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Resolving Phylogenetic Relationships Within the *Trichophyton mentagrophytes* Complex: A RADseq Genomic Approach Challenges Status of 'Terbinafine-Resistant' *Trichophyton indotineae* as Distinct Species

Michaela Švarcová[1,2] | Miroslav Kolařík[2] | Yuanjie Li[3] | Clement Kin Ming Tsui[4,5,6] | Vít Hubka[2,7]

[1]Department of Genetics and Microbiology, Faculty of Science, Charles University, Prague, Czech Republic | [2]Laboratory of Fungal Genetics and Metabolism, Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic | [3]State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China | [4]Infectious Diseases Research Laboratory, National Centre for Infectious Diseases, Tan Tock Seng Hospital, Singapore | [5]LKC School of Medicine, Nanyang Technological University, Singapore | [6]Faculty of Medicine, University of British Columbia, Vancouver, Canada | [7]Department of Botany, Faculty of Science, Charles University, Prague, Czech Republic

Correspondence: Vít Hubka (vit.hubka@gmail.com; vit.hubka@natur.cuni.cz)

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ABSTRACT

Background: The *Trichophyton mentagrophytes* complex encompasses common dermatophytes causing superficial mycoses in humans and animals. The taxonomy of the complex is unstable, with conflicting views on the species status of some taxa, particularly *T. indotineae* and *T. interdigitale*. Due to the presence of intermediate genotypes, neither MALDI-TOF MS nor ITS rDNA sequencing can accurately distinguish all taxa in the complex, potentially contributing to clinical misdiagnoses.

Objectives: This research resolves phylogenetic relationships within the *T. mentagrophytes* complex. Based on these data, the taxonomical recommendations are suggested.

Methods: In order to resolve the phylogenetic relationship of the *T. mentagrophytes* complex, we employed Restriction Site-Associated DNA Sequencing (RADseq) to produce a high-resolution single nucleotide polymorphism (SNP) dataset from 95 isolates. The SNP-based analyses indicated the presence of two major genetic clusters corresponding to *T. mentagrophytes* (including *T. indotineae*) and *T. interdigitale*.

Results: Our results challenge the species status of *T. indotineae* because of insufficient genetic divergence from *T. mentagrophytes*. Therefore, we propose designating *T. indotineae* as *T. mentagrophytes* var. *indotineae* (or *T. mentagrophytes* ITS genotype VIII) to avoid further splitting of the complex and taxonomic inflation. Although *T. interdigitale* shows clearer genetic differentiation, its separation is incomplete and identification of some isolates is ambiguous when using routine methods, leading us to consider it a variety as well: *T. mentagrophytes* var. *interdigitale*.

Conclusions: We recommend using *T. mentagrophytes* as the overarching species name for all complex isolates. Where precise molecular identification is possible, the use of variety ranks is encouraged. Since identical resistance mechanisms are not specific to any genotype or dermatophyte species, identifying antifungal resistance is more important than differentiating closely related genotypes or populations.

1 | Introduction

Trichophyton mentagrophytes is a key pathogen within the diverse dermatophyte group, responsible for causing superficial mycoses affecting skin, nails and hair in humans and animals. These infections pose a clinical concern for the global population with a high prevalence [1–3]. The complexity and variety within the *T. mentagrophytes* complex underscore the challenges in dermatophyte taxonomy, necessitating precise species identification for effective clinical management and the introduction of preventive measures against the spread of dermatophytoses.

Routine identification of dermatophytes in a clinical setting still often relies on morphological characters or molecular methods such as sequencing of the ITS rDNA region or MALDI-TOF mass spectrometry. However, in some cases, these methods may falter when discerning closely related dermatophyte species. Such limitations can lead to misdiagnosis and suboptimal treatment, highlighting the need for a more robust understanding of dermatophyte genetics and taxonomy [3–5].

Historically, the species delimitation within the *T. mentagrophytes* complex has undergone many revisions with a rich tapestry of renaming and reclassification efforts [5–9]. Yet the most recent taxonomic study [5] recommends treating *T. mentagrophytes* as a single species with three varieties, including *T. mentagrophytes* var. *mentagrophytes*, *T. mentagrophytes* var. *interdigitale* and *T. mentagrophytes* var. *indotineae*. Based on this study, there are few taxonomic arguments for preserving these populations as separate species names due to the lack of monophyly and unique morphology, among others. This study also challenges the distinctiveness of *T. indotineae*, a widely used name in clinical practice in recent years, known for its association with an Indian dermatophytosis epidemic and a propensity for terbinafine resistance [10–12]. Thus, the *T. mentagrophytes* complex presents a substantial challenge in species identification and clinical management due to its diverse morphology, genetic variability, clinical presentation and spread of resistance.

Our study addresses the pressing question of taxonomic classification and population genetic relatedness within the *T. mentagrophytes* complex. As traditional methods such as ITS barcoding, MLST approaches, morphological analysis and biochemical testing have limitations to conclusively resolve these complex questions, we used Restriction Site Associated DNA Sequencing (RADseq), a powerful tool that provides comprehensive SNP data and insights into genetic variability and evolutionary relationships. This cost-effective and powerful approach of producing comprehensive single nucleotide polymorphism (SNP) data across the entire genome has been extensively employed in diverse organisms [13–18] but has never been used in dermatophytes. Our findings based on the high-resolution SNP dataset offer a comprehensive re-evaluation of species boundaries, particularly challenging the distinct species status of *T. indotineae* and propose a simplified and cohesive taxonomic framework.

2 | Material and Methods

2.1 | Isolates

The examined strains originated from Czech patients with various manifestations of dermatophytosis or from foreign culture collections. These strains were previously identified using the DNA sequencing of the ITS rDNA region, translation elongation factor 1-α (*tef1-α*) and β-tubulin gene (*tubb*) in the study of Švarcová et al. [5]. A subset of 95 isolates examined in this study represents major clades identified in the *T. mentagrophytes* complex from the aforementioned research.

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as the research in this article related to microorganisms.

2.2 | DNA Extraction

Cultures were grown in liquid Sabouraud's glucose medium (Thermo Fisher Scientific, Waltham, MA, USA), shaking at 100 rpm for 10 days. DNA was extracted using the QuickDNA Miniprep Kit (Zymo Research, Irvin, CA), following the manufacturer's protocol. The quality and quantity of the DNA were measured using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). The DNA was normalised to a concentration of 20 ng/μL.

2.3 | Sequencing, Variant Calling and Genome Assembly

Library preparation, enzyme selection and RAD sequencing reaction were performed by Floragenex Inc. (9590 SW Gemini Dr., Beaverton, OR, USA). The PstI enzyme was used for digestion. From a total of 95 samples, 409.8 million reads were obtained, achieving an average coverage of 20,187.5× per variant. This allowed identification of 16,795 variable sites. The reference genome for strain CCF 6682 (=ME 517/15) was assembled using VELVET software v. 1.2.10 [19]. The remaining samples were aligned to this reference genome and processed into variant call format (vcf) using BOWTIE v. 1.1.1 [20], BWA v. 0.6.1 [21] and SAMTOOLS v. 0.1.16 [22].

2.4 | Population Structure

The vcf file generated above was used for SNP data processing. TASSEL v. 5.2.91 [23] was used for quality control, with initial filtering removing taxa with more than 10% missing sites, reducing the dataset from 95 to 87 individuals. Further filtering eliminated missing sites, parameter Site Minimum Count of alleles not Unknown was set to 80 and Site Minimum Minor Allele Frequency to 0.1 (10%), reducing the number of sites from 16,795 to 6996.

For performing population structure analysis and SNP phylogeny analysis, only individuals with unique haplotypes were

included ($n = 18$). The population structure analysis was performed using the set of scripts STRUCTURE multi-PBS Pro [24], which enables parallel execution of the STRUCTURE software on a high-performance computing (HPC) cluster using the Portable Batch System (PBS) Pro job scheduler. This method allows for efficient and simultaneous analysis of multiple datasets or multiple runs of the STRUCTURE program [25]. The analysis spanned a range of clustering numbers (K) from 1 to 10, with each subset run 10 times using 1,000,000 replicates and a burn-in of 500,000. Computational resources were provided by MetaCentrum. The Evanno ΔK statistic was calculated using STRUCTURE HARVESTER [26]. Filtered SNPs were also used to construct a TCS haplotype network using the PopART program [27].

2.5 | Discriminant Analysis of Principal Components (DAPC)

The genetic structure among the isolates was also investigated using DAPC. First, the vcf file containing the variant information was imported using vcfR and converted into a genlight object [28]. Second, the find.cluster function in the R package adegenet v2.0.1 was used to identify clusters and the optimal K was determined by the lowest value of Bayesian Information Criterion (BIC) [29]. We set max.n.clust = 40 and n.iter = 1×10^9. The optimal number of principal components was determined by 'a score' using the optim.a.score function in the adegenet package. The final DAPC was performed using the optimal K with the best score and the scatter plot was plotted using ggplot2 v3.3.5 [30].

2.6 | Phylogeny

SNAPP v. 1.6.1 [31], an add-on package to BEAST 2 software [32], was utilised for SNP tree computation. The Markov Chain Monte Carlo (MCMC) chain length was set to 10^7 and samples were collected every 1000 iterations. The results obtained from the SNAPP analysis indicated that the effective sample size (ESS) for the parameters estimated by the MCMC chains was greater than 200, suggesting effective convergence. The log files were reviewed using Tracer to ensure the accuracy and reliability of the analysis. To visualise the posterior distribution of species trees, which represents the most likely tree topology, we utilised the DensiTree v. 2.2.7. This software overlays all the sampled tree topologies onto a single plot, providing a visual representation of the uncertainty and variation in the estimated species tree. A total of 10,000 trees were processed using TreeAnnotator v. 2.7.4, with the initial 20% of trees discarded. The consensus topology was generated from the remaining 8000 trees and visualised using Figtree v. 1.4.4 [33].

