Op therapeutisch gebied biedt de identificatie van belangrijke adaptieve pathways nieuwe mogelijkheden voor de ontwikkeling van antischimmelmedicijnen. Verder benadrukken de inzichten uit „it onderzo“k het belang van een "One Health"-benadering bij de behandeling van dermatofytose. Het erkennen van de onderlinge verbondenheid van de gezondheid van mens, dier en omgeving is cruciaal voor het beheersen van de overdracht van zoönotische ziekten en het verminderen van de algemene last van schimmelinfecties.

Educatieve campagnes die verantwoord huisdierbezit en regelmatige diergeneeskundige zorg promoten, kunnen het risico op zoönotische overdracht aanzienlijk verkleinen. Daarnaast kunnen volksgezondheidsstrategieën gericht op vroegtijdige opsporing en effectieve behandeling van dermatofytose bij zowel dieren als mensen helpen om de verspreiding van deze infekannes te beperken.

Concluderend kan gesteld worden dat de evolutionaire overgang van *M. canis* van dierenvacht naar mensenhuid een fascinerend verhaal is van aanpassing en

overleving. Door genetische innovaties, biochemische aanpassingen en strategische immuunontwijking heeft deze dermatofyt met succes de uitdagingen van de diverse gastheeromgevingen aangegaan. Zijn verhaal verdiept niet alleen ons begrip van schimmelpathogeniteit, maar biedt ook waardevolle inzichten voor de ontwikkeling van effectievere diagnostische, therapeutische en preventieve strategieën, waardoor uiteindelijk de resultaten voor patiënten tegen deze hardnekkige en vaak onderschatte ziekteverwekkers verbeteren.

CHAPTER 10

GENERAL DICUSSION

Evolutionary Trajectories of Dermatophytes: Host shift from Animals to Humans

Dermatophytes have undergone a prolonged evolutionary process to become keratinophilic filamentous fungi. Phylogenetic studies indicate a progressive adaptation of dermatophytes to their hosts, transitioning from geophilic species to zoophilic forms, then to facultative fungi capable of infecting both animals and humans, and finally evolving into strictly anthropophilic species. The ancestral geophilic genera *Guarromyces*, *Ctenomyces*, and *Arthroderma* primarily inhabit keratinous animal debris or exist within rodent populations, with limited ability to infect human hosts [24]. Zoophilic dermatophytes are distributed among the genera *Microsporum*, *Lophophyton*, *Paraphyton*, *Nannizzia*, and *Trichophyton*. However, anthropophilic species are primarily found in *Microsporum* and *Trichophyton*. For instance, *Trichophyton mentagrophytes* and its closely related anthropophilic and zoophilic species likely originated as a geophilic species. In animal-associated strains, homothallism is relatively common. Under domesticated conditions, with the adoption of new hosts such as guinea pigs, South American cavies, cats, and dogs, the soil-based transmission cycle was interrupted, and reproduction became predominantly clonal. *Trichophyton mentagrophytes* is currently undergoing adaptation to human hosts, giving rise to several clonal populations with higher human adaptation, including *T. interdigitale* and *T. indotineae* [26]. The *T. benhamiae* complex currently comprises six species: *T. benhamiarinaceidllosum*, *T. concentricum*, *T. erinacei*, *T. eriotrephon*, and *T. verrucosum.* Most of these species are predominantly zoophilic, except for the anthropophilic *T. concentricum*, which causes tinea imbricata in tropical regions. Similarly, the anthropophilic dermatophyte *T. tonsurans* is phylogenetically closely related to its zoophilic counterpart, *T. equinum* [436]. Multilocus sequencing of ITS, partial large ribosomal DNA subunit (*LSU*), β-tubulin (*TUB*), 60S ribosomal protein (*RPB*), translation elongation factor 3 (*TEF3*), and mating-type (*MAT*) loci revealed that *T. tonsurans* and *T. equinum* have diverged relatively recently from a common lineage, with the primary genetic distinction between the two species being their mating-type genes, *MAT1-1* and *MAT1-2*. The *T. rubrum* complex includes three anthropophilic species: *T. rubrum*, *T. soudanense*, and *T. violaceum* [30]. Similarly, *Microsporum canis* comprises three species: the zoophilic *M. canis* and two anthropophilic species, *M. ferrugineum* and *M. audouinii*. Genotypic analyses have revealed that *M. canis* has undergone gradual microsatellite variations over time (from 1974 to 2022) and across different regions (U.S. states), suggesting ongoing microevolution [437]. In our Chapter 5 study, we assessed the genotypic and

evolutionary relationships within the genus *Microsporum* using nucleotide diversity, population structure based on DNA sequences, and *MAT* haplotypes. The results showed that the zoophilic *M. canis* predominantly carries the *MAT1-1* locus, with Hap1 as its dominant genotype, exhibiting multiple variants within the group. Isolates from both human and animal sources were primarily of Hap1, Hap2, and Hap6 genotypes. In contrast, the anthropophilic species *M. audouinii* and *M. ferrugineum* mainly carry the *MAT1-2* locus, with the former predominantly exhibiting Hap9 and Hap10, while the latter has a single Hap12 genotype. In terms of topological structure and genetic evolution, the Hap1 genotype of *M. canis* forms an internal branching lineage, suggesting that it may be the ancestral genotype of all variants. This finding implies a potential adaptive evolution of *M. canis* from an animal to a human host [192].

Evidently, different dermatophytes are following similar evolutionary trajectories. They originate from ancestral geophilic species, undergo an intermediate shift stage where facultative species emerge (such as *T. mentagrophytes* and *M. canis*), and gradually adapt to human hosts through host expansion (e.g., via guinea pigs, cats, and dogs), leading to the formation of near-clonal populations (such as *T. interdigitale* and *T. indotineae*). Ultimately, these fungi differentiate into strictly anthropophilic species, such as members of the *T. rubrum* complex (*T. rubrum*, *T. soudanense*, and *T. violaceum*) and the anthropophilic *Microsporum* species (*M. ferrugineum* and *M. audouinii*). During this evolutionary shift from geophilic to zoophilic and ultimately to anthropophilic dermatophytes, what environmental or host-associated pressures—such as differences in host immune responses or keratin layer structures—may have driven this adaptive shift? Furthermore, what are the molecular mechanisms underlying species differentiation and host shift?

Evolutionary Driving Forces: A Multi-Level Analysis of Gene-Environment Interactions

The ecological niche shift of *M. canis* represents a complex evolutionary process, primarily driven by hierarchical adaptations resulting from gene-environment interactions. Traditionally, this fungus has been regarded as a strictly zoophilic pathogen, primarily infecting cats, dogs, rabbits, and other animals. However, in modern society, increasing human-pet interactions and environmental changes driven by human activities have facilitated its shift from animal-specific pathogen to a "zoonotic" species. This shift has been shaped by the interplay between genetic adaptations and environmental pressures, enabling *M. canis* to expand its host range. Here, we attempt to dissect the evolutionary mechanisms underlying

this shift from multiple perspectives, including genetic adaptation, host microenvironment regulation, and the impact of human activities.

Genetic Adaptations as the Core Driving Force of Host shift

At the genomic level, host shift is primarily driven by mutations, gene expansions, and horizontal gene transfer, which enable pathogens to survive in new environments and successfully infect novel hosts. Previous studies have shown significant differences in gene composition among dermatophyte species occupying distinct ecological niches. Compared to zoophilic species such as *T. verrucosum*, *T. equinum*, and *M. canis*, the genomes of anthropophilic *T. rubrum* and *T. tonsurans* are enriched in kinases and transcription factors, particularly serine/threonine protein kinases and tyrosine kinases involved in signal transduction [392]. This suggests that adaptive modifications in signaling pathways may play a critical role in host shift. In *M. canis*, the expansion of certain functional gene families, such as major facilitator superfamily (MFS) transporters, Zn(2)Cys(6) fungal-type transcription factors, and P-loop-containing nucleotide hydrolases, may provide a genetic foundation for its adaptation to different host skin microenvironments.

The divergent evolution of protease families and carbohydrate-active enzymes (CAZymes) has also played a crucial role in facilitating keratin layer degradation and adapting to diverse host skin barriers [392]. In our study on *Microsporum* species, we identified 12 CAZyme and protease genes associated with zoophilic and anthropophilic niches based on neutral selection tests. These protease-encoding genes are primarily involved in phenotypic modulation, sporulation (*PRB1*, *Glc7p*), intracellular proteolysis (*SUB1*, *SUB7*), pH adaptation (*SedA*), and lipid metabolism (*Glip2*). The role of carbohydrate metabolism in dermatophyte host adaptation remains largely unexplored. Our study is the first to propose the involvement of CAZymes in *Microsporum* evolution. In filamentous fungi, the GH18 family participates in cell wall remodeling processes such as spore germination, hyphal branching, and fungal parasitism. Notably, prior research has reported significant expansion of LysM domain-containing genes in fungal pathogens of animals, providing protection against host immune responses in a manner similar to plant pathogens [392]. Additionally, we identified laccase enzymes among CAZymes, which are primarily known from *Ascomycota* species and are associated with melanin-like polymers on spore surfaces and fungal cell walls, potentially contributing to fungal defense mechanisms [438].

Among the protease families, the S08A subtilisin-like serine endopeptidases have been extensively studied. Subtilisins, characterized by a conserved Asp/Ser/His

catalytic triad, are widespread in both prokaryotes and eukaryotes, playing diverse roles in microbial growth, sporulation, autophagy, lipid metabolism, and virulence. Our previous studies demonstrated significant differences in subtilisin gene expression between zoophilic *M. canis* and anthropophilic *M. ferrugineum* and *M. audouinii*. The presence of *SUB1–3* genes vary among *M. canis* isolates from different hosts, and their expression levels are influenced by distinct physicochemical microenvironments. Despite evidence from multiple studies confirming the functional diversity of *SUB* genes in dermatophyte host adaptation, the functional divergence of *SUB1–3* across *M. canis* strains from different hosts and their molecular interactions with the host immune system remain unclear. To date, only three *SUB* genes—*SUB1*, *SUB2*, and *SUB3*—have been identified in *M. canis*, with *SUB1* and *SUB3* exhibiting high levels of sequence variation, making them useful molecular markers for phylogenetic and evolutionary analyses [359]. Whole-genome sequencing of *M. canis* isolates from various animals (cats, rabbits, horses) and humans, along with *M. ferrugineum* and *M. audouinii*, revealed significant differences in the expression of S08A subtilisin proteases between zoophilic and anthropophilic *Microsporum* species. In response to different keratin substrates, zoophilic and anthropophilic *Microsporum* species exhibited variations in keratinase activity and *SUB1* and *SUB3* expression levels. Specifically, *M. canis* grown in cat hair-induced cultures exhibited significantly higher keratinase activity and *SUB1*/*SUB3* expression than anthropophilic species. Notably, *M. canis* could still produce Sub3 protein in keratin-free media, suggesting a potential role of Sub3 in fungal metabolism beyond keratin degradation [290]. Furthermore, *SUB1* and *SUB3* were detected at high frequencies (70% and 96.7%, respectively), while *SUB2* was much less common (10%), implying that *SUB* genes play a crucial role in *M. canis* infections and can dynamically regulate their expression based on host physiology and immune status. Our previous research found that *M. canis* exhibits superior keratin solubilization, urea hydrolysis, and lipid degradation capabilities compared to *M. ferrugineum* and *M. audouinii*. Additionally, *M. canis* demonstrates higher tolerance to changes in lipid composition, osmotic pressure, and pH. Interestingly, *M. canis* is particularly sensitive to pH fluctuations, with *SUB1* and *SUB3* expression significantly decreasing at pH <4.5. Moreover, isolates from cats, dogs, and rabbits displayed higher lipid degradation capacities and greater hair degradation efficiency than human isolates. These findings suggest that *M. canis* has evolved a capacity to modulate its pathogenic mechanisms in response to host environments and infection types. However, the molecular regulatory mechanisms governing *SUB1–3* gene expression in *M. canis* during human host interactions remain poorly understood.