An ITS rDNA-based tree was constructed for comparison with phylogeny based on SNP data. To determine the partitioning schemes and suitable substitution models (Bayesian Information Criterion), PartitionFinder 2 was used [34]. The ITS region was segmented into ITS1, 5.8S and ITS2 regions, which were considered as independent datasets. The optimal partitioning schemes identified for the dataset were TrNef+I for ITS1 and ITS2 regions and JC for the 5.8S region. A maximum likelihood (ML) phylogenetic tree was constructed in IQ-TREE v. 2.1.2 [35], with nodal support assessed through nonparametric bootstrapping (BS) with 1000 replicates. The comparison of two phylogenetic trees was visualised using a tanglegram, with the samples connected by lines based on their names. The tanglegram visualisation was generated using R Studio [36] and R packages ape and phytools [37, 38].

3 | Results

3.1 | Population Structure

Analysis of the population structure using Evanno ΔK statistics revealed that the scenario $\Delta K = 2$ was the most probable, while the scenario $\Delta K = 4$ was the second most likely. This suggests that our dataset can be classified into either two or, less likely, four distinct populations. Figure 1 illustrates these findings, showing the Evanno ΔK statistics graph (Figure 1B) alongside pophelper-generated bar plots from STRUCTURE runs for $\Delta K = 2$, $\Delta K = 3$ and $\Delta K = 4$ (Figure 1A).

In the most probable scenario ($\Delta K = 2$), the isolates were distributed into two populations corresponding to *T. mentagrophytes* var. *mentagrophytes* and *T. mentagrophytes* var. *interdigitale*, as previously identified using multilocus sequence typing (MLST) [5]. *Trichophyton mentagrophytes* var. *indotineae* was not recognised in this scenario and was included in *T. mentagrophytes* var. *mentagrophytes*. However, a significant number of haplotypes showed a high level of admixture between these two populations, indicating possible recombination and incomplete separation of *T. mentagrophytes* var. *mentagrophytes* and *T. mentagrophytes* var. *interdigitale*.

The second most probable scenario ($\Delta K = 4$) recognises *T. mentagrophytes* var. *interdigitale* as a distinct group and divides *T. mentagrophytes* var. *mentagrophytes* into three groups: menta1 (green), menta2 (pale blue) and menta3 (red) (Figure 1). *Trichophyton mentagrophytes* var. *indotineae* was again not recognised as a separate population and was included in group menta1 of *T. mentagrophytes* var. *mentagrophytes* along with some other isolates not belonging to *T. mentagrophytes* var. *indotineae* (ITS genotype VIII). There was a significant level of admixture between *T. mentagrophytes* var. *interdigitale* and population menta3, as seen in the example of strain ME 918/14, and also between populations menta1 and menta3, as seen in the example of strain CCF 6580 (Figure 1A). This indicates that these populations are not fully separated.

3.2 | Phylogeny and Haplotype Network

A total of 87 isolates meeting the quality control (QC) criteria were selected for analysis. We initially constructed a Bayesian tree using BEAST 2 based on 6996 SNPs extracted from examined strains that remained after QC and filtering (see Section 2). Figure 1C presents a DensiTree visualisation of the consensus species tree, showcasing the confidence level across various trees topologies. The Consensus topology tree,

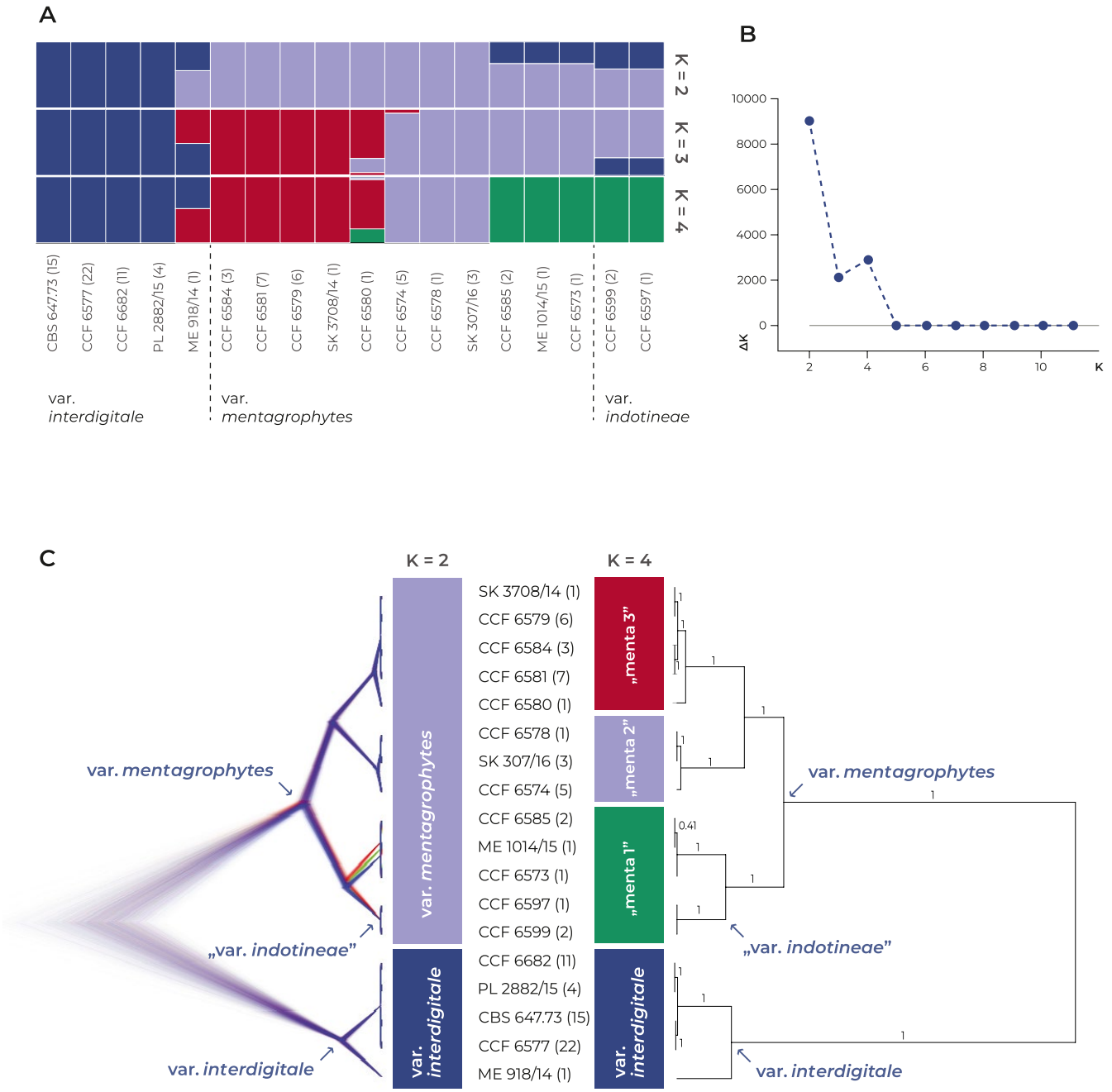
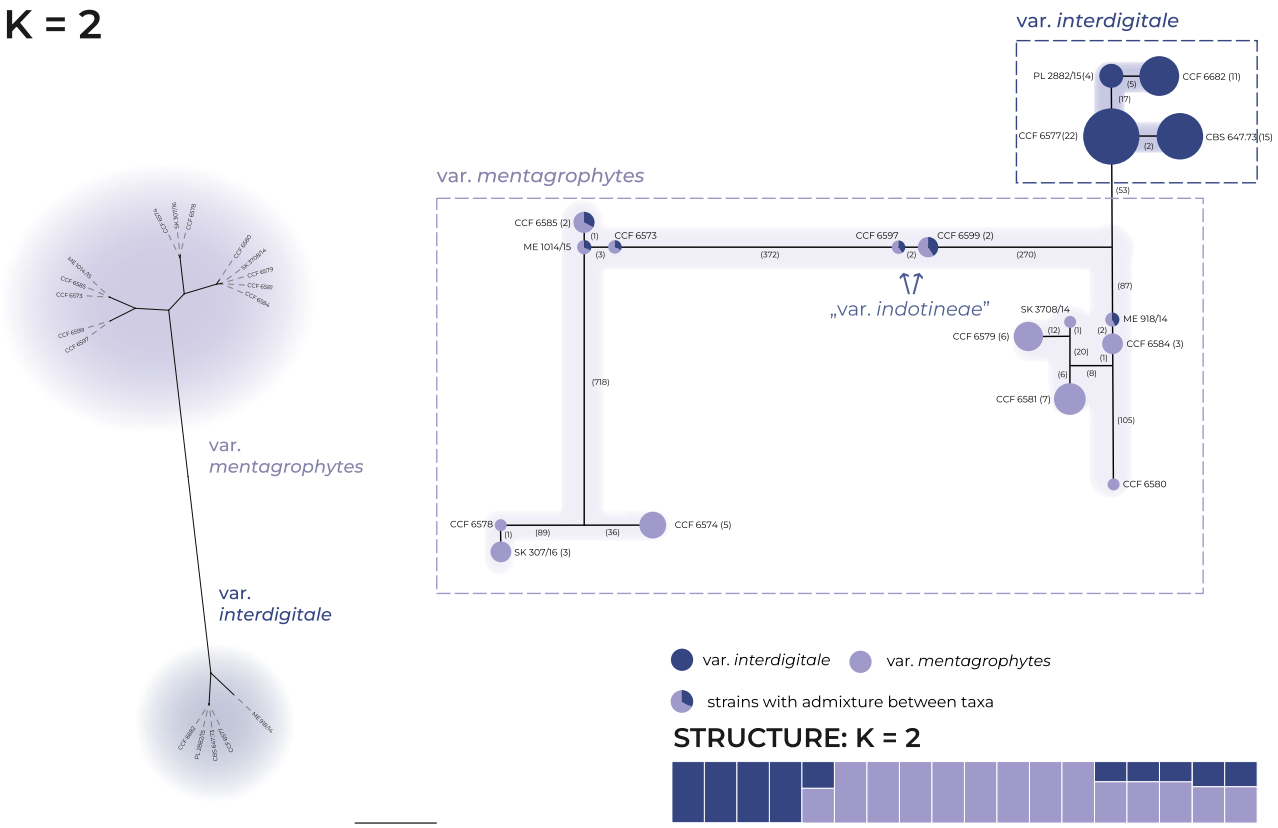


FIGURE 1 | Population structure and phylogenetic relationships of populations within the *Trichophyton mentagrophytes* complex based on SNP data. (A) Bar plots displaying the population structure based on STRUCTURE analysis, showing clustering for $K=2$, $K=3$ and $K=4$. Each vertical bar represents a unique haplotype (the numbers in brackets indicate the number of identical strains), with colours corresponding to the proportion of ancestry from each inferred population. (B) The Evanno ΔK statistic indicates that $K=2$ is the most likely scenario, while $K=4$ represents the second most probable scenario. (C) Phylogenetic tree generated using BEAST based on SNP data. The left side displays the BEAST tree visualised using DensiTree; trees with the most common topology are highlighted in blue, trees with the second most common topology in red and trees with the third most common topology in green. On the right side, the consensus phylogenetic tree (posterior probabilities supporting branches are appended to nodes) is shown. Populations identified in the STRUCTURE in scenarios $K=2$ and $K=4$ are indicated by coloured bars in the middle.

depicted to the right in Figure 1C, highlights the consensus topology across sampled trees. Predominantly high posterior probabilities (pp) denote strong support (mostly pp. = 1.00), with only one branch having a pp of 0.41, reflecting poorly resolved relationships between some members of the menta1 population. The coloured bars inserted between the trees show the corresponding distribution of populations according to the STRUCTURE analysis (most probable scenarios $\Delta K=2$, and $\Delta K=4$).

The haplotype network (PopART) and unrooted SNP-based tree (BEAST 2) illustrated in Figure 2 provide detailed insights into the relationships among *T. mentagrophytes* complex strains and populations. The unrooted phylogenetic tree



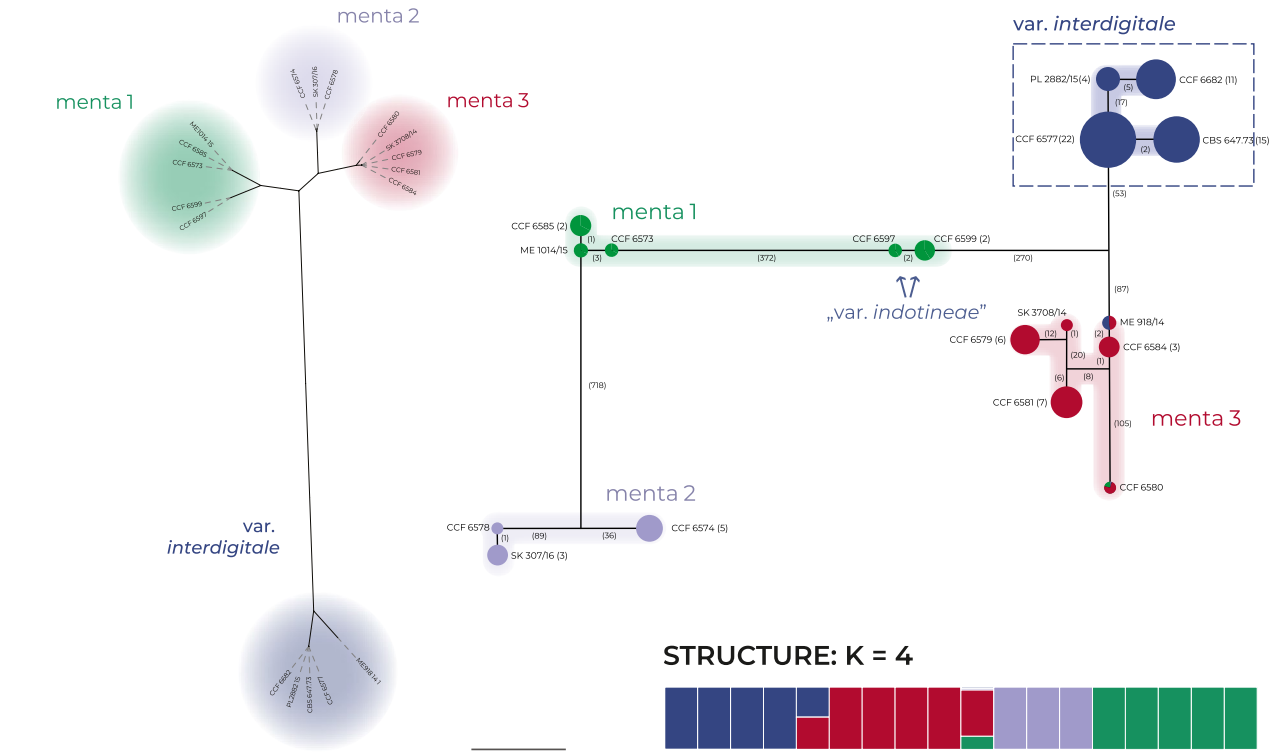


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FIGURE 2 | Clustering patterns of the *Trichophyton mentagrophytes* complex based on SNP data. The figure shows population structure and genetic relationships for $K=2$ and $K=4$ scenarios inferred from STRUCTURE. The relationships are illustrated on a haplotype network (right parts of panel, PopART software) and an unrooted phylogenetic tree (left parts of panel, BEAST 2 software). In the haplotype network, the haplotypes are represented by circles with size corresponding to the number of isolates with identical haplotype, numbers on connecting lines indicate the number of substitutions between haplotypes (indels excluded), the colours represent the clusters from STRUCTURE analysis and the presence of a multiple colours in one circle indicates admixture for a given haplotype and scenario (bar plot below network; for details see Figure 1). The unrooted phylogenetic trees based on SNPs (left parts of panels), depicting the phylogenetic distances among the unique haplotypes and their affiliation to clusters from STRUCTURE analysis.

illustrates the phylogenetic distances between isolates and clearly distinguishes *T. mentagrophytes* var. *interdigitale* from *T. mentagrophytes* var. *mentagrophytes*, highlighting their relatively high genetic divergence compared to divergences between subpopulations present within *T. mentagrophytes* var. *mentagrophytes*. However, *T. mentagrophytes* var. *indotineae* is included within *T. mentagrophytes* var. *mentagrophytes* (scenario $\Delta K=2$) or within its subpopulation menta1 (scenario $\Delta K=4$).

3.3 | Comparison of SNP-Based and ITS-Based Phylogenies

There is a good congruence between ITS genotypes and the SNP tree (Figure 3). In the SNP-based phylogeny, the populations of *T. mentagrophytes* var. *interdigitale* and *T. mentagrophytes* var. *mentagrophytes* are well separated and monophyletic, while in the ITS tree, they are paraphyletic. In both phylogenies, isolates of *T. mentagrophytes* var. *indotineae* are placed within *T. mentagrophytes* var. *mentagrophytes* (subpopulation menta1). In the SNP-based phylogeny, the isolate CCF 6580 is part of subpopulation menta3 of *T. mentagrophytes*, whereas the ITS phylogeny positions this isolate separately from other *T. mentagrophytes* strains/genotypes.

3.4 | DAPC

To gain insights into the population structure of *T. mentagrophytes* complex, we performed DAPC analysis using SNP markers. DAPC plots revealed a high level of population differentiation and relatively consistently clustered samples that correspond to the *T. mentagrophytes* var. *interdigitale*, as well as menta1, menta2 and menta3 (Figure 4). *Trichophyton mentagrophytes* var. *indotineae*, as currently circumscribed, was differentiated from the other four strains of the menta1 subpopulation. Notably, isolates CCF 6580 (menta3) and ME 918/14 (var. *interdigitale*) were clearly distinct from the remaining strains in the respective populations, potentially representing genotypes or populations that require additional sampling.

4 | Discussion

This study provides substantial new insights into the taxonomy and species delimitation within the *Trichophyton mentagrophytes* complex using a RADseq genomic approach. These findings challenge the current status of *T. indotineae* as a distinct species, suggesting it should instead be considered a variety or integral part of *T. mentagrophytes*.

The primary goal of this study was to address the taxonomic uncertainties within the *T. mentagrophytes* complex, which has historically been plagued by numerous taxonomic changes and misidentifications [5–8, 39]. The methods commonly used for identification, such as phenotype, ITS region sequencing and MALDI-TOF MS, often fail to distinguish related taxa within the complex due to their limitations or the presence of transitional forms and genotypes [39–41]. The ambiguous or challenging identification leads to situations where the authors, despite using molecular data for species identification, prefer to use only the designations '*T. mentagrophytes* complex' and '*T. mentagrophytes/T. interdigitale*' to refer to all strains from the complex [42–48].