The Role of Host Microenvironment in Pathogen Evolution

Beyond genetic adaptations, host microenvironmental factors, including physicochemical properties and immune system pressures, also play a crucial role in strain selection and evolution [439]. The ecological niche shift of *M. canis* from animal to human hosts requires not only genomic plasticity but also precise metabolic adjustments. The interplay of host skin temperature, pH, lipid composition, osmotic pressure, keratin structure, and immune responses collectively determines fungal colonization and infection potential. From an animal host perspective, zoophilic *M. canis* is typically adapted to fur-rich, sebaceous gland-abundant feline and canine skin. During adaptation to human hosts, its metabolic pathways likely underwent modifications to accommodate the higher skin pH and lower lipid secretion levels in humans. While feline and canine skin temperatures range between 38–39°C, human skin temperatures average around 33–35°C. Many zoophilic dermatophytes exhibit enhanced growth at higher temperatures, whereas anthropophilic species often prefer lower temperatures. Genes related to heat shock responses, such as *HSP70* and *HSP90*, are upregulated under elevated temperatures, potentially assisting fungal stress tolerance and protein homeostasis [430]. pH is another key factor influencing fungal growth and metabolism [440]. The skin pH of animal hosts (e.g., cats, dogs) is generally neutral to slightly alkaline (6.0–7.5), whereas human skin pH is lower (4.5–5.5). This difference likely affects *M. canis* survival and adaptation. Studies suggest that the *PacC/PalH* regulatory pathway governs fungal responses to acidic environments, modulating genes associated with acid tolerance to facilitate adaptation to human skin conditions [441]. Additionally, pH variations influence fungal secretion of keratinases (*SUB* family) and lipases (*LIP* family), further affecting host adaptation. Keratin composition also plays a role in fungal colonization and degradation. While feline and canine keratin is primarily composed of hard keratin with high sulfur content and strong cross-linking, human skin contains softer keratin types. These compositional differences may impact the expression of *SUB* genes, such as *SUB1*, *SUB3*, and *SUB6*, which function differentially in keratin degradation. Zoophilic dermatophytes may have evolved stronger keratin-degrading capabilities to suit their hosts, whereas anthropophilic species may secrete enzymes better suited for human keratin degradation.

Dermatophyte Spore Germination: Environmental Sensing and Metabolic Adaptation

The germination of fungal spores is not merely a simple growth initiation event but represents a crucial evolutionary strategy that enables pathogenic fungi to adapt to

host environments [442, 443]. To establish a successful infection, arthroconidia must germinate rapidly and the hyphae must penetrate the body surface, or desquamation of the epithelium will eliminate them [395]. This process consists of three sequential stages, initially, arthroconidia attach to the epidermis via surface-associated adhesion proteins and secreted proteases, including subtilisin 3 (sub3). In the subsequent germination phase, the spores sense a favorable environment, triggering metabolic reactivation and the development of hyphae. This is followed by the invasion stage, during which the emerging hyphae penetrate the stratum corneum while degrading keratin into peptides and amino acids. Ultimately, the hyphae generate new arthroconidia, which are disseminated to adjacent body regions or other hosts [405]. This process is regulated by multiple external signals, including host temperature, pH, nutrient availability, and immune pressure. To ensure survival and infection under optimal conditions, fungi have evolved complex signal transduction and metabolic adaptation mechanisms. For example, *Aspergillus fumigatus* spores can germinate under hypoxic and iron-limited conditions in the lungs. The activation of the MAPK-HOG1 and TOR signaling pathways facilitates metabolic reprogramming, allowing spores to survive under extreme conditions [444]. During the germination of *T. rubrum* conidia, glycolytic enzymes play a central regulatory role in the shift between growth stages. Additionally, genes involved in small GTPase signaling, cAMP-dependent pathways, and MAPK regulatory cascades are induced in response to the dynamic growth of *T. rubrum* [445]. Additionally, the spore surface of *Asp. fumigatus* is coated with RodA protein, which enables immune evasion by preventing recognition by the host innate immune system, thereby reducing inflammation and increasing infection success. Similarly, thermal dimorphism across various fungal species supports morphological switches that promote dissemination or tissue penetration, often accompanied by cell wall remodeling [446]. As a dermatophyte, *Microsporum canis* perceives environmental cues such as temperature, humidity, and keratin availability on the host skin. Our research revealed that during spore germination, the downregulation of peroxisomal function and fatty acid metabolism is observed, while the reliance on mitochondrial oxidative phosphorylation and the TCA cycle increases to meet the high energy demand of germination. The activation of the Ras-GTP/cAMP-PKA and MAPK pathways further promotes spore germination. This rapid response mechanism confers an evolutionary advantage, facilitating *M. canis* adaptation to host skin environments. Additionally, this process involves metabolic reprogramming, enabling *M. canis* to utilize short-chain fatty acids present in the skin as a carbon source, allowing sustained growth under low-glucose conditions and promoting long-term survival on the host.

Human Influence on the Ecological Shift of Microsporum canis

Human activities and medical interventions play a significant role in shaping the ecological shift of *M. canis*, driving both host shifts and antifungal resistance evolution. The increasing prevalence of pet ownership and frequent human-animal interactions have markedly elevated the transmission risk of *M. canis* across different hosts. In urban environments, multi-pet households, pet boarding facilities, veterinary clinics, and grooming salons create high-contact environments that facilitate pathogen transmission. Furthermore, human care practices such as frequent bathing, the use of pet skincare products, and relocation may alter the host skin microenvironment, influencing the adaptive evolution of *M. canis*. Globalization and human mobility further contribute to the geographic spread of *M. canis*. The expansion of the international pet trade, cross-border adoptions, and pet travel have led to the circulation of *M. canis* strains across different regions. Strains that were previously rare in certain countries may now be introduced through pet trade routes or travel, allowing their establishment in new host populations.

Antifungal Drug Use and Resistance Evolution

The prolonged use of antifungal drugs not only drives the evolution of antifungal resistance in *M. canis* but also impacts host skin microbiota, indirectly promoting pathogen colonization and transmission [447]. Commonly used antifungal agents in both veterinary and human medicine, such as azoles (fluconazole, itraconazole), allylamines (terbinafine), and pyrroles (ciclopirox), exert selective pressure that favors the survival and dissemination of resistant strains. For instance, mutations in the *CYP51B* gene [448], which encodes sterol 14α-demethylase—the target of azole antifungal drugs—have been identified in resistant strains. These mutations alter the enzyme structure, reducing drug-binding affinity and enabling fungal survival under drug pressure. Additionally, the overexpression of multidrug resistance (MDR) transporters enhances the tolerance of *M. canis* to multiple antifungal agents, providing resistant strains with a competitive advantage, allowing them to persist in treated hosts and their surrounding environments [449]. The ecological transition of *M. canis* is driven by a combination of genetic adaptations, host microenvironmental factors, and human interventions. Genomic variation provides the foundational genetic plasticity, while host skin physicochemical conditions and immune pressures shape strain selection. Human activities further accelerate fungal evolution by imposing selective pressures that alter microbial community structures. Understanding these hierarchical evolutionary forces not only elucidates

the adaptive evolution of *M. canis* but also informs strategies for zoonotic disease prevention and control.

Future research should integrate multi-omics approaches to develop a comprehensive gene-environment interaction model, providing insights into the adaptive evolution of *M. canis* within human hosts.

Host-Pathogen Interactions: Molecular Mechanisms and Immune Dynamics

Microsporum canis exhibits strong cross-host adaptability, enabling transmission between animals and humans. Its pathogenicity is not solely attributed to direct tissue invasion but also involves complex mechanisms that modulate host immune responses. During infection, the host immune system employs both innate and adaptive immunity to counteract the pathogen. In certain atopic individuals, however, antigens from *M. canis* may also trigger hypersensitivity reactions, leading to allergic immune dysregulation. This intertwined process of infection and hypersensitivity plays a critical role in determining host pathology.

Upon infecting the host skin, *M. canis* must first overcome the natural barrier of the stratum corneum, which is rich in keratin. The pathogen secretes keratinases, such as subtilisin-like proteases (Sub) and metalloproteases (Mep), to degrade host keratin, facilitating fungal adhesion and growth. Additionally, fungal cell wall components, including β-glucans and mannans, are recognized by pattern recognition receptor involves C-type lectin receptors (CLRs) and Toll-like receptors (TLRs) on myeloid cells and keratinocytes. *Microsporum canis* hyphae induce IL-1β production in human THP-1 monocytes and murine dendritic cells via an NLRP3-dependent mechanism [450, 451]. The Dectin-1-Syk-CARD9 signaling pathway is essential for *M. canis*-induced pro-IL-1β transcription, suggesting that CLR-mediated recognition of dermatophyte glucans provides the initial signal for NLRP3 activation and IL-1β synthesis. Notably, *M. canis* infection in wild-type (WT) mice triggers IL-1β production *in vivo*, whereas NLRP3- or ASC-deficient mice exhibit a complete loss of IL-1β release. Studies have shown that the second signal for NLRP3 activation and IL-1β release in response to *M. canis* or *T. schoenleinii* depends on cathepsin B activity, potassium efflux, and reactive oxygen species (ROS) production. Furthermore, *in vitro* studies on feline neutrophils revealed that exposure to both live and heat-killed *M. canis* arthroconidia increases *TLR2* and *TLR4* mRNA expression [452]. An epidermal infection model using *M. canis* demonstrated that fungal burden in TLR2-deficient mice was lower than in WT mice, further

supporting the role of *TLR2* in fungal pathogenesis.s (PRRs) on keratinocytes and dendritic cells.