Our population structure analysis using data from RAD sequencing revealed that the most probable scenario with the presence of two populations in the *T. mentagrophytes* complex ($\Delta K=2$), corresponds to *T. mentagrophytes* (including *T. indotineae*) and *T. interdigitale* (Figure 1). This finding suggests that *T. indotineae* does not possess sufficient genetic divergence to be considered a separate taxonomic entity. Its recognition at species level could lead to taxonomic inflation and further unnecessary segregation of *T. mentagrophytes* into multiple species to retain monophyly of recognised taxa. In the second most probable scenario with four populations ($\Delta K=4$), *T. interdigitale* is recognised as a separate population and *T. mentagrophytes* is divided into three populations (menta1, menta2 and menta3), none of which corresponds to *T. indotineae*, reinforcing the notion that it should not be recognised as an independent species.

The use of approaches based on whole-genome sequencing (WGS) is still uncommon in dermatophytes and only a few studies performed WGS to address various hypotheses [39, 49–52]. From a taxonomic point of view, the work of Pchelin et al. [39] is particularly noteworthy. Based on phylogenomic data and species delimitation analyses, they concluded that *T. mentagrophytes* and *T. interdigitale* belong to the same phylogenetic species. The authors, however, argued that both species should be retained due to the correlation of epidemiological data with ITS genotypes and taxonomic stability. Other well-established methods for population analysis in dermatophytes involve in particular microsatellite genotyping and MLST (multilocus sequence typing). The MLST approaches reached the same conclusion that *T. mentagrophytes* and *T. interdigitale* and/or *T. indotineae* are not monophyletic as reviewed by Švarcová et al. [5]. Although

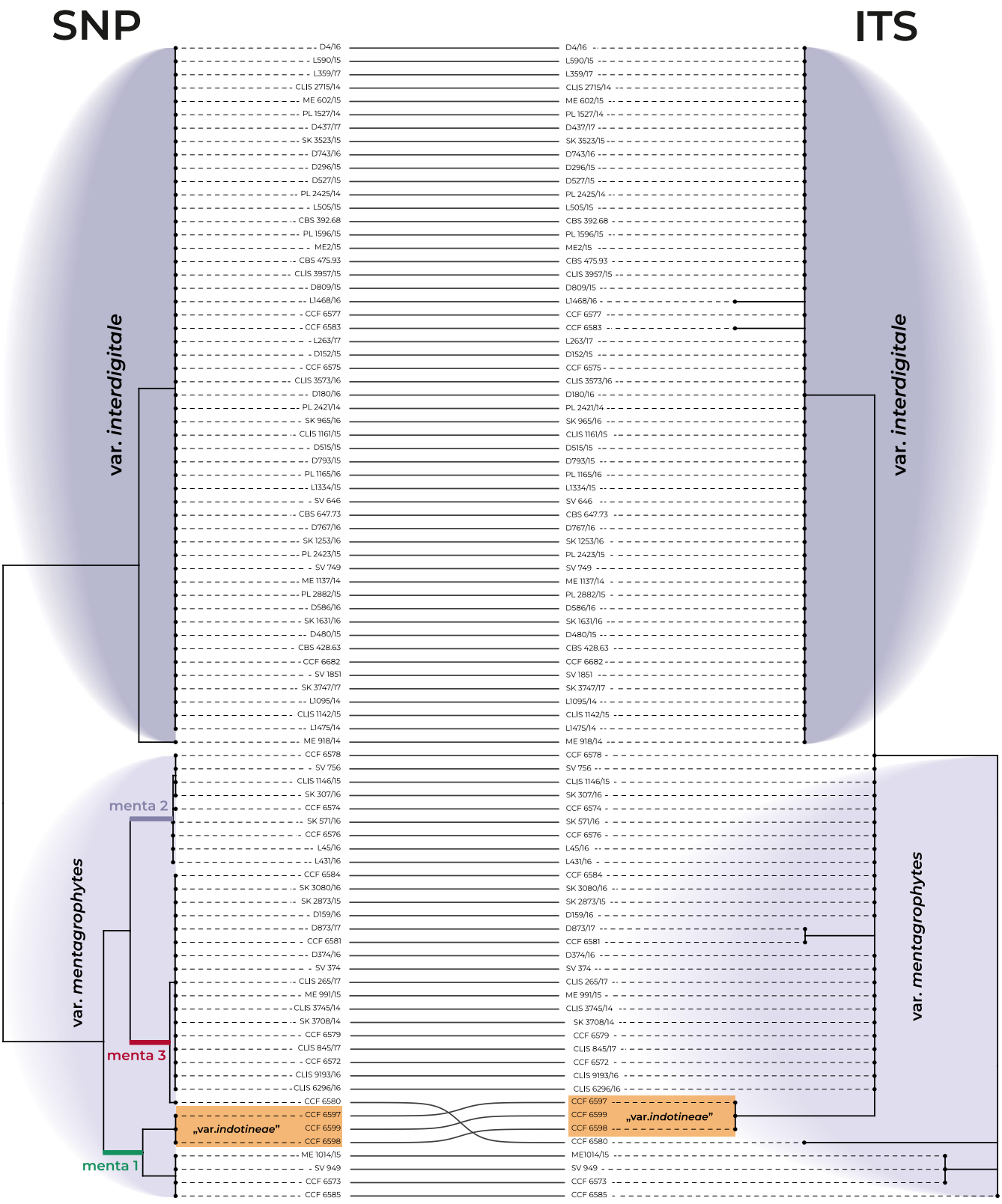

FIGURE 3 | Comparison of phylogenetic tree topologies based on SNP data (RADseq) and ITS rDNA region within the *Trichophyton mentagrophytes* complex, connected by tanglegram. Lines connect corresponding isolates between the trees for visual clarity. The SNP-based phylogeny provides better resolution within *T. mentagrophytes* var. *mentagrophytes*, identifying three main subpopulations (menta1, menta2 and menta3). Both trees show low resolution within *T. mentagrophytes* var. *interdigitale*, which is resolved as monophyletic in the SNP-based tree but paraphyletic in the ITS-based tree. Additionally, *T. mentagrophytes* var. *indotineae* is integrated within *T. mentagrophytes* var. *mentagrophytes* in both analyses.

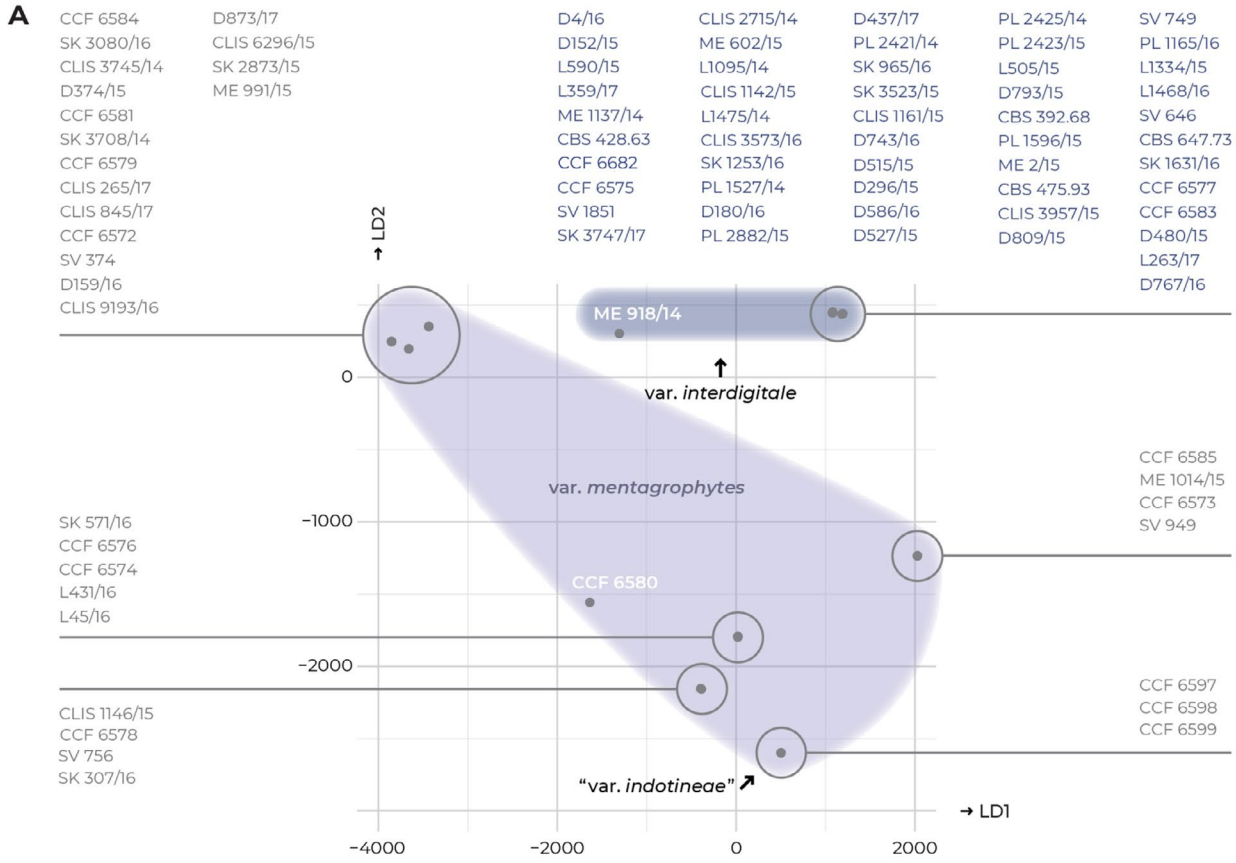

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FIGURE 4 | Discriminant analysis of principal components (DAPC) based on SNP data showing clustering of strains within *Trichophyton mentagrophytes* complex. Coloured clouds represent clustering from STRUCTURE analysis with $K = 2$ (A) and $K = 4$ (B). The analysis does not show clear clustering of isolates based on taxonomic varieties (var. *mentagrophytes*, var. *interdigitale* and var. *indotineae*).

microsatellite markers were used in many clinically important species complexes, Including *T. rubrum* [53, 54], *T. benhamiae* [55–57] and *Microsporum canis* [58–60], they are not available in the *T. mentagrophytes* complex.