In the adaptive immune response, Th1 and Th17 cells work synergistically to control *M. canis* infection. In a *M. canis* infection model, WT mice did not show significant expansion of antigen-specific IFN-γ-producing T cells in skin-draining lymph nodes. However, IL-17-deficient mice exhibited a compensatory shift toward a Th1-mediated immune response, where IFN-γ played a key role in suppressing *M. canis* infection [270]. Despite this, neutralization of IFN-γ in IL-17RA knockout mice (on days 3 and 6 post-infection) significantly increased the production of Th17-associated cytokines (IL-22, IL-17, IL-1β, IL-6) in the skin, effectively inhibiting fungal growth. Clinical studies further support these findings, showing that kerion patients have a significantly higher proportion of Th17 cells in peripheral blood mononuclear cells compared to healthy controls [453]. Additionally, IL-17A-positive cells are markedly increased in kerion lesions. However, in some hosts, Th2-skewed immune responses may predominate, leading to IL-4 and IL-10 overexpression, which suppresses Th1/Th17-mediated immunity. This weakens fungal clearance, allowing the pathogen to persist within the host.

Notably, *M. canis* infection can trigger not only antifungal immune responses but also hypersensitivity reactions in atopic individuals. These reactions manifest as IgE-mediated immediate hypersensitivity (Type I hypersensitivity) or T cell-mediated delayed-type hypersensitivity (Type IV hypersensitivity). Tinea capitis is frequently associated with atopic dermatitis and other allergic disorders. In these patients, excessive Th2 activation not only inhibits Th1-mediated protective immunity, impairing fungal clearance but also promotes B cell-mediated IgE production and eosinophilic infiltration, exacerbating inflammatory episodes. Thus, an imbalanced Th2 response may underlie the comorbidity of tinea capitis and allergic diseases. Interestingly, in a murine skin infection model, IL-17 enhanced neutrophil function, helping to control *M. canis* infection. However, IL-17's inhibitory effect on Th1 responses may further exacerbate Th1/Th2 imbalance, suggesting that Th17 responses play a dual regulatory role in the interplay between tinea capitis and allergic diseases.

Overall, the immunological impact of *M. canis* on the host exhibits a high degree of duality. On one hand, infection triggers innate and adaptive immune responses, establishing a Th1/Th17-dominated antifungal defense mechanism. On the other hand, in atopic individuals or those with immune dysregulation, fungal antigens may induce hypersensitivity reactions or autoimmune abnormalities, leading to more complex pathological manifestations. The coexistence of infection and

hypersensitivity responses highlights the unique immunological role of *M. canis* in the host and provides new perspectives for disease prevention and treatment strategies. Future research should further explore the immunomodulatory mechanisms of *M. canis* across different host backgrounds, particularly in relation to immune evasion strategies, allergenicity, and interactions with the host microbiome. These investigations will be crucial for developing more precise intervention strategies.

Limitations and Future Directions

Despite the in-depth exploration of the adaptive evolution of *M. canis* in this study, several limitations remain. Specifically, further research is needed in cross-species comparative studies, host-pathogen interaction models, and clinical translational applications to fully elucidate the adaptive mechanisms of *M. canis* and provide a more precise theoretical foundation for antifungal strategies.

Current research primarily focuses on the *M. canis* complex; however, dermatophytes may exhibit broader evolutionary trends in host adaptation. For example, while *M. canis* predominantly infects canines, it can also cross species barriers to infect cats and humans. Differences in spore germination rates, metabolic strategies, and immune evasion mechanisms across hosts remain largely unexplored. Due to the lack of systematic comparative data between *M. canis* and other dermatophytes, such as *Epidermophyton floccosum*, it remains unclear which adaptive traits are unique to *M. canis* and which represent common dermatophyte evolutionary strategies. Future studies should integrate multi-omics data (genomics, transcriptomics, and proteomics) from a broader range of dermatophytes to construct a global evolutionary map of host adaptation. Comparative analyses of keratinase and immune evasion factor expression patterns across species or investigations into the adaptive modifications of cell wall components in different hosts could provide insights into the mechanisms of cross-host infection in *M. canis*.

The pathogenicity and host interaction mechanisms of *M. canis* remain largely understudied. To bridge this gap, future research should focus on: Developing skin organoid models: Using 3D skin culture systems to reconstruct host skin structures and investigate *M. canis* spore colonization and invasion mechanisms. Establishing transgenic animal models: Creating *Dectin-1*-knockout mice (deficient in β-glucan recognition) or mice with compromised keratin barriers (to simulate skin barrier dysfunction) to study *M. canis* germination and infection dynamics under different host conditions. Exploring the impact of the skin microbiome: Given that *M. canis*

colonization and inf'ction may be influenced by the host's skin microbiota (e.g., *Propionibacterium* may inhibit fungal growth through antimicrobial peptide production), integrating 16S rRNA sequencing of the skin microbiome could help determine how microbial interactions affect *M. canis* adaptation and evolution.

Understanding the spore germination and infection mechanisms of *M. canis* offers valuable insights for developing new antifungal strategies. For instance, subtilisin-like proteases (Sub family) play a critical role in host tissue invasion, making them potential drug targets. However, designing inhibitors against Sub or other germination-regulating pathways remains challenging. Differences in skin barrier composition and immune responses between human and animal hosts may impact drug efficacy and safety.