4.1 | Summary of Reasons for Not Recognising Populations in the Complex as Separate Species

4.1.1 | Lack of Monophyly

Numerous studies performing phylogenies based on the ITS region, multiple genes [6, 11, 61] and phylogenomic data [39, 52] demonstrated that taxa in the complex are not monophyletic. Overuse of the rank of a species leads to taxonomic inflation and could lead to the subdivision of the *T. mentagrophytes* complex into additional species without biological relevance so that monophyly is maintained. These situations are well known in many fungal genera such as *Alternaria* [62], *Aspergillus* [63, 64], *Bryoria* [65], *Diaporthe* [66], *Flammulina* [67] and others, and led to significant species reduction in recent years. The use of taxonomic levels below the species level, such as variety, subspecies and others, is appropriate for the aforementioned cases when we want to describe biologically or clinically relevant populations [46]. For these units, there is no pressure to meet all the usual criteria used to define species under study, including monophyly.

4.1.2 | Lack of Unique Morphology

Reliable differentiation of *T. mentagrophytes*, *T. interdigitale* and *T. indotineae* is practically impossible using phenotypic features [5, 10, 11]. Although some statistically significant differences were found between these species, there is an important overlap in the presence/absence of features or range of values, making these characters unusable for accurate identification in clinical laboratories. For instance, Švarcová et al. [5] demonstrated that *T. interdigitale* strains had significantly slower growth after 7 days at 37°C (6–24 mm; 15 mm on average) than *T. mentagrophytes* strains (8–32; 22 mm on average). Tang et al. [11] reported statistically significant differences in urease activity between *T. mentagrophytes* (67% strains hydrolysed urea) and *T. interdigitale* (75%) versus *T. indotineae* (27% strains hydrolysed urea). The hair perforation showed similar results, with 92.5% of *T. mentagrophytes* and 98.5% of *T. interdigitale* having positive results, while only 27% of *T. indotineae* showed. As is evident, none of these characteristics are diagnostic for mentioned populations/species.

4.1.3 | Antifungal Resistance and Treatment

The treatment of dermatophytosis is guided by the recommendations specific to a particular clinical unit (such as tinea capitis, tinea corporis and tinea unguium), along with an understanding of the local situation regarding resistance levels. Terbinafine resistance, which has been increasingly documented in recent years in the genus *Trichophyton* [68–73], is most commonly due to non-synonymous point mutations in the squalene epoxidase gene (SQLE). The amino acid substitution F397L in SQLE is one of the most commonly observed substitutions worldwide associated with a high level of terbinafine resistance [74]. This mechanism of resistance is not specific to any *Trichophyton* species, let alone genotype or population in the *T. mentagrophytes* complex. The same mechanism has been described in *T. mentagrophytes* [69], *T. interdigitale* [75] and *T. rubrum* [70–72]. Although the ITS genotype VIII of *T. mentagrophytes* (*T. indotineae*) is often labelled as 'terbinafine-resistant', its resistance level varies greatly. In other words, resistance is not an intrinsic characteristic of *T. indotineae* and only a portion of its isolates display resistance. Although some studies reported resistance in all strains collected, for example, study from the Greece (9/9 strains) [74] and North America (21/21 *T. indotineae* strains) [76], the majority of epidemiological studies showed lower levels of resistance. Namely, 16.7% (1/6) in France (isolates collected in 2021 only) [77], 45% (13/29) in Germany (2016–2019) [78], 50% (2/4 strains) in Vietnam (2020–2021) [79], 53% (34/64) in India (2018–2019) [80], 72% (202/279) in India (2017–2019) [81] and 78% (7/9) in the Czech Republic (2018–2022) [69]. Identical resistance mechanism is also known in other ITS genotypes of *T. mentagrophytes*, including ITS genotype VII [82] and ITS genotype XVII [73]. This demonstrates that arguments advocating the recognition of *T. indotineae* because of its resistance are unfounded, and that antifungal susceptibility testing is superior to species/genotype identification. It is true that the level of resistance in this genotype is higher than in other genotypes and species, likely due to positive selection pressure, which confers a survival advantage to resistant strains, allowing them to spread more effectively because of their resistance to treatment. This scenario can happen in any resistant genotype or population. Species identification as 'terbinafine-susceptible' or 'terbinafine-resistant' *T. mentagrophytes* provides more clinically meaningful information than '*T. indotineae*' only, because strains classified under *T. indotineae* may exhibit both susceptibility and resistance.

Similar situations, where resistance is predominantly associated with a specific subpopulation of a clinically relevant species, can be found in other clinically significant fungi. For example, azole resistance is primarily associated with MLST clade 4 of *Candida tropicalis* [83] and clade A/population A of *Aspergillus fumigatus* [84, 85]. Although these subpopulations can be clearly identified using DNA sequencing or subtyping methods, the resistant population has not been reclassified as a new species, unlike *T. indotineae*.

4.1.4 | Clinical and Ecological Definition

It has repeatedly been confirmed that onychomycosis and tinea pedis are more commonly linked to *T. interdigitale* than *T. mentagrophytes* [5, 39, 86–89] and patients infected with

T. interdigitale are significantly older [5] as onychomycosis is more prevalent in patients in older age groups. While *T. interdigitale* is usually considered anthropophilic and *T. mentagrophytes* a zoophilic dermatophyte, there are some other sublineages/genotypes in *T. mentagrophytes sensu lato* that primarily spread among humans, including ITS genotype VIII (*T. indotineae*) and ITS genotype VII [90]. This may partly reflect the lack of research in the veterinary field, as evidenced by isolations of the ITS genotype VIII [91–93] and VII [94, 95] from animals. These findings suggest that *T. mentagrophytes* cannot be easily classified into an anthropophilic or zoophilic ecological group but as a species with a broad host spectrum that includes a wide range of mammals, including humans, with certain lineages more or less specialised to specific hosts. The designation of some clinically relevant lineages as varieties (or genotypes) can raise awareness of these lineages and maintain the integrity of this species as a whole.

Although *T. indotineae* is usually ascribed to an agent of severe cases of tinea corporis, tinea cruris and tinea faciei [78], some recent studies from Switzerland [96] and the Czech Republic [69] revealed that this genotype is most frequently associated with onychomycosis, making it challenging to use the clinical picture as a taxonomic criterion to define *T. indotineae*.

4.1.5 | Lack of Simple Identification Molecular Tools and Identification Ambiguities

The ability to easily and accurately identify pathogens is crucial in clinical practice. Differentiating *T. mentagrophytes* and *T. interdigitale* using MALDI-TOF, a method that has gained popularity due to its speed and efficiency, usually fails [40, 47, 97]. In contrast, MALDI-TOF probably enables the identification of *T. indotineae* from *T. mentagrophytes/T. interdigitale* with an accuracy rate of 96% or higher [40, 47]. However, it does not differentiate terbinafine-susceptible and terbinafine-resistant strains [98]. A key limitation of these studies is that they analysed a limited number of strains representing restricted variability within *T. mentagrophytes* (many ITS genotypes/clades were missing). As a result, the identification accuracy of *T. interdigitale* and *T. mentagrophytes* is expected to decline even more in broader studies. The same concern may apply to *T. indotineae* strains, where further limitations could arise when comparing it to other closely related genotypes of *T. mentagrophytes*.

The differentiation of *T. indotineae* from *T. mentagrophytes*/*T. interdigitale* relies only on one to two unique substitutions in the ITS region (depending on the alignment length and intraspecific diversity included) [5, 40], and no unique substitutions were found in other commonly used markers such as *tef1-α* and *tubb* [5]. This highlights how poorly defined these populations are, aligning with our results based on SNPs obtained through the RADseq method, where *T. indotineae* is located inside the menta1 subpopulation of *T. mentagrophytes* var. *mentagrophytes*.

The ITS region genotyping, increasingly used to characterise isolates of the *T. mentagrophytes* complex [12, 89, 99, 100], is relatively time-consuming and requires expertise. From a practical standpoint, it may be advisable to differentiate ITS genotypes that predominantly spread via human-to-human contact to carry out an epidemiological investigation, that is, I, II, X, XI and XII (*T. mentagrophytes* var. *interdigitale*), VII (mostly associated with sexually transmitted dermatophytosis) and VIII (*T. mentagrophytes* var. *indotineae*). However, animal reservoirs cannot be ruled out (discussed above), and ecological classification is further complicated by the existence of intermediate genotypes II* and III* [12, 39, 86, 101] between *T. mentagrophytes* var. *interdigitale* and *T. mentagrophytes* var. *mentagrophytes* that more frequently originate from animals [11, 12, 39, 86].

5 | Conclusions and Recommendations

This study reexamines the taxonomy of the *T. mentagrophytes* complex, particularly challenging the species status of *T. indotineae* and addressing broader taxonomic issues. Our phylogenetic and population-genetic analysis, based on whole-genome SNP data generated through RADseq, revealed insufficient genetic divergence to support the recognition of *T. indotineae* as a distinct species. In contrast to *T. indotineae*, the population of *T. interdigitale* appears better separated from *T. mentagrophytes* based on whole-genome SNP data. Despite this, due to the ambiguous definition of this species and the difficulty of identification by routine methods, we recommend using the name *T. mentagrophytes* for all isolates within the complex. When molecular identification of *T. interdigitale* and *T. indotineae* is clear and unambiguous, we suggest optionally assigning them variety ranks: *T. mentagrophytes* var. *interdigitale* and *T. mentagrophytes* var. *indotineae* (alternative: *T. mentagrophytes* ITS genotype VIII). This practice will contribute to cohesive taxonomy, reduce confusion in both clinical and research contexts, and simplify identification efforts in clinical practice. By adopting this taxonomic framework, we ensure that historical and current epidemiological data remain comparable while maintaining clinical distinctions of some subpopulations. This reclassification will also prevent unnecessary taxonomic inflation and streamline diagnostic processes, allowing clinicians to focus on identifying antifungal resistance rather than differentiating taxa and genotypes with limited clinical relevance.

Author Contributions

Michaela Švarcová: conceptualization, investigation, writing – original draft, methodology, validation, visualization, writing – review and editing, software, formal analysis, data curation. **Miroslav Kolařík:** writing – original draft, funding acquisition, validation, writing – review and editing, project administration, resources, supervision, conceptualization, investigation. **Yuanjie Li:** investigation, writing – review and editing, visualization, validation, methodology, software, formal analysis, data curation. **Clement Kin Ming Tsui:** writing – review and editing, visualization, validation, methodology, software, data curation, investigation, formal analysis. **Vít Hubka:** conceptualization, investigation, funding acquisition, writing – original draft, methodology, validation, visualization, writing – review and editing, project administration, supervision, resources.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The filtered dbSNP VCF dataset used is available through DRYAD digital repository https://doi.org/10.5061/dryad.zpc866tjn. Selected isolates used in this study are available in the CCF collection of fungi (Charles University, Prague, Czechia).

References

1. R. C. Summerbell, I. Weitzman, and A. A. Padhye, *Trichopyton, Microsporum, Epidermophyton and Agents of Superficial Mycoses*, vol. 2, 9th ed. (ASM Press, 2007).

2. B. Havlickova, V. A. Czaika, and M. Friedrich, "Epidemiological Trends in Skin Mycoses Worldwide," *Mycoses* 51 (2008): 2–15.

3. A. Chowdhary, A. Singh, P. K. Singh, A. Khurana, and J. F. Meis, "Perspectives on Misidentification of *Trichophyton interdigitale*/*Trichophyton mentagrophytes* Using Internal Transcribed Spacer Region Sequencing: Urgent Need to Update the Sequence Database," *Mycoses* 62 (2019): 11–15.

4. C. Cafarchia, R. Iatta, M. S. Latrofa, Y. Graser, and D. Otranto, "Molecular Epidemiology, Phylogeny and Evolution of Dermatophytes," *Infection, Genetics and Evolution* 20 (2013): 336–351.

5. M. Svarcova, T. Vetrovsky, M. Kolarik, and V. Hubka, "Defining the Relationship Between Phylogeny, Clinical Manifestation, and Phenotype for *Trichophyton mentagrophytes*/*interdigitale* Complex; a Literature Review and Taxonomic Recommendations," *Medical Mycology* 61 (2023): myad042.

6. G. S. de Hoog, K. Dukik, M. Monod, et al., "Toward a Novel Multilocus Phylogenetic Taxonomy for the Dermatophytes," *Mycopathologia* 182 (2017): 5–31.

7. Y. Gräser, A. F. A. Kuijpers, W. Presber, and G. S. De Hoog, "Molecular Taxonomy of *Trichophyton mentagrophytes* and *T. tonsurans*," *Medical Mycology* 37 (1999): 315–330.

8. P. Nenoff, J. Herrmann, and Y. Graser, "*Trichophyton mentagrophytes Sive interdigitale*? A Dermatophyte in the Course of Time," *Journal der Deutschen Dermatologischen Gesellschaft* 5 (2007): 198–202.

9. H. Beguin, N. Pyck, M. Hendrickx, C. Planard, D. Stubbe, and M. Detandt, "The Taxonomic Status of Trichophyton Quinckeanum and *T. interdigitale* Revisited: A Multigene Phylogenetic Approach," *Medical Mycology* 87 (2012): 871–882.

10. R. Kano, U. Kimura, M. Kakurai, et al., "*Trichophyton indotineae* sp. Nov.: A New Highly Terbinafine-Resistant Anthropophilic Dermatophyte Species," *Mycopathologia* 185 (2020): 947–958.

11. C. Tang, X. Kong, S. A. Ahmed, et al., "Taxonomy of the *Trichophyton mentagrophytes*/*T. interdigitale* Species Complex Harboring the Highly Virulent, Multiresistant Genotype *T. indotineae*," *Mycopathologia* 186 (2021): 315–326.

12. S. Uhrlaß, S. B. Verma, Y. Gräser, et al., "*Trichophyton indotineae*—An Emerging Pathogen Causing Recalcitrant Dermatophytoses in India and Worldwide—A Multidimensional Perspective," *Journal of Fungi* 8, no. 7 (2022): 757, https://doi.org/10.3390/jof8070757.

13. T. L. Parchman, J. P. Jahner, K. A. Uckele, L. M. Galland, and A. J. Eckert, "RADseq Approaches and Applications for Forest Tree Genetics," *Tree Genetics & Genomes* 14 (2018): 1–25.

14. A. Lasalle, P. Cáceres, T. Montenegro, C. Araneda, J. Yáñez, and D. Vizziano-Cantonnet, "Development of a Dense SNP Panel for the Siberian Sturgeon (*Acipenser baerii*) Using High-Depth RAD-Seq," *Conservation Genetics Resources* 14 (2021): 37–39.

15. J. G. Martinez, J. D. Rangel-Medrano, A. J. Yepes-Acevedo, N. Restrepo-Escobar, and E. J. Marquez, "Species Limits and Introgression in Pimelodus From the Magdalena-Cauca River Basin," *Molecular Phylogenetics and Evolution* 173 (2022): 107517.

16. O. Razkin, G. Sonet, K. Breugelmans, M. J. Madeira, B. J. Gomez-Moliner, and T. Backeljau, "Species Limits, Interspecific Hybridization and Phylogeny in the Cryptic Land Snail Complex Pyramidula: The Power of RADseq Data," *Molecular Phylogenetics and Evolution* 101 (2016): 267–278.

17. M. P. Sain, J. Norrell-Tober, K. Barthel, et al., "Multiple Complementary Studies Clarify Which Co-Occurring Congener Presents the Greatest Hybridization Threat to a Rare Texas Endemic Wildflower (*Hibiscus dasycalyx*: Malvaceae)," *Journal of the Botanical Research Institute of Texas* 15 (2021): 283–308.

18. H. C. Wagner, A. Gamisch, W. Arthofer, K. Moder, F. M. Steiner, and B. C. Schlick-Steiner, "Evolution of Morphological Crypsis in the *Tetramorium caespitum* Ant Species Complex (*Hymenoptera: Formicidae*)," *Scientific Reports* 8 (2018): 12547.

19. D. R. Zerbino and E. Birney, "Velvet: Algorithms for de Novo Short Read Assembly Using de Bruijn Graphs," *Genome Research* 18 (2008): 821–829.

20. B. Langmead, C. Trapnell, M. Pop, and S. L. Salzberg, "Ultrafast and Memory-Efficient Alignment of Short DNA Sequences to the Human Genome," *Genome Biology* 10 (2009): 1–10.

21. H. Li, "Aligning Sequence Reads, Clone Sequences and Assembly Contigs With BWA-MEM," arXiv, (2013), https://doi.org/10.48550/arXiv.1303.3997.

22. H. Li, B. Handsaker, A. Wysoker, et al., "The Sequence Alignment/Map Format and SAMtools," *Bioinformatics* 25 (2009): 2078–2079.

23. P. J. Bradbury, Z. Zhang, D. E. Kroon, T. M. Casstevens, Y. Ramdoss, and E. S. Buckler, "TASSEL: Software for Association Mapping of Complex Traits in Diverse Samples," *Bioinformatics* 23 (2007): 2633–2635.

24. V. Zeisek, "STRUCTURE Multi PBS Pro Scripts, on github.com," (2021), accessed January 10, 2021, https://github.com/V-Z/structure-multi-pbspro.git.

25. B. C. Carstens, T. A. Pelletier, N. M. Reid, and J. D. Satler, "How to Fail at Species Delimitation," *Molecular Biology and Evolution* 22 (2013): 4369–4383.

26. D. A. Earl and B. M. Von Holdt, "STRUCTURE HARVESTER: A Website and Program for Visualizing STRUCTURE Output and Implementing the Evanno Method," *Conservation Genetics Resources* 4 (2012): 359–361.

27. J. W. Leigh and D. Bryant, "POPART: Full-Feature Software for Haplotype Network Construction," *Methods in Ecology and Evolution* 6 (2015): 1110–1116.

28. B. J. Knaus and N. J. Grünwald, "Vcfr: A Package to Manipulate and Visualize Variant Call Format Data in R," *Molecular Ecology Resources* 17 (2017): 44–53.

29. T. Jombart and I. Ahmed, "Adegenet 1.3-1: New Tools for the Analysis of Genome-Wide SNP Data," *Bioinformatics* 27 (2011): 3070–3071.

30. H. Wickham, *ggplot2: Elegant Graphics for Data Analysis New York. Applied Spatial Data Analysis* (Springer New York, 2009), 784–785.

31. D. Bryant, R. Bouckaert, J. Felsenstein, N. A. Rosenberg, and A. RoyChoudhury, "Inferring Species Trees Directly From Biallelic Genetic Markers: Bypassing Gene Trees in a Full Coalescent Analysis," *Molecular Biology and Evolution* 29 (2012): 1917–1932.

32. R. Bouckaert, T. G. Vaughan, J. Barido-Sottani, et al., "BEAST 2.5: An Advanced Software Platform for Bayesian Evolutionary Analysis," *PLoS Computational Biology* 15 (2019): e1006650.