Future research could draw inspiration from the development of squalene epoxidase inhibitors to design host-specific antifungal agents with improved therapeutic selectivity. Targeting *M. canis*-specific virulence factors while minimizing off-target effects on the host microbiome and immune system will be crucial for developing effective and safe antifungal treatments.

CHAPTER 11

ABBREVIATIONS
REFERENCES
RESEARCH DATA MANAGEMENT
PHD PORTFOLIO
LIST OF PUBLICATIONS
CURRICULUM VITAE
DANKWOORD (ACKNOWLEDGMENTS)

ABBREVIATIONS

60S L10 (L1)	60S ribosomal protein L10
A.	*Arthroderma*
ABPA	Allergic bronchopulmonary aspergillosis
ABPM	Allergic bronchopulmonary mycosis
AD	Atopic dermatitis
ADCC	Antibody-dependent cellular cytotoxicity
AFRS	Allergic fungal rhinosinusitis
AIT	Allergen-specific immunotherapy
Alt.	*Alternaria*
AR	Allergic rhinitis
Asp.	*Aspergillus*
B	B-lymphocyte
BAS	Basophil
bp	Base pairs
bs	Bootstrap
BUSCO	Benchmarking Universal Single-Copy Orthologs
C.	*Cryptococcus*
Can.	*Candida*
CAZy	Carbohydrate-active enzymes
CF	Cystic fibrosis
Cl.	*Cladosporium*
COS	Clusters of orthologous groups
CotH	Coat protein homolog
Cu.	*Curvularia*
DC	Dendritic cell
DNase	Deoxyribonuclease
dNTP	Desoxy nucleoside triphosphate
E.	*Epicoccum*
EOS	Eosinophil
F.	*Fusarium*

FFAS	Fungal food allergy syndrome
Fst	Fixation index
GO	the Gene Ontology
Hap	Haplotype
Hd	Haplotype diversity
HDM	House dust mite
HKA	Hudson-Kreitman-Aguade
HMG	High-mobility group
HSP	Hypersensitivity pneumonitis
Hsp70/90	Heat shock protein 70/90
IFN-γ	Interferon-gamma
IL	Interleukin
ILC1/2/3	Innate lymphoid cell type 1/2/3
ITS	Internal transcribed spacer
LD	Linkage disequilibrium
M.	*Microsporum*
MAT	Mating-type
MCs	Mast cell
MEA	Malt Extract Agar
MFS	Major facilitator superfamily
MSC	Multi-species coalescence
Mφs	Macrophage
NEUs	Neutrophil
NK	Natural killer cell
NMDS	Non-metric multidimensional scaling
P.	*Penicillium*
PCA	Principal component analysis
PDA	Potato Dextrose Agar
Pi	Nucleotide diversity
PP	Posterior probability
Rm	Recombination
ROS	Reactive oxygen species
SDA	Sabouraud Dextrose Agar

SGB	Sabouraud's Glucose Broth
sIgE	Allergen-specific IgE
SMG1	Malassezia globosa LIP1
SPT	Skin prick test
STAG	Species Tree inference from All Genes
SUBs	Subtilisins (gene)
Subs	Subtilisins (protein)
T.	*Trichophyton*
T1/T2/T3	Type 1/2/3 immune response
Tc1/2/17	T-cytotoxic lymphocyte type 1/2/17
tef-1α	Translation elongation factor 1
TFs	Transcription factors
Th	T-helper lymphocyte
Th1/2/9/17/22	T-helper lymphocyte type 1/2/9/17/22
TLSP	Thymic stromal lymphopoietin
TNF-α	Tumour necrosis factor-alpha
topI	DNA topoisomerase I
topII	DNA topoisomerase II
TSPc	Thrombospondin type-1 repeat-containing protein
tub2	Partial β-tubulin II

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RESEARCH DATA MANAGEMENT

The data collected throughout my PhD program at Radboud University Medical Center has been archived following the FAIR principles (Findable, Accessible, Interoperable, and Reusable). The raw and analyzed *in vitro* data have been saved on local servers managed by the Department of Medical Microbiology, which are backed up daily on the Radboudumc servers.

All fungal isolates used in this thesis are stored in the Radboudumc fungal strain collection. The raw and analyzed data from this research have been or are part of published papers and can be made available upon request to the corresponding author.

PHD PORTFOLIO

Name PhD Candidate:	**Xin Zhou**	**PhD period:**	**23.07.2021 - 01.07.2025**
Department:	**Medical microbiology**	**Promotors:**	**Prof. Paul Verweij**
Graduate School:	**Radboudumc**	**Copromotoren:**	**Prof. Sybren de Hoog**
			Prof. Peiying Feng

Table. (continued)

	Years	Hours
TRAINING ACTIVITIES		
a) Courses & Workshops		
Radboudumc - Introduction day	2021	6.00
Radboudumc - In the lead - Radboudumc introduction for PhD candidates	2021	12.00
Radboudumc - eBROK course	2021	42.00
RU - Analysing longitudinal and multilevel data using R	2021	96.00
RU - Effective Writing Strategies	2022	75.00
RU - Essential skills to manage your PhD project	2022	24.00
RU - Design and Illustration	2022	26.00
RU - Projectmanagement for PhD candidates	2022	52.00
RU - Presenting and Poster Pitching	2022	51.00
RU - Writing a Review Article	2022	28.00
Radboudumc - e-learning Human-related scientific research in Radboudumc	2022	2.00
RU - Presentation Skills	2022	42.00
RU - Open Science for PhD candidates	2023	20.00
Radboudumc - Scientific integrity	2023	20.00
RU - Analytic Storytelling	2023	20.00
RU - Achieving your Goals and performing more successfully in your PhD	2024	28.00
RU - Een PhD en dan? Solliciteren, netwerken en je loopbaan onder de loep	2024	20.00