33. A. Rambaut, "FigTree," (2024), accessed November, 12, 2024, http://tree.bio.ed.ac.uk/software/figtree/.

34. R. Lanfear, P. B. Frandsen, A. M. Wright, T. Senfeld, and B. Calcott, "PartitionFinder 2: New Methods for Selecting Partitioned Models of Evolution for Molecular and Morphological Phylogenetic Analyses," *Molecular Biology and Evolution* 34 (2017): 772–773.

35. B. Q. Minh, H. A. Schmidt, O. Chernomor, et al., "IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era," *Molecular Biology and Evolution* 37 (2020): 1530–1534.

36. R_Development_Core_Team, "R: A Language and Environment for Statistical Computing," (2021), Vienna, Austria: R Foundation for Statistical Computing, http://www.R-project.org.

37. E. Paradis, J. Claude, and K. Strimmer, "APE: Analyses of Phylogenetics and Evolution in R Language," *Bioinformatics* 20 (2004): 289–290.

38. L. J. Revell, "Phytools: An R Package for Phylogenetic Comparative Biology (And Other Things)," *Methods in Ecology and Evolution* 3 (2012): 217–223.

39. I. M. Pchelin, D. V. Azarov, M. A. Churina, et al., "Species Boundaries in the *Trichophyton mentagrophytes* / *T. interdigitale* Species Complex," *Medical Mycology* 57 (2019): 781–789.

40. C. Tang, S. A. Ahmed, S. Deng, et al., "Detection of Emerging Genotypes in *Trichophyton mentagrophytes* Species Complex: A Proposal for Handling Biodiversity in Dermatophytes," *Frontiers in Microbiology* 13 (2022): 960190, https://doi.org/10.3389/fmicb.2022.960190.

41. P. Nenoff, S. Uhrlaß, S. B. Verma, and S. Panda, "*Trichophyton mentagrophytes* ITS Genotype VIII and *Trichophyton indotineae*: A Terminological Maze, or Is It?," *Indian Journal of Dermatology, Venereology and Leprology* 88 (2022): 586–589.

42. D. Shaw, S. Singh, S. Dogra, et al., "MIC and Upper Limit of Wild-Type Distribution for 13 Antifungal Agents Against a *Trichophyton mentagrophytes-Trichophyton interdigitale* Complex of Indian Origin," *Antimicrobial Agents and Chemotherapy* 64 (2020): e0196419, https://doi.org/10.1128/AAC.01964-19.

43. F. Symoens, O. Jousson, C. Planard, et al., "Molecular Analysis and Mating Behaviour of the *Trichophyton mentagrophytes* Species Complex," *International Journal of Medical Microbiology* 301 (2011): 260–266.

44. O. Bontems, M. Fratti, K. Salamin, E. Guenova, and M. Monod, "Epidemiology of Dermatophytoses in Switzerland According to a Survey of Dermatophytes Isolated in Lausanne Between 2001 and 2018," *Journal of Fungi* 6 (2020): 1–8.

45. M. G. Frías-De-León, R. Hernández-Castro, T. Vite-Garín, et al., "Antifungal Resistance in *Candida auris*: Molecular Determinants," *Antibiotics* 9, no. 9 (2020): 568.

46. S. de Hoog, T. J. Walsh, S. A. Ahmed, et al., "A Conceptual Framework for Nomenclatural Stability and Validity of Medically Important Fungi: A Proposed Global Consensus Guideline for Fungal Name Changes Supported by ABP, ASM, CLSI, ECMM, ESCMID-EFISG, EUCAST-AFST, FDLC, IDSA, ISHAM, MMSA, and MSGERC," *Journal of Clinical Microbiology* 61 (2023): e00873-23.

47. A.-C. Normand, A. Moreno-Sabater, A. Jabet, et al., "MALDI-TOF Mass Spectrometry Online Identification of *Trichophyton indotineae* Using the MSI-2 Application," *Journal of Fungi* 8 (2022): 1103.

48. C. Tang, X. Zhou, J. Guillot, et al., "Dermatophytes and Mammalian Hair: Aspects of the Evolution of Arthrodermataceae," *Fungal Diversity* 125 (2024): 139–156.

49. P. Kumar, S. Ramachandran, S. Das, S. Bhattacharya, and B. Taneja, "Insights Into Changing Dermatophyte Spectrum in India Through Analysis of Cumulative 161,245 Cases Between 1939 and 2021," *Mycopathologia* 188 (2023): 1–20.

50. M. M. Alshahni, T. Yamada, A. Yo, et al., "Insight Into the Draft Whole-Genome Sequence of the Dermatophyte *Arthroderma vanbreuseghemii*," *Scientific Reports* 8 (2018): 15127, https://doi.org/10.1038/s41598-018-33505-9.

51. G. F. Persinoti, D. A. Martinez, W. Li, et al., "Whole-Genome Analysis Illustrates Global Clonal Population Structure of the Ubiquitous Dermatophyte Pathogen *Trichophyton rubrum*," *Genetics* 208 (2018): 1657–1669.

52. A. Singh, A. Masih, J. Monroy-Nieto, et al., "A Unique Multidrug-Resistant Clonal *Trichophyton* Population Distinct From *Trichophyton mentagrophytes*/*Trichophyton interdigitale* Complex Causing an Ongoing Alarming Dermatophytosis Outbreak in India: Genomic Insights and Resistance Profile," *Fungal Genetics and Biology* 133 (2019): 103266, https://doi.org/10.1016/j.fgb.2019.103266.

53. Y. Gräser, J. Fröhlich, W. Presber, and S. de Hoog, "Microsatellite Markers Reveal Geographic Population Differentiation in *Trichophyton rubrum*," *Journal of Medical Microbiology* 56 (2007): 1058–1065.

54. T. Ohst, S. De Hoog, W. Presber, V. Stavrakieva, and Y. Graser, "Origins of Microsatellite Diversity in the *Trichophyton rubrum-T. violaceum* Clade (Dermatophytes)," *Journal of Clinical Microbiology* 42 (2004): 4444–4448.

55. A. Čmoková, M. Kolařík, R. Dobiáš, et al., "Resolving the Taxonomy of Emerging Zoonotic Pathogens in the Trichophyton Benhamiae Complex," *Fungal Diversity* 104 (2020): 333–387.

56. A. Čmoková, M. Kolařík, J. Guillot, et al., "Host-Driven Subspeciation in the Hedgehog Fungus, *Trichophyton erinacei*, an Emerging Cause of Human Dermatophytosis," *Persoonia* 48 (2022): 203–218.

57. A. Čmoková, A. Rezaei-Matehkolaei, I. Kuklová, et al., "Discovery of New *Trichophyton* Members, *T. persicum* and *T. spiraliforme* spp. Nov., as a Cause of Highly Inflammatory Tinea Cases in Iran and Czechia," *Microbiology Spectrum* 9 (2021): e00284-21.

58. C. I. Aneke, A. Čmoková, V. Hubka, W. Rhimi, D. Otranto, and C. Cafarchia, "Subtyping Options for *Microsporum canis* Using Microsatellites and MLST: A Case Study From Southern Italy," *Pathogens* 11 (2021): e10004, https://doi.org/10.3390/pathogens11010004.

59. M. Pasquetti, A. Peano, D. Soglia, et al., "Development and Validation of a Microsatellite Marker-Based Method for Tracing Infections by *Microsporum canis*," *Journal of Dermatological Science* 70 (2013): 123–129.

60. T. Mochizuki, T. Futatsuya, K. Anzawa, et al., "Multilocus Microsatellite Analysis of the Molecular Epidemiology of *Microsporum canis* Isolated in Japan," *Medical Mycology Journal* 64 (2023): 63–72.

61. I. M. Pchelin, V. V. Zlatogursky, M. V. Rudneva, et al., "Reconstruction of Phylogenetic Relationships in Dermatomycete Genus *Trichophyton* Malmsten 1848 Based on Ribosomal Internal Transcribed Spacer Region, Partial 28S rRNA and Beta-Tubulin Genes Sequences," *Mycoses* 59 (2016): 566–575.

62. J. Woudenberg, M. Seidl, J. Groenewald, et al., "*Alternaria* Section *Alternaria*: Species, Formae Speciales or Pathotypes?," *Studies in Mycology* 82 (2015): 1–21.

63. F. Sklenar, K. Glassnerova, Z. Jurjevic, et al., "Taxonomy of *Aspergillus* Series *versicolores*: Species Reduction and Lessons Learned About Intraspecific Variability," *Studies in Mycology* 102 (2022): 53–93.

64. C. Bian, Y. Kusuya, F. Sklenář, et al., "Reducing the Number of Accepted Species in *Aspergillus* Series *Nigri*," *Studies in Mycology* 102 (2022): 95–132.

65. C. G. Boluda, V. Rico, P. Divakar, et al., "Evaluating Methodologies for Species Delimitation: The Mismatch Between Phenotypes and Genotypes in Lichenized Fungi (*Bryoria Sect. Implexae*, *Parmeliaceae*)," *Persoonia* 42 (2019): 75–100.

66. S. Hilário, M. F. Gonçalves, and A. Alves, "Using Genealogical Concordance and Coalescent-Based Species Delimitation to Assess Species Boundaries in the *Diaporthe eres* Complex," *Journal of Fungi* 7 (2021): 507, https://doi.org/10.3390/jof7070507.

67. P. M. Wang, X. B. Liu, Y. C. Dai, E. Horak, K. Steffen, and Z. L. Yang, "Phylogeny and Species Delimitation of *Flammulina*: Taxonomic Status of Winter Mushroom in East Asia and a New European Species Identified Using an Integrated Approach," *Mycological Progress* 17 (2018): 1013–1030.

68. A. Singh, A. Masih, A. Khurana, et al., "High Terbinafine Resistance in *Trichophyton interdigitale* Isolates in Delhi, India Harbouring Mutations in the Squalene Epoxidase Gene," *Mycoses* 61 (2018): 477–484.