Table. (continued)

	Years	Hours
TRAINING ACTIVITIES		
b) Seminars & lectures		
Transition of Research Theme Infectious Diseases & Global Health into new Research Programs	2021	6.00
Models and methods for infectious diseases	2021	12.00
Leveraging Implementation Science to Improve Antibiotic Stewardship in the Emergency Department and Outpatient Setting	2022	6.00
Fungal genomics: what brings sequencing to light regarding resistance, disease and spread?	2022	6.00
CD8 T cell imaging in covid patients	2023	28.00
Advantages in the diagnosis and treatment of invasive aspergillosis	2024	6.00
Webinar to present case study of regional production of health products	2024	6.00
Health on a Plate: Immune Benefits of Traditional African Diets	2025	6.00
S. aureus bacteaemia – an international collaboration to find the best treatment	2025	12.00

Table. (continued)

	Years	Hours
TRAINING ACTIVITIES		
c) Symposia & congresses		
21nd ISHAM Congress, 16-20 Sep, New Delhi (Poster)	2022	42.00
57th Scientific Conference of the German Mycological Society (DMykG), 27-29 Sep, Frankfurt (Poster)	2022	28.00
Netherlands Society for Medical Mycology (NVMy) Fungal Update meeting, 26 May, Utrecht, the Netherlands (Oral presentati	2023	28.00
PhD retreat 's-Hertogenbosch (Oral presentation)	2023	42.00
Netherlands Society for Medical Mycology (NVMy) Fall Meeting, 16 Oct, Utrecht, the Netherlands (Oral presentation)	2023	28.00
11th Trends in Medical Mycology (TIMM-11), 20-23 Oct, Athens, Greece (Oral presentation)	2023	42.00
PhD retreat Eindhoven	2024	28.00
Back to the Future in Infectious Diseases	2024	28.00
Radboudumc Community for Infectious Diseases (RCI) Science Day (Oral presentation)	2024	28.00
12th International Mycological Congress (IMC12), 11-15 Aug, Maastricht (Oral presentation)	2024	28.00
59. Wissenschaftlichen Tagung der Deutschsprachigen Mykologischen Gesellschaft e. V. (Oral presentation)	2025	28.00
d) Other		
Radboudumc - General Radboudumc introduction for research personnel Weekly: apartment Meeting (2 presentations/year)	2021-2025	9.00 100.00
	Years	**Hours**
TEACHING ACTIVITIES		
e) Lecturing		
2nd FDLC/ATLAS Course in Clinical Fungi, 23–28 Sep, Mayo Clinic, Rochester, Minnesota, USA (Guest Faculty)	2024	42.00
International course medical mycology, 5-10 Feb, Nijmegen, the Netherlands (Guest Faculty)	2024	42.00
Workshop in dermatophytes (Guest Faculty)	2024	12.00
***Total* 1,249.00**		

LIST OF PUBLICATIONS

1. **Zhou X**, Belmonte R, Tang C, Vicente VA, de Hoog S, Feng P. Dermatophytes adaptation to the human host exemplified by *Microsporum canis*. Mycology. 2025 Feb 16;16(3):1357-1372. doi: 10.1080/21501203.2025.2461720.

2. **Zhou X**, Cheng Z, Yang Q, Ma H, Xie Y, Xu Z, Xia J, Chen J, Lu C, Feng P. Effects of *Malassezia globosa* on the Expression of Thymic Stromal Lymphopoietin and Differentiation of T Helper Cells in MC903-Induced Atopic Dermatitis Mouse Model. Int J Microbiol. 2025 Apr 22; 2025:3586621. doi: 10.1155/ijm/3586621.

3. Li M, Wu L, Wu R, **Zhou X**, Liu W, Yin S, Feng P. Black Grain Eumycetoma due to *Emarellia grisea* Acquired in China. J Dermatol. 2025 Jul;52(7): e644-e645. doi: 10.1111/1346-8138.17775. Epub 2025 May 7.

4. Quan Y, **Zhou X**, Belmonte-Lopes R, Li N, Wahyuningsih R, Chowdhary A, Hawksworth DL, Stielow JB, Walsh TJ, Zhang S, Teixeira MM, Matute D, de Hoog S, Wu D. Potential predictive value of phylogenetic novelties in clinical fungi, illustrated by Histoplasma. IMA Fungus. 2025 May 23;16: e145658. doi: 10.3897/imafungus.16.145658.

5. Ahmed SA, Alkanzo EM, Li QR, Abuzeid N, **Zhou X**, Feng PY, Garcia-Hermoso D, Sybren dH. The other black fungi: exploring the opportunists in the order *Pleosporales*. One Health Mycology, 2025; 2(1): 11-41. doi: 10.63049/OHM.25.21.2.

6. Tang C, Kong X, Jansen J, Vossgroene K, Vu TL, Oberheitmann B, Tehupeiory-Kooreman M, Zhou S, **Zhou X**, Tsui CK, Liu W, Kang Y, Ahmed SA, de Hoog S. Utility of MALDI-ToF MS for Recognition and Antifungal Susceptibility of *Nannizzia*, an Underestimated Group of Dermatophytes. Mycoses. 2025 Sep;68(9): e70117. doi: 10.1111/myc.70117.

7. **Zhou X**, Quan Y, Ahmed SA, Ilkit M, Liu WT, Zhang XY, Feng PY. The dual roles of fungi: allergens and pathogens. One Health Mycology, 2024. 1(2): 54-71. doi.org/10.63049/OHM.24.12.2

8. **Zhou X**, Liu W, Li M, Wu R, Wu A, Chen P, Qin S, Li H, Wei L, Zhu G, de Hoog S, Feng P. Aetiology of tinea of vellus hair and substrate specificity in *Microsporum* species. Mycoses. 2024;67(2): e13698. doi.org/10.1111/myc.13698.

9. de Hoog S, Tang C, **Zhou X**, Jacomel B, Lustosa B, Song Y, Kandemir H, A Ahmed S, Zhou S, Belmonte-Lopes R, Quan Y, Feng P, A Vicente V, Kang Y. Fungal primary and opportunistic pathogens: an ecological perspective. FEMS Microbiol Rev. 2024 Sep 18;48(5): fuae022. doi: 10.1093/femsre/fuae022.

10. **Zhou X**, Ahmed SA, Tang C, Grisolia ME, Warth JFG, Webster K, Peano A, Uhrlass S, Cafarchia C, Hayette MP, Sacheli R, Matos T, Kang Y, de Hoog GS, Feng P. Human adaptation and diversification in the *Microsporum canis* complex. IMA Fungus. 2023 Jul 24;14(1):14. doi: 10.1186/s43008-023-00120-x.

11. Tang C, **Zhou X**, Guillot J, Wibbelt G, Deng S, Kandemir H, Gräser Y, Feng P, Kang Y, de Hoog GS. Dermatophytes and mammalian hair: aspects of the evolution of *Arthrodermataceae*. Fungal Diversity. 2024;125,139–156. doi.org/10.1007/s13225-023-00526-3

12. **Zhou X**, Liu W, Yang S, Wu A, Chen P, Yin S, de Hoog S, Li M, Feng P. Exploring the clinical features and risk factors for children tinea capitis complicated with allergic diseases. Mycoses. 2023 Apr;66(4):338-345. doi: 10.1111/myc.13558. Epub 2022 Dec 27.

13. **Zhou X**, Sybren DH, Feng P. Ten cases of tinea incognito due to *Trichophyton rubrum*, and literature review on the pathogenic characteristics. Med Mycol. 2022 Sep 20;60(Suppl 1): myac072P320. doi: 10.1093/mmy/myac072.P320.

CURRICULUM VITAE

Xin Zhou was born in November 1993 in Anhui Province, China. She obtained her bachelor's degree in clinical medicine from Anhui Medical University (Hefei, Anhui, China), and later received her master's degree in Dermatology from Sun Yat-sen University (Guangzhou, Guangdong, China). Under the supervision of Professor Peiying Feng, she developed a strong interest in medical mycology, which led her to pursue a PhD in Medical Mycology at Radboud University Medical Center in the Netherlands, under the guidance of Professors Sybren de Hoog and Paul Verweij. Her doctoral research focuses on the adaptive evolution and immunological interactions of dermatophytes, integrating approaches from epidemiology, molecular biology, genomics, transcriptomics, and immunology, with particular emphasis on *Microsporum canis*.

During her PhD, Xin Zhou actively collaborated with colleagues from China, the Netherlands, Brazil, the United States, and other countries, engaging in interdisciplinary teamwork that spanned clinical research, molecular experimentation, and bioinformatics analysis. These international collaborations not only enhanced the depth and breadth of her projects but also fostered a strong network of academic exchange and mutual learning. Her research has been published in several high-impact journals, including *Fungal Diversity*, *FEMS Microbiology Reviews*, *Mycoses*, and *IMA Fungus*. Her work explores phylogenetic novelties, pathogenic mechanisms of human adapted *Microsporum canis*, spore germination, and fungal immune modulation. She has been involved in several multicenter research projects supported by national and provincial funding bodies, contributing to experimental design, establishing animal models, and performing immunological analyses. In September 2025, her research titled "Dermatophytes adaptation to the human host exemplified by *Microsporum canis*" was awarded the Scientific Prize by the DMykG Foundation (Deutschsprachigen Mykologischen Gesellschaft).

Xin Zhou has presented her findings at numerous international conferences, including the ISHAM Congress, TIMM Congress, the IMC Congress, and IUMS *et al.* She serves as a member of Yong ISHAM and ECMM, acts as the guest lecturer for the International Clinical Mycology Course. In addition to her research, she regularly reviews manuscripts for journals such as *Mycoses*, *Mycopathologia*, and *Frontiers in Fungal Biology*. She has supervised undergraduate and graduate students, assisting them in project planning, experimental protocols, and academic writing. Her experimental expertise includes fungal culture, quantitative PCR, genome and transcriptome sequencing, spatial transcriptomics, fluorescence in situ

hybridization (FISH), scanning and transmission electron microscopy (SEM/TEM), flow cytometry, *in vivo* infection models, and immunohistochemistry, encompassing a broad range of techniques in cell and molecular biology.

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This thesis is not just the result of experiments and analyses; it is a collective story of kindness, collaboration, and resilience. I carry with me not only a degree but also profound gratitude to all of you who walked this path with me.

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