69. D. Kolarczyková, P. Lysková, M. Švarcová, et al., "Terbinafine Resistance in *Trichophyton mentagrophytes* and *Trichophyton rubrum* in The Czech Republic: A Prospective Multicentric Study," *Mycoses* 67 (2024): e13708.

70. S. M. Rudramurthy, S. A. Shankarnarayan, S. Dogra, et al., "Mutation in the Squalene Epoxidase Gene of *Trichophyton interdigitale* and *Trichophyton rubrum* Associated With Allylamine Resistance," *Antimicrobial Agents and Chemotherapy* 62 (2018): e02522-17, https://doi.org/10.1128/aac.02522-17.

71. R. Kano, U. Kimura, H. Noguchi, and M. Hiruma, "Clinical Isolate of a Multi-Antifungal-Resistant *Trichophyton rubrum*," *Antimicrobial Agents and Chemotherapy* 66 (2022): e02393-21.

72. C. S. Osborne, I. Leitner, B. Favre, and N. S. Ryder, "Amino Acid Substitution in *Trichophyton rubrum* Squalene Epoxidase Associated With Resistance to Terbinafine," *Antimicrobial Agents and Chemotherapy* 49 (2005): 2840–2844.

73. H. R. Mahmood, M. Shams-Ghahfarokhi, Z. Salehi, and M. Razzaghi-Abyaneh, "Epidemiological Trends, Antifungal Drug Susceptibility and SQLE Point Mutations in Etiologic Species of Human Dermatophytosis in Al-Diwaneyah, Iraq," *Scientific Reports* 14 (2024): 12669.

74. M. Siopi, I. Efstathiou, K. Theodoropoulos, S. Pournaras, and J. Meletiadis, "Molecular Epidemiology and Antifungal Susceptibility of *Trichophyton* Isolates in Greece: Emergence of Terbinafine-Resistant *Trichophyton mentagrophytes* Type VIII Locally and Globally," *Journal of Fungi* 7 (2021): 419.

75. G. Blanchard, B. Amarov, M. Fratti, et al., "Reliable and Rapid Identification of Terbinafine Resistance in Dermatophytic Nail and Skin Infections," *Journal of the European Academy of Dermatology and Venereology* 37 (2023): 2080–2089.

76. C. F. Cañete-Gibas, J. Mele, H. P. Patterson, et al., "Terbinafine-Resistant Dermatophytes and the Presence of *Trichophyton indotineae* in North America," *Journal of Clinical Microbiology* 61 (2023): e00562-23.

77. A. Moreno-Sabater, A. C. Normand, A. L. Bidaud, et al., "Terbinafine Resistance in Dermatophytes: A French Multicenter Prospective Study," *Journal of Fungi* 8 (2022): 220.

78. P. Nenoff, S. B. Verma, A. Ebert, et al., "Spread of Terbinafine-Resistant *Trichophyton mentagrophytes* Type VIII (India) in Germany–'the Tip of the Iceberg?'," *Journal of Fungi* 6 (2020): 1–20.

79. T. M. C. Ngo, A. Santona, P. A. Ton Nu, et al., "Detection of Terbinafine-Resistant *Trichophyton indotineae* Isolates Within the *Trichophyton mentagrophytes* Species Complex Isolated From Patients in Hue City, Vietnam: A Comprehensive Analysis," *Medical Mycology* 62 (2024): myae088.

80. X. Kong, C. Tang, A. Singh, et al., "Antifungal Susceptibility and Mutations in the Squalene Epoxidase Gene in Dermatophytes of the *Trichophyton mentagrophytes* Species Complex," *Antimicrobial Agents and Chemotherapy* 65 (2021): e0005621.

81. A. Ebert, M. Monod, K. Salamin, et al., "Alarming India-Wide Phenomenon of Antifungal Resistance in Dermatophytes: A Multicentre Study," *Mycoses* 63 (2020): 717–728.

82. L. Z. Mohammadi, M. Shams-Ghahfarokhi, Z. Salehi, and M. Razzaghi-Abyaneh, "Increased Terbinafine Resistance Among Clinical Genotypes of *Trichophyton mentagrophytes*/*T. interdigitale* Species Complex Harboring Squalene Epoxidase Gene Mutations," *Journal of Medical Mycology* 34 (2024): 101495.

83. X. Fan, R.-C. Dai, S. Zhang, et al., "Tandem Gene Duplications Contributed to High-Level Azole Resistance in a Rapidly Expanding *Candida tropicalis* Population," *Nature Communications* 14 (2023): 8369.

84. J. Rhodes, A. Abdolrasouli, K. Dunne, et al., "Population Genomics Confirms Acquisition of Drug-Resistant *Aspergillus fumigatus* Infection by Humans From the Environment," *Nature Microbiology* 7 (2022): 663–674.

85. K. A. Etienne, E. L. Berkow, L. Gade, et al., "Genomic Diversity of Azole-Resistant *Aspergillus fumigatus* in the United States," *MBio* 12, no. 4 (2021): e0180321, https://doi.org/10.1128/mBio.01803-21.

86. S. Heidemann, M. Monod, and Y. Graser, "Signature Polymorphisms in the Internal Transcribed Spacer Region Relevant for the Differentiation of Zoophilic and Anthropophilic Strains of *Trichophyton interdigitale* and Other Species of *T. mentagrophytes Sensu Lato*," *British Journal of Dermatology* 162 (2010): 282–295.

87. I. Dhib, I. Khammari, A. Yaacoub, et al., "Relationship Between Phenotypic and Genotypic Characteristics of *Trichophyton mentagrophytes* Strains Isolated From Patients With Dermatophytosis," *Mycopathologia* 182 (2017): 487–493.

88. S. Taghipour, I. M. Pchelin, A. Zarei Mahmoudabadi, et al., "*Trichophyton mentagrophytes* and *T. interdigitale* Genotypes Are Associated With Particular Geographic Areas and Clinical Manifestations," *Mycoses* 62 (2019): 1084–1091.

89. M. Klinger, M. Theiler, and P. Bosshard, "Epidemiological and Clinical Aspects of *Trichophyton mentagrophytes*/*Trichophyton interdigitale* Infections in the Zurich Area: A Retrospective Study Using Genotyping," *Journal of the European Academy of Dermatology and Venereology* 35 (2021): 1017–1025.

90. A. Jabet, S. Dellière, S. Seang, et al., "Sexually Transmitted *Trichophyton mentagrophytes* Genotype VII Infection Among Men Who Have Sex With Men," *Emerging Infectious Diseases* 29 (2023): 1411.

91. S. M. Rudramurthy, D. Shaw, S. A. Shankarnarayan, Abhishek, and S. Dogra, "Comprehensive Taxonomical Analysis of *Trichophyton mentagrophytes*/*interdigitale* Complex of Human and Animal Origin From India," *Journal of Fungi* 9, no. 5 (2023): 577.

92. M. N. Kumar, P. Thomas, V. A, et al., "Molecular Epidemiology of *Trichophyton* Infections Among Canines From Northern India," *Journal of Medical Mycology* 33, no. 1 (2022): 101352.

93. V. Oladzad, A. N. Omran, I. Haghani, M. Nabili, S. Seyedmousavi, and M. T. Hedayati, "Multi-Drug Resistance *Trichophyton indotineae* in a Stray Dog," *Research in Veterinary Science* 166 (2024): 105105.

94. S. Nikkholgh, I. M. Pchelin, A. Zarei Mahmoudabadi, et al., "Sheep Serve as a Reservoir of *Trichophyton mentagrophytes* Genotype V Infection," *Medical Mycology* 61 (2023): myad066.

95. C. Kupsch, V. A. Czaika, C. Deutsch, and Y. Graser, "*Trichophyton mentagrophytes* – a New Genotype of Zoophilic Dermatophyte Causes Sexually Transmitted Infections," *Journal der Deutschen Dermatologischen Gesellschaft* 17 (2019): 493–501.

96. T. Yamada, M. Maeda, M. M. Alshahni, et al., "Terbinafine Resistance of *Trichophyton* Clinical Isolates Caused by Specific Point Mutations in the Squalene Epoxidase Gene," *Antimicrobial Agents and Chemotherapy* 61 (2017): e00115-17, https://doi.org/10.1128/aac.00115-17.

97. S. O. Suh, K. M. Grosso, and M. E. Carrion, "Multilocus Phylogeny of the *Trichophyton mentagrophytes* Species Complex and the Application of Matrix-Assisted Laser Desorption/Ionization-Time-Of-Flight (MALDI-TOF) Mass Spectrometry for the Rapid Identification of Dermatophytes," *Mycologia* 110 (2018): 118–130.

98. R. De Paepe, A.-C. Normand, S. Uhrlaß, P. Nenoff, R. Piarroux, and A. Packeu, "Resistance Profile, Terbinafine Resistance Screening and MALDI-TOF MS Identification of the Emerging Pathogen *Trichophyton indotineae*," *Mycopathologia* 189 (2024): 29.

99. S. Hainsworth, V. Hubka, A. C. Lawrie, D. Carter, T. Vanniasinkam, and D. Grando, "Predominance of *Trichophyton interdigitale* Revealed in Podiatric Nail Dust Collections in Eastern Australia," *Mycopathologia* 185 (2020): 175–185.

100. P. Nenoff, S. B. Verma, R. Vasani, et al., "The Current Indian Epidemic of Superficial Dermatophytosis due to *Trichophyton mentagrophytes*-A Molecular Study," *Mycoses* 62 (2019): 336–356.

101. S. Uhrlaß, S. Mey, D. Koch, et al., "Dermatophytes and Skin Dermatophytoses in Southeast Asia—First Epidemiological Survey From Cambodia," *Mycoses* 67 (2024): e13718